FATTY ACIDS IN NEUTRAL LIPIDS AND PHOSPHOLIPIDS FROM CHICKEN TISSUES

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ABSTRACT

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by Michael Alexander Katz

Lipids from skin, depot fat, dark and white meat from broiler type male chickens were fractionated into neutral and phospholipids by column chromatography. Lipid fractions were measured gravimetrically as a percentage of total lipids. The fatty acids of each fraction were methylated and analyzed using gas-liquid chromatography. Fatty acid content was calculated as a percentage of the total fatty acids in a fraction.

Lipids in muscle tissues were found to contain relatively large quantities of phospholipids, while lipids in skin and depot fat contained small amounts. The phospholipid content of dark meat, white meat, skin, and depot fat, was 21, 48, 2, and 0.9 percent respectively.

The neutral lipid fraction contained at least 18 different fatty acids. The percentage distribution of the fatty acids in the neutral lipids was similar in all four tissues. Palmitic, palmitoleic, stearic, oleic, and linoleic acids dominated the fatty acids in the neutral lipids, and amounted to 94 percent of the total.

The phospholipid fraction contained at least 22 fatty acids. The predominant acids in this fraction were palmitic, stearic, oleic, linoleic, and arachidonic acid. These acids accounted for about 75 percent of the total

fatty acids. The total fatty acid content of the phospholipids varied among the different tissues. Generally, the dark and white muscle tissues had a similar fatty acid composition, which was different from that of skin and depot fat in the content of C_{20} to C_{24} fatty acids.

The percentage of total molecules of unsaturated fatty acids was approximately the same in the lipids from the four tissues examined. However, the phospholipids contained more long chain fatty acids with four or more double bond, and therefore had a higher degree of unsaturation than the neutral lipids.

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By

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Michael Alexander Katz

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INTRODUCTION

Poultry meat consumption in the United States has increased from 24.7 pounds per capita in 1950, to over 38.2 pounds in 1964 (2). A large portion of poultry meat has been utilized as boneless meat, processed meat, or in commercial freeze dried products. Since the processed products may be used in combination with other foods and may require a longer shelf life than fresh meat, the needs for detailed compositional data of the meat have increased to aid preservation.

The main problem in processed and stored meat is flavor stability. Oxidative rancidity is a major cause for flavor deterioration. Therefore, the lipids present in fatty tissues as well as lipids present in muscle tissues, may affect flavor quality, and be responsible for problems related to product utilization and stability.

The susceptability of natural fat to oxidative rancidity depends largely upon its degree of unsaturation and its fatty acid composition. Fatty acid composition and metabolism have received considerable research attention, although major emphasis has been on problems related to laying hens, eggs, body fluids and organs, and depot fat. Detailed fatty acid analysis of broiler muscle tissues has received little research attention.

The great variety of fatty acids present in meat makes a detailed analysis difficult. Many of the fatty acids are present in small quantities, and being homologous compounds, they do not separate readily. For such related

compounds, chromatographic analysis has become a suitable means for separation and identification. Gas chromatography, a recent and sensitive tool in this area, enables rapid identification and quantification of fatty acids.

The objective of this research was to determine the actual amounts and percentages of fatty acids present in the neutral and phospholipid fractions from lipids found in chicken skin, muscle, and depot fat.

LITERATURE REVIEW

Chicken Fat:

In general, animal fats may contain as many as 35 different fatty acids, however, myristic, palmitic, palmitoleic, stearic, oleic, and linoleic acids comprise 90 percent of the total amounts present (26). Fats from different animal species differ in their rate of oxidative rancidity. Unsaturated fatty acids tend to oxidize faster than saturated acids (39), and the susceptability of fat to rancidity is therefore related to its fatty acids composition.

Poultry fat is more unsaturated than beef, lamb and pork fat (5), and has a total unsaturation of 60-70 percent (5, 19, 20). Because of its unsaturated nature, poultry meat tends to become rancid faster than beef or lamb. Dietary fat affects and reflects the composition of body fat in the chicken (16, 35), and generally body tissues tend to assume the fatty acid composition of the fat in the diet (39). Triglycerides in blood plasma and adipose tissue reflect more closely dietary fatty acids than do the phospholipid fractions (27).

Many fatty acids can be synthesized in the body, and polyunsaturated acids such as arachidonic, originate from linoleic and linolenic in mammals (55). Linoleic acid is considered to be an essential fatty acid for chickens, and is involved in the synthesis of arachidonic acid (35, 34, 48). Linolenic acid is involved in the synthesis of several polyunsaturated fatty acids (48).

Oxidative rancidity of fat is influenced greatly by the unsaturated fatty

acids and natural antioxidants present. Phospholipids were reported to oxidize in freeze dried beef prior to neutral lipids (13). The phospholipids contain more unsaturated fatty acids than neutral lipids, and their presence renders lipids more susceptible to oxidation, which is the major factor in deteriorative reactions leading to flavor degradation (33, 56). The majority of arachidonic acid in chicken muscle was found in the phospholipids (44), thus suggesting this fraction to be an important source of rancidity. Research has shown that more long chain polyunsaturated fatty acids are found in muscle than in skin or adipose tissues (39).

Depot fat was reported to be similar in composition to body fat in chickens, but different in other animals (49, 8). Recently, differences in composition of muscle tissues from skin and adipose fat were reported (39). However, since skin and adipose fat are low in phospholipids and polyunsaturated fatty acids (56, 27), compositional difference between these tissues and muscle tissue may be quantitative rather than qualitative. Lipid fractionation, therefore, is important for the detection of polyunsaturated fatty acids which are present mainly in the phospholipid fraction. Only a trace amount of these acids is found in unfractionated poultry fat (44).

Lipid Extraction:

Different solvents can be used to extract lipids from a tissue. Comparative investigations of different solvent systems for lipid extraction (10) showed that the method in which total lipids are extracted with chloroform and methanol 2:1 (v/v) is preferred (15). The removal of non-lipid material is accomplished by adding water to a final volume of 8:4:3 chloroform-methanol-

water. The same solvent system, with different proportions, was used for a rapid lipid extraction which involves a mild and short treatment to minimize oxidative decomposition (3).

Fractionation of Neutral Phospholipids:

Phospholipids are usually more satisfactorily adsorbed on adsorbents than are neutral lipids and fatty acids. The problem, however, was to find an adsorbent from which it is possible to elute the phospholipids quantitatively (4). After investigating several adsorbents, silicic acid was found to be "the best one" (4). Neutral lipids and free fatty acids can be eluted from a silicic acid column with chloroform, while the phospholipids are adsorbed on the column at the rate of 30 mg/g of silicic acid. The phospholipids are then eluted quantitatively with methanol.

Many workers since have used silicic acid for the fractionation of neutral and phospholipids (51, 9, 13, 17, 32). This separation is possible because of polarity differences of eluting solvents. The polarity of chloroform can be increased by mixing with methanol or water to further fractionate phospholipids (51).

Non-lipid material present in the lipid fraction, is not eluted from the silicic acid column with chloroform or methanol. Such impurities may consist of pigments, oxidation products, or denatured protein material, and can be eluted with acetone (32, 13, 25, 9).

Although ninhydrin is used mainly for the detection of protein material, it can also be used to detect certain phospholipids (6). Ninhydrin test was used extensively in the detection of phospholipids such as phosphatidyl and

lysophosphatidyl ethanolamine, phosphatidyl and lysophosphatidyl serine, sphingomyelin, and others (9, 32, 52, 51, 46, 6).

Methyl Esterification of Fatty Acids:

This procedure involves the liberation of fatty acids from the lipids by saponification, acid hydrolysis, or enzymatic hydrolysis. Esters of the fatty acids may then be prepared by a variety of methods which require acid catalysis of the esterification reaction (41). The chief reaction of fatty acids with sulfuric acid involves the formation of acylium ion-sulfuric acid complex. Acylium ion is then combined with methanol to form methyl esters.

Saponification of lipids followed by methyl esterification with acidified methanol was reported with various modifications (36, 21, 28). Acid hydrolysis of fats and their methyl esterification was accomplished by refluxing with dry HCl methanol solution (12, 28, 54). Hydrolysis of fats with H_2SO_4 and esterification with methanol HCl solution on a basic ion exchange resin was also reported (24). A rapid method for the hydrolysis and esterification of fatty acids with excess H_2SO_4 at low temperature was described recently (41). Methylation of fatty acids has also been accomplished with boron trifluoride-methanol solution (43), and with diazomethane (53).

Direct interesterification of triglycerides with methanol in ether containing KOH was also reported (28).

Chromatography:

Chromatography is an analytical procedure frequently used for the separation of closely related compounds. Fundamentally the technique uses a

two phase system: a stationary phase and a mobile phase. The stationary phase may be a solid (adsorption chromatography) or a liquid (partition chromatography) (50). Adsorption chromatography includes column and thin layer chromatography, and partition chromatography includes gas-liquid chromatography. One advantage of gas chromatography over other analytical procedures is the rapidity of identification and quantification, which are accomplished simultaneously.

A. Adsorption chromatography:

Adsorption chromatography consists of a stationary solid (adsorbent) and a mobile liquid. In column chromatography, the solid is held in a tube, while the liquid moves past it carrying the sample. Thin layer chromatography is essentially an open column chromatography, in which the solid stationary phase is coated over glass plates.

The adsorbents used in adsorption chromatography adsorb polar compounds to their surface by electrostatic forces (the same forces which hold the crystal lattice together). These electrical surface forces induce dipole moment in non-polar compounds, and increase the existing dipole moments of the polar compound (47). The rate of migration of a compound on a given adsorbent, depends upon the solvent used. Solvents have different elutive power (47) and polar solvents have greater elution power than non-polar solvents (38). A mixture of solvents gives a better separation than one solvent (38, 47).

The detection of a compound separated by adsorption chromatography

is accomplished colorimetrically by special applications during the developing (migration) stage. Fractions resolved by adsorption chromatography may be isolated and further analyzed by complementary methods such as gas-liquid chromatography (GLC).

B. Partition chromatography:

Partition chromatography is accomplished by partition between two immiscible liquids, one of which is held stationary while the other liquid moves past it. In gas-liquid chromatography, the mobile phase is an inert gas which is compressible to produce a gradient of gas velocity down the column. The sorbent is a non-volatile liquid coated on a finely divided inert solid support which is enclosed in a column. The sample is introduced as a narrow band of vapor into the continuous stream of carrier gas. The volatilized sample is distributed between the moving gas and the stationary liquid, and the individual components move down the column at a rate which depends on their partition coefficient. Stationary phases differ in holding capacity (or retention value) of the dissolved sample molecules, and those which hold the dissolved molecules by Van de Waals forces (induced dipoles) have a lower retention value than those permitting hydrogen bonding (50). The relative retention value of homologous series give a linear relation when plotted, whose slope depends on the amount of hydrogen bonding (29).

The various components of the sample are detected by passing the carrier gas, with the eluted sample, through a suitable flame ionization detector. Detection is based on the measurement of electrical conductivity of gases in hydrogen flame. The conductivity of hydrogen burning in air is low, but,

when an organic substance is fed into the flame, the conductivity increases (23). The detector is connected to a recorder pen which records, by deflection, peaks corresponding to concentration of the eluate over its retention time. Since retention value is specific to a substance, both quality and quantity are recorded simultaneously.

Gas-liquid chromatography is a most valuable tool for the analysis of fatty acids. The theory of gas chromatography was introduced in 1952 (29) as a method for separating carboxylic acids up to 12 carbon atoms. Later the method was extended to the separation of methyl esters of fatty acids (7). Methyl esters have lower boiling points than fatty acids, thus they vaporize at lower temperatures and minimize the danger of decomposition (45). Today GLC has been adapted for use in many diversified areas.

Initially non-specific liquid phases, such as silicone oil or apiezon L greases, were used for column separation. With these liquid phases it is difficult to separate isomers with the same carbon number. The polyester type liquid phases gave the necessary separation efficiency. Of these types, adipate and succinate polyesters of diethelene glycol are the most widely used (14). The addition of phosphoric acid to the liquid phase proved to be useful in retaining symmetrical peaks for long chain fatty acids (42).

Retention value in GLC depends on the type and amount of liquid phase, temperature, gas flow, and type of compound analyzed (45). Satisfactory application of GLC to fatty acid analysis, however, depends also on careful control of temperature and sample size (22), making it a delicate operation.

The measurement of peak area gives a direct weight percentage measurement for methyl esters of fatty acids with over eight carbon atoms (22, 14). Several ways to measure the peak area have been suggested (22), but using the integrator recordings for the measurement is simplest.

Identification of the different peaks of a chromatograph can be accomplished by comparing their retention value to that of a standard. Since all standards are not readily available, a standard curve can be plotted, in which the log retention time is plotted against carbon number (28). This method of identifying components is widely used. A more accurate identification is achieved by using different column packings, and by plotting the log retention time of the standards on a polar column vs the log retention time on a non-polar column (28). On both curves, homologous compounds with the same degree of saturation form a linear relation.

Some compounds may have the same retention value at a given operating condition, thus appearing in a common peak. These compounds may be resolved by programming the temperature, using different isothermal temperature (1), and using new and old columns (37).

EXPERIMENTAL PROCEDURE

Meat Preparation:

Twelve 14 weeks old male broilers obtained from a commercial farm were killed, defeathered and eviscerated in the usual manner, and chilled in crushed ice for four hours. From this group, four uniform birds weighing 2 kg each were selected for analysis. Muscle samples (100 g each) were removed from the corresponding one-half of each carcass. Samples of white meat were taken from the breast muscles and dark meat samples were taken from the thigh and drumstick. Fifty g of skin were used for the skin sample, and all removable depot fat from the abdominal cavity, after the removal of the viscera, was used for depot fat sample. Muscles and skin tissues were ground prior to lipid extraction.

Lipid Extraction:

Total lipids were extracted with chloroform-methanol-water solution 8:4:3 (V/V/V) (15, 3). A 100 g ground sample (76 percent water content) was blended in a Waring Blender for two minutes with 100 ml chloroform, 200 ml methanol, and 4 ml water. One hundred ml chloroform was added and the mixture blended for 30 minutes after which 100 ml water was added and the mixture blended for an additional 30 minutes. The resulting mixture was then filtered on a Buchner funnel, and the residue plus the filter paper was blended for one minute in 100 ml chloroform, filtered, and rinsed with 180 ml chloroform and 40 ml methanol.

The filtrates were collected in a separatory funnel and held at 30°C

overnight to facilitate separation. A biphase liquid system was formed. The upper phase of water and methanol was discarded, and the lower phase, containing the lipids in chloroform and some methanol, was saved for analysis.

Separation of Neutral Lipids from Phospholipids:

Fractionation of the lipids was accomplished on a silicic acid column (4). A multiple column system (31) was used, where four columns were connected in a series to a nitrogen source by a rubber hose. A special valve, which eliminated high pressure buildup in the column, was attached to the rubber hose (see Appendix E).

A. Preparation and packing of the column:

Silicic acid (80-100 mesh) was washed with distilled water to remove the fines. Water was then removed from the silicic acid on a Buchner funnel, and this was followed by a methanol rinse. The washed silicic acid was activated by holding overnight at 105°C.

A 10 g sample of activated silicic acid-chloroform slurry was poured into a glass column (1 cm in diameter), fitted with a glass wool stopper in its lower end. The silicic acid was allowed to settle and the chloroform drained under slight nitrogen pressure. After the silicic acid settled, a 2-3 cm layer of granular anhydrous sodium sulfate was placed on top of the silicic acid. The column was rinsed several times with chloroform and was ready for use.

B. Neutral and phospholipid fractions:

A solvent column containing about 0.2 g crude lipids was placed in the column. The sample was eluted from the column under nitrogen pressure, at the rate of two drops per second, into drying flasks. The chloroform eluant of the sample was taken as neutral lipids, and the methanol eluant as phospholipids. Neutral lipids were eluted until a negative Salkowski test was achieved, and phospholipids were eluted until a negative ninhydrin test was reached. Purity was further tested by checking the neutral lipids with ninhydrin, and the phospholipids by micro thin layer chromatography (TLC). New column packing material was used for each sample, and the non-lipid material present in the lipid fraction was discarded.

The neutral and phospholipid fractions were then prepared for fatty acids esterification, or dried for the gravimetric determination of total neutral and phospholipids.

C. Spot tests used in the fractionation of the lipids:

Salkowski test. A sample dissolved in chloroform was carefully added to an equal volume of concentrated sulfuric acid in a test tube. The development of a characteristic yellow band in the test tube indicated the presence of lipids in the chloroform.

Ninhydrin test. Ninhydrin was added to the methanol eluant in a test tube, placed in a sand bath at 150°C, and shaken vigorously periodically. The development of a bluish color indicated the presence of phospholipids.

Micro TLC. Micro slides (2.5 x 7 cm) were covered with adsorbent by dipping them into a suspension of silica gel G in chloroform. The chromoslides

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were allowed to dry, spotted with phospholipid fraction, and eluted in an ascending manner with petroleum ether, ethyl ether, and acetic acid (90: 10:1 by volume). The slides were sprayed with sulfuric acid followed by charring. Migration of the original spot or streaking, indicated presence of neutral lipids.

D. Percent of neutral and phospholipids:

A known quantity of crude lipids was fractionated on a silicic acid column. The fractions were evaporated in tared flasks on a Rinco vacuum evaporator at 50°C, and dried for 10 minutes in an oven at 110°C. The flasks were then placed in a dessicator and allowed to equilibrate overnight, and then weighed. The combined weight of the dried neutral and phospholipids was taken as 100 percent of the lipid content.

Preparation of Methyl Esters:

Fatty acids were esterified at low temperature (41). A solvent volume containing not more than 200 mg neutral or phospholipids was concentrated in a flask on a Rinco vacuum evaporator in a 40°C water bath. The lipids were dissolved in 20 ml ethyl ether and the flask placed in a dry ice acetone bath on a magnetic stirrer. When the dissolved lipids reached the bath temperature, 2 ml of concentrated H_2SO_4 were added dropwise to the stirred sample. The flask was then corked and stirred for 10 minutes in the bath. Fifteen ml of absolute methanol was added followed by 22 ml of 25 percent methanolic KOH. The mixture was then removed from the bath, phenolpthalein added to ascertain the presence of enough base, and stirred to red color.

The mixture was quantitatively transferred to a 250 ml separatory funnel with 200 ml distilled water and the fatty acids extracted with 20 and 15 ml petroleum ether (30-60°C B.P.). The ether extracts were dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated in a 15 ml graduated centrifuge tube over 60°C water bath, under a nitrogen atmosphere. Neutral lipid solutions were concentrated to 0.2 ml, and phospholipid solution to 0.1 ml.

The concentrated solutions of methyl esters of fatty acids were transferred, with a syringe, to small tubes, made from No. 4 glass tubing by heat sealing one end, and stoppered with parafilm. These samples were kept at 0°C in the dark for not more than 12 hours prior to GLC analysis.

The completeness of methyl esterification was checked by TLC. Chromatographic plates were prepared with a Desaga applicator by making a slurry of 25 g silica gel G with 50 ml distilled water. The chromoplates were activated at 110°C for 35 minutes. Samples were spotted and developed in an ascending manner in petroleum ether-ethyl ether-glacial acetic acid solution 90:10:1 (by volume). The plates were sprayed with sulfuric acid and charred to visualize the methyl esters and non-esterified material.

Gas-liquid Chromatography:

Fatty acid methyl esters were fractionated in an F & M (Model 810) dual column gas chromatograph, equipped with a flame ionization detector and a disc chart integrator. Helium was used as the carrier gas at a flow rate of 35 ml/min. The hydrogen flame was fed by using 60 ml/min hydrogen and 170 ml/min compressed air. A 72 x 1/4" copper column was packed

with 15 percent diethylene glycol succinate and 3 percent phosphoric acid as liquid phase and chromosorb-W as solid support. The column temperature was run isothermally at 190°C, with detector temperature at 260°C and injector at 250°C. Attenuation was 10^2 x 32 for neutral lipids and 10^2 x 4 for phospholipids.

A. Preparation of column:

Three g of phosphoric acid were dissolved in distilled water and mixed with 32 g acid washed, 80-100 mesh, chromosorb-W. The compounds were mixed in a round bottom flask, on a Rinco rotator, and dried in an oven. Fifteen g of diethylene glycol succinate (DEGS) dissolved in chloroform were added to the dried mixture and enough chloroform to assure proper mixing was added. After mixing and evaporation on the Rinco evaporator, the residual chloroform was removed in an air oven, and the dried mixture was ready for packing.

Copper column tubings were packed with the aid of an electrical vibrator. Glass wool plugs were placed in both ends of the column to prevent loss of packing. The packed columns were conditioned for two days at 220°C and 35 ml/min helium flow before using.

B. Calculation:

Percentage composition of the fatty acids was calculated from the peak area measurement associated with each fatty acid. Peak area was calculated from the pen traces made under the peak by the disc chart integrator, taking the total tracings as 100 percent.

Two isothermal runs at different sensitivities were made, and the final results were calculated with a reference to methyl myristate which was well defined in each run.

C. Identification:

Methyl esters of C 8:0,[1] 10:0, 12:0, 14:0, 14:1, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:0, 20:4, 20:5, 22:0, and 22:6 were identified by comparing the retention time to that of pure samples. Using the above standards, the log of retention time was plotted against carbon number to give a standard curve which was used in the identification of other fatty acids.

Identification of the extremely short and long fatty acids was aided with programming, use of old and new columns at the same operating conditions, use of the same column at different temperatures, and the use of a non-polar apiezon L column.

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[1]The first number indicates the carbon chain length. The colon is followed by the number of double bonds per molecule, if any (11).

RESULTS AND DISCUSSION

Total, Neutral, and Phospholipids:

The percentage of total lipids in dark and white meat, skin, and depot fat varied considerably as expected. Total lipids varied on a wet weight basis from 1 percent in white meat to 60-80 percent in depot fat, as shown in Table 1. In agreement with other work (5), dark meat had twice as much lipid as did white meat. Generally, the lipid content of skin varied with the apparent amount of fat deposited.

Table 1. Total lipid, phospholipid, and neutral lipid content of poultry tissues

Tissue	Lipids		
	Total[1]	Phospholipids[2]	Neutral[2]
		percent	
White meat	1.0	48	52
Dark meat	2.5	21	79
Skin	25	2	98
Depot fat	60-80	0.9	99.1

[1]As percentage of raw tissue.

[2]As percentage of total lipids.

Separation of the lipids into neutral and phospholipids, revealed additional differences among tissues (Table 1). White meat, the lowest in total lipids, contained almost equal amounts of neutral and phospholipids. As the total lipid content in tissues increased, the ratio of neutral to

phospholipids increased. Depot fat tissue, the richest in total lipids, contained 99 percent neutral lipids and only 1 percent phospholipids. These results are in general agreement with other reports. A large content of phospholipids in skeletal muscle of birds was reported (9,17) where lipids of pigeon breast muscle contained over 40 percent phospholipids. Lipids of adipose tissue in hens were reported to have only 2 percent phospholipids (27), and skin lipids were reported to be similar to abdominal adipose tissue (40).

Many workers (8, 16, 20, 36) who studied the composition of fatty acids in chicken tissues, concentrated their investigations on fat rich tissues such as skin and depot fat. These tissues contain relatively small amounts of phospholipids, and are therefore relatively low in polyunsaturated acids. Due to such minute quantities present, it is probable that the long chain polyunsaturated fatty acids, above arachidonic acid, were not detected, and thus not reported. The presence of long chain polyunsaturated fatty acids in muscle tissue lipids, and their absence in skin and depot fat has been reported (39). However, this observation may have been affected by the high concentration of phospholipids in muscle tissues and the low concentration of these lipids in skin and depot fats as shown in Table 1. For a detailed study of polyunsaturated fatty acids in poultry tissues, the phospholipids should be fractionated before analysis, since more of the polyunsaturated fatty acids and those with carbon number greater than C_{20}, are present in this fraction, as shown in Tables 2 and 3. The low level of the phospholipids and consequently of some of the long chain

polyunsaturated fatty acids in poultry tissues which are rich in fat, may account for the apparent lack of compositional changes in lipids from these tissues, due to oxidative deterioration (5, 10). It is doubtful that an examination of the unfractionated lipids could detect compositional changes.

Table 2. Fatty acids of the neutral lipids[1]

Fatty[2] acid	Source of fatty acids			
	Dark meat	White meat	Skin	Depot fat
	percent			
8:0	tr	tr	tr	tr
10:0	tr	tr	tr	tr
12:0	tr	tr	tr	tr
14:0	0.8	1.0	0.7	0.7
14:1	0.3	0.3	0.3	0.2
15:0	0.2	0.2	0.2	0.2
16:0	22.4	24.2	23.1	22.8
16:1	6.7	5.5	5.7	5.7
16:2	0.5	0.5	0.4	0.5
17:0	0.2	0.3	0.2	0.2
18:0	6.4	5.9	5.9	6.5
18:1	34.6	34.6	37.0	37.0
18:2	24.7	24.7	24.0	23.7
18:3	1.5	1.3	1.3	1.3
20:1	0.5	0.5	0.6	0.6
20:2	0.3	0.2	0.2	0.2
20:3	0.2	0.3	0.1	0.1
20:4	0.5	0.5	0.2	0.2

[1]Calculated as percentage of the total fatty acids in the neutral lipids.

[2]Number of carbons : number of double bonds.

Table 3. Fatty acids of the phospholipids[1]

Fatty[2] acid	Source of fatty acids: Dark meat	White meat	Skin	Depot fat
	percent			
10:0	tr	tr	tr	tr
12:0	tr	tr	tr	tr
14:0	3.2	0.1	3.65	1.8
14:1	0.3	0.1	0.5	1.1
15:0	0.7	0.3	0.7	0.8
16:0	14.6	23.0	22.1	18.7
16:1	1.6	0.9	3.1	5.0
16:2	0.5	0.3	0.7	0.7
17:0	0.4	0.4	1.0	0.6
18:0	16.6	9.8	11.8	9.0
18:1	13.5	16.3	18.5	25.5
18:2	19.8	17.0	14.8	22.3
18:3	0.6	0.5	1.0	1.7
20:1	0.6	0.6	0.8	1.2
20:2	0.8	0.8	0.9	0.7
20:3	1.2	1.5	1.6	0.6
20:4	16.8	15.1	11.4	4.6
22:2	0.4	0.7	1.1	0.9
22:3	0.3	0.7	0.6	0.7
22:4	2.5	3.0	2.7	1.3
24:1	0.9	1.0	1.7	1.6
22:5-24:2	1.5	1.7	0.9	0.2
22:6-24:4	3.3	3.9	1.4	1.3

[1]Calculated as percentage of total fatty acids in the phospholipids.

[2]Number of carbons : number of double bonds.

Fatty Acids from Neutral Lipids:

The fatty acid composition of the neutral lipid fractions from the different tissues was calculated as a percentage of the total fatty acids in these fractions. Results are shown in Table 2. Eighteen different fatty acids were identified and quantified from the neutral lipid fraction from dark and white meat, skin, and depot fat tissues. The similarity in the relative amount of the fatty acids found in neutral lipids from these four tissues is of major significance. Such similarity in the triglyceride content has been previously reported (17). The most prevalent fatty acids in these fractions had 16 carbon atoms (palmitic and palmitoleic), and 18 carbon atoms (stearic, oleic, and linoleic acid). These C_{16} and C_{18} fatty acids amounted to 94 percent of the total fatty acids in the neutral lipids. Palmitic acid accounted for a major portion of the saturated fatty acids (about 23 percent out of 30 percent). A large percentage of the unsaturated acids were oleic and linoleic acids which amounted to about 60 percent. This distribution of fatty acids is quite typical and was reported by other workers (27, 26). Saturated fatty acids with odd numbers of carbon atoms were minor components in all the tissues, and their presence was previously reported in depot fat (35, 27). However, since the identification of these acids was established indirectly by a comparison to the standard plot, these odd carbon peaks possibly may correspond to dimethylacetals of aldehydes of other fatty acids.

Fatty Acids from the Phospholipids:

Twenty-two fatty acids were identified and quantified in the phospholipid

fraction from the lipids obtained from the dark and white meat, skin and depot fat of broilers. The predominant fatty acids in this fraction were palmitic, stearic, oleic, linoleic, and arachidonic acids. These acids accounted for about 75 percent of the total fatty acids. The fatty acid content of the phospholipids varied among the different tissues. Although fatty acid composition from the white and dark meat phospholipids were similar, they differed from that found in skin and depot fat. The composition of fatty acids from skin phospholipids, resembled phospholipids from depot fat more than the phospholipids from muscle tissue.

The most apparent difference in the phospholipid fatty acid composition of the different tissues was a variation in the arachidonic acid content. The percentage of arachidonic was lower in the lipid rich tissues (skin and depot fat) than in the muscle lipids: only 4 percent arachidonic acid was found in the depot fat as compared to 16 percent in the dark muscle meat. Phospholipids from dark meat contained the lowest percentage of palmitic, and the highest percentage of stearic and arachidonic acids. Phospholipids from the white meat lipids were low in palmitoleic, high in arachidonic, and contained equal amounts of oleic and linoleic acids. Depot fat phospholipids were highest in palmitoleic, oleic, and linoleic, and lowest in arachidonic acid. The fatty acid composition from the skin phospholipids resembled those from depot fat, with the exception of arachidonic acid.

All tissues examined contained small amounts of saturated fatty acids with 15 and 17 carbons. Such acids were previously found in phospholipids of pigeon (17), and chicken (44, 27). Detailed comparative quantitative

investigation of total fatty acids in the neutral lipids and the phospholipids from poultry tissues has not been reported. However, similar work on total fatty acids in the phospholipids confirmed the presence of the acids reported here. Investigation of the fatty acids in specific phospholipids (17, 46) indicated that arachidonic acid is a major component of several phospholipids, and that 19.2 and 16.3 percent arachidonic acid was present in the phospholipid fraction of pork and beef, respectively (25).

Fatty acids from the phospholipids had a larger variation in their relative distribution in the same tissue of different birds, than those from the neutral lipids. These differences are due to biological variability and are shown in Appendix C. Similar differences were observed previously (18, 25).

Total Unsaturation:

The weight percentage of total molecules of unsaturated fatty acids was approximately the same in the lipids from the four tissues examined, as shown in Table 4. The fatty acids in the neutral lipids had only slightly more unsaturated molecules than the fatty acids in the phospholipids. Similar fatty acid characteristics were found in plasma and adipose lipids of laying hens (27). However, the phospholipids contained more long chain fatty acids with four or more double bonds than the neutral lipids. These fatty acids make the phospholipids more susceptible to oxidative deterioration, which is more severe in polyunsaturated than in saturated fats (33, 56).

The results obtained in this experiment support previous suggestion

that flavor deterioration may result from fat oxidation in muscle tissue (39), and not only from oxidation in fatty tissues. The relatively large quantities of highly unsaturated fatty acids, helps to provide an explanation for the relative ease with which oxidative rancidity occurs in poultry meat. Based on the polyunsaturated fatty acids present in the phospholipids, muscle tissues have the same potential for oxidation as does depot fat.

Table 4. Degree and type of unsaturation of the fatty acids in poultry tissues

No. of double bonds	Source of fatty acids			
	Dark meat	White meat	Skin	Depot fat
a.) Fatty acids of the phospholipids				
		Weight percent		
0	34.5	34.6	37.3	30.2
1	16.9	19.0	25.1	32.8
2	21.4	19.1	17.4	23.9
3	2.1	2.1	3.2	2.6
4	19.8	18.3	14.1	5.9
5	1.5	1.7	0.9	0.3
6	3.3	3.9	1.4	1.3
0 (odd C#)	1.0	1.3	1.7	1.5
Unsaturated fatty acids	64.5	64.1	61.0	68.2
b.) Fatty acids of the neutral lipids				
		Weight percent		
0	28.9	31.2	29.7	30.1
1	42.2	40.1	43.5	43.6
2	25.5	25.5	24.7	24.4
3	1.7	1.6	1.5	1.4
4	0.5	0.5	0.2	0.2
0 (odd C#)	0.4	0.5	0.3	0.3
Unsaturated fatty acids	70.7	68.3	69.0	69.3

SUMMARY AND CONCLUSIONS

Lipids from poultry skin, depot fat, and muscle tissues, contain essentially the same fatty acids. Differences in the fatty acid content are quantitative rather than qualitative, and are due to the variation in total lipids and phospholipids content of the tissues.

The lipids from muscle tissues contained a large percentage of phospholipids, while lipids from skin and depot fat contained a small percentage of phospholipids. At least 22 fatty acids, varying in chain length from C_{10} to C_{24}, were found in the phospholipid fraction. The predominant acids in this fraction were palmitic, stearic, oleic, linoleic, and arachidonic acid. Arachidonic acid accounted for the main difference in the fatty acids content of the phospholipids in the tissues examined. The percentage of arachidonic acid was high in muscle tissues, and decreased with the increase of total lipids in depot fat and skin tissues.

The relative distribution of the fatty acids in the neutral lipids, were similar percentagewise in all the tissues examined. Eighteen fatty acids, varying in chain length from C_8 to C_{20}, were found in this fraction. The predominant fatty acids in this fraction were palmitic, palmitoleic, stearic, oleic, and linoleic acid.

About 60 to 70 percent of the fatty acids in poultry tissues were unsaturated. However, based on the number of double bonds present, fatty acids from the phospholipids showed a much higher degree of unsaturation

than those from the neutral lipids. The relatively large quantities of highly unsaturated fatty acids, provide an explanation for the relative ease in which oxidative rancidity occurs in poultry meat.

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APPENDICES

APPENDIX A

Table 1. The main fatty acids of the phospholipids and the neutral lipids in the diet

Fatty acid[1]	Lipid fraction	
	Phospholipids	Neutral lipids
	Percent	
14:0	0.3	0.3
16:0	20.4	12.4
16:1	0.8	0.7
18:0	3.5	2.9
18:1	15.4	26.9
18:2	49.5	53.6
18:3	9.5	3.0
20:4	0.5	tr.

[1]Carbon number : number of double bonds.

Table 2. Composition of ration fed to broilers used in experiment[1]

Component	Age				
	1-10 days	10 days - 6 wks	6-10 wks	10-13 wks	13 wks & up
Peter Hand Custom	50	-	-	-	-
Peter Hand FW poultry	-	50	50	38	40
Ground yellow corn	1100	1072	1175	1455	1425
Soybean oil meal (50%)	675	475	325	150	2000
Meat & bone scraps (50%)	50	100	100	100	100
Dehydrated alfalfa meal	38	50	50	50	50
Wheat standard midds	-	200	250	150	150
Salt	5	8	8	5	8
Dicalcium phosphate	20	23	25	-	18
Ground limestone	-	13	10	10	10
Defluorinated phosphate	-	-	-	18	-
PH trace mineral mix	-	1/2	1/2	-	1/2
CCC trace mineral mix	1	-	-	1/2	-
Fat	40	-	-	-	-
Aurofac 10	20	-	-	-	-
Zoamix	1	10	10	10	10
% protein	24	22	19	15.6	16

[1]Expressed as pounds per ton.

APPENDIX B

Table 1. Fatty acids of the neutral lipids in dark meat

Fatty acid[1]	Bird number 1	2	3	4	Average
	percent of total fatty acids				
8:0	tr.	tr.	tr.	tr.	tr.
10:0	tr.	tr.	tr.	tr.	tr.
12:0	tr.	tr.	tr.	tr.	tr.
14:0	0.8	0.8	0.9	0.8	0.8
14:1	0.3	0.3	0.4	0.2	0.3
15:0	0.1	0.2	0.1	0.2	0.2
16:0	22.1	20.7	22.8	24.1	22.4
16:1	7.6	6.9	7.2	5.3	6.7
16:2	0.5	0.6	0.5	0.5	0.5
17:0	0.2	0.2	0.2	0.2	0.2
18:0	4.8	6.3	6.1	8.2	6.4
18:1	37.2	35.8	33.1	32.3	34.6
18:2	24.0	24.2	25.7	24.9	24.7
18:3	1.5	1.6	1.4	1.4	1.5
20:1	0.4	0.8	0.4	0.6	0.5
20:2	0.1	0.4	0.2	0.4	0.3
20:3	0.3	0.3	0.2	0.3	0.3
20:4	0.3	0.7	0.6	0.6	0.5

[1]Number of carbons : number of double bonds.

Table 2. Fatty acids of the neutral lipids in white meat

Fatty acid[1]	Bird number 1	2	3	4	Average
	percent of total fatty acids				
8:0	tr.	tr.	tr.	tr.	tr.
10:0	tr.	tr.	tr.	tr.	tr.
12:0	tr.	tr.	tr.	tr.	tr.
14:0	0.8	1.0	1.0	1.1	1.0
14:1	0.4	0.2	0.3	0.4	0.3
15:0	0.2	0.2	0.2	0.3	0.2
16:0	24.1	23.5	24.3	24.7	24.2
16:1	6.0	5.5	5.0	5.6	5.5
16:2	0.4	0.7	0.5	0.6	0.5
17:0	0.2	0.3	0.3	0.4	0.3
18:0	5.0	6.7	6.4	5.4	5.9
18:1	36.1	35.0	32.7	34.7	34.6
18:2	24.1	24.0	25.9	24.7	24.7
18:3	1.5	1.1	1.5	1.2	1.3
20:1	0.5	0.6	0.5	0.4	0.5
20:2	0.2	0.3	0.2	0.3	0.2
20:3	0.2	0.2	0.3	0.3	0.3
20:4	0.3	0.4	0.6	0.5	0.5

[1]Number of carbons : number of double bonds.

Table 3. Fatty acids of the neutral lipids in skin

Fatty acid[1]	Bird number 1	2	3	4	Average
	percent of total fatty acids				
8:0	tr.	tr.	tr.	tr.	tr.
10:0	tr.	tr.	tr.	tr.	tr.
12:0	tr.	tr.	tr.	tr.	tr.
14:0	0.8	0.5	0.8	0.8	0.7
14:1	0.3	0.1	0.4	0.1	0.2
15:0	0.2	0.1	0.1	0.2	0.2
16:0	22.7	22.9	24.0	22.7	23.1
16:1	7.2	3.8	6.5	5.1	5.7
16:2	0.4	0.4	0.4	0.5	0.4
17:0	0.3	0.1	0.2	0.1	0.2
18:0	4.9	6.4	5.8	6.6	5.9
18:1	37.0	39.4	35.5	35.9	37.0
18:2	23.2	24.4	23.7	24.9	24.0
18:3	1.8	0.9	1.4	1.4	1.3
20:1	0.7	0.4	0.5	0.6	0.5
20:2	0.2	0.1	0.2	0.3	0.2
20:3	0.2	tr.	0.1	0.2	0.1
20:4	0.2	0.1	0.2	0.3	0.2

[1]Number of carbons : number of double bonds.

Table 4. Fatty acids of the neutral lipids in depot fat

Fatty acid[1]	Bird number 1	2	3	4	Average
	percent of total fatty acids				
8:0	tr.	tr.	tr.	tr.	tr.
10:0	tr.	tr.	tr.	tr.	tr.
12:0	tr.	tr.	tr.	tr.	tr.
14:0	0.7	0.7	0.8	0.7	0.7
14:1	0.3	0.2	0.2	0.2	0.2
15:0	0.2	0.1	0.2	0.2	0.2
16:0	21.7	22.5	23.3	23.7	22.8
16:1	7.7	4.0	6.9	4.3	5.7
16:2	0.4	0.5	0.4	0.4	0.4
17:0	0.2	0.2	0.2	0.2	0.2
18:0	6.4	8.2	4.4	7.1	6.5
18:1	37.3	37.5	37.0	36.2	37.0
18:2	22.7	23.2	24.3	24.6	23.7
18:3	1.4	1.1	1.3	1.2	1.3
20:1	0.6	0.7	0.5	0.6	0.6
20:2	0.1	0.3	0.2	0.2	0.2
20:3	tr.	0.1	0.1	0.1	0.1
20:4	0.1	0.2	0.1	0.2	0.1

[1]Number of carbons : number of double bonds.

APPENDIX C

Table 1. Fatty acids of the phospholipids in dark meat

Fatty acid[1]	Bird number 1	2	3	4	Average
	percent of total fatty acids				
10:0	tr.	tr.	tr.	tr.	tr.
12:0	tr.	tr.	tr.	tr.	tr.
14:0	3.3	2.6	1.9	4.4	3.2
14:1	0.1	0.1	0.1	0.1	0.1
15:0	1.4	0.2	0.6	0.4	0.6
16:0	13.7	13.5	14.6	16.5	14.6
16:1	2.6	1.0	1.1	1.6	1.6
16:2	0.3	0.4	0.3	0.6	0.4
17:0	0.9	0.1	0.4	0.2	0.4
18:0	14.4	19.0	17.5	15.7	16.6
18:1	14.6	10.1	13.5	15.8	13.5
18:2	18:0	20.6	21.7	18.6	19.8
18:3	0.6	0.4	0.7	0.7	0.6
20:1	0.7	0.4	0.4	0.9	0.6
20:2	0.7	0.6	0.6	1.3	0.8
20:3	1.4	0.9	1.2	1.4	1.2
20:4	14.0	19.1	18.6	15.5	16.8
22:2	1.1	0.4	0.1	0.1	0.4
22:3	0.6	0.3	0.2	0.1	0.3
22:4	3.1	2.5	2.5	1.8	2.5
24:1	1.2	1.2	0.6	0.5	0.9
22:5-24:2	2.0	2.0	1.2	0.9	1.5
22:6-24:4	4.1	4.4	2.4	2.4	3.3

[1]Number of carbons : number of double bonds.

Table 2. Fatty acids of the phospholipids in white meat

Fatty acid[1]	Bird number 1	2	3	4	Average
	percent of total fatty acids				
10:0	tr.	tr.	tr.	tr.	tr.
12:0	tr.	tr.	tr.	tr.	tr.
14:0	2.3	1.9	1.7	1.4	1.8
14:1	2.3	tr.	0.1	tr.	0.1
15:0	1.2	0.6	0.2	1.3	0.8
16:0	24.9	23.5	24.5	18.9	23.0
16:1	1.8	1.0	0.8	0.2	0.9
16:2	0.4	0.3	0.2	0.1	0.3
17:0	0.4	0.4	tr.	0.7	0.4
18:0	8.8	9.3	11.9	9.4	9.8
18:1	19.6	14.5	15.2	15.8	16.3
18:2	17.2	16.4	17.0	17.4	17.0
18:3	0.3	1.3	0.1	0.3	0.5
20:1	0.3	1.8	0.2	0.3	0.6
20:2	0.7	1.1	0.6	0.9	0.8
20:3	1.4	1.4	1.6	1.6	1.5
20:4	12.1	15.1	17.1	15.9	15.0
22:2	0.3	0.7	0.7	1.1	0.7
22:3	0.8	0.7	0.1	1.0	0.7
22:4	3.7	2.1	2.8	3.3	3.0
24:1	0.8	1.0	0.5	1.8	1.0
22:5-24:2	1.0	2.0	1.0	2.6	1.7
22:6-24:4	3.3	4.1	3.4	4.7	3.9

[1]Number of carbons : number of double bonds.

Table 3. Fatty acids of the phospholipids in skin

Fatty acid[1]	Bird number 1	2	3	4	Average
	percent of total fatty acids				
10:0	tr.	tr.	tr.	tr.	tr.
12:0	tr.	tr.	tr.	tr.	tr.
14:0	3.3	5.0	2.9	2.7	3.0
14:1	0.8	0.3	0.7	0.7	0.5
15:0	0.5	0.7	0.3	1.2	0.7
16:0	24.1	18.7	25.5	19.7	22.0
16:1	4.6	2.8	1.2	3.2	3.1
16:2	0.7	0.9	0.5	0.7	0.7
17:0	tr.	0.6	0.3	2.0	1.0
18:0	11.4	15.4	9.4	10.9	11.8
18:1	16.8	15.7	21.7	20.0	18.5
18:2	17.9	11.0	14.4	15.9	14.8
18:3	1.8	0.6	0.4	1.2	1.0
20:1	1.3	0.6	0.3	1.0	0.8
20:2	0.9	0.7	0.8	1.0	0.9
20:3	2.1	1.3	1.7	1.1	1.6
20:4	9.8	13.9	12.2	9.6	11.4
22:2	0.8	0.6	0.4	2.5	1.1
22:3	tr.	0.5	0.3	1.1	0.6
22:4	1.9	3.7	3.4	1.8	2.7
24:1	0.6	3.6	0.6	1.8	1.7
22:5-24:2	0.6	1.4	1.2	0.5	0.9
22:6-24:4	1.0	1.7	1.6	1.3	1.4

[1]Number of carbons : number of double bonds.

Table 4. Fatty acids of the phospholipids in depot fat

Fatty acid[1]	Bird number 1	2	3	4	Average
	percent of total fatty acids				
10:0	tr.	tr.	tr.	tr.	tr.
12:0	tr.	tr.	tr.	tr.	tr.
14:0	2.1	1.3	1.2	2.7	1.8
14:1	1.4	1.1	0.6	1.1	1.1
15:0	0.8	0.8	0.5	1.2	0.8
16:0	17.7	19.1	21.0	17.0	18.7
16:1	7.3	3.0	5.8	3.8	5.0
16:2	0.7	tr.	0.6	0.7	0.7
17:0	0.4	0.9	0.6	0.6	0.6
18:0	10.9	6.5	8.7	9.8	9.0
18:1	21.2	29.2	28.6	23.0	25.5
18:2	25.4	20.3	22.4	20.1	22.3
18:3	1.4	1.7	1.6	2.0	1.7
20:1	1.0	1.3	0.9	1.4	1.8
20:2	0.5	0.9	0.7	0.7	0.7
20:3	0.6	0.2	0.5	1.2	0.6
20:4	3.1	5.7	3.6	5.0	4.6
22:2	0.8	1.0	0.5	1.8	0.9
22:3	0.6	1.3	tr.	0.9	0.7
22:4	0.9	1.5	0.8	1.7	1.3
24:1	1.2	2.0	1.0	2.3	1.6
22:5-24:2	tr.	0.2	1.0	0.4	0.2
22:6-24:4	1.2	1.4	0.6	1.9	1.3

[1]Number of carbons : number of double bonds.

APPENDIX D

Table 1. Percentage of phospholipids found in broiler tissues[1,2]

Bird No.	Source of phospholipids			
	Dark meat	White meat	Skin	Depot fat
	percent			
1	22.0	32.1	1.5	0.5
2	21.8	55.0	2.3	1.1
3	23.2	50.3	2.2	0.6
4	17.0	51.0	1.8	1.4
5	20.5	40.0	-	-
Average	21.0	48.0	2.0	0.9

[1]Phospholipids plus neutral lipids equal 100 percent.

[2]Each figure represents at least two determinations.

APPENDIX E

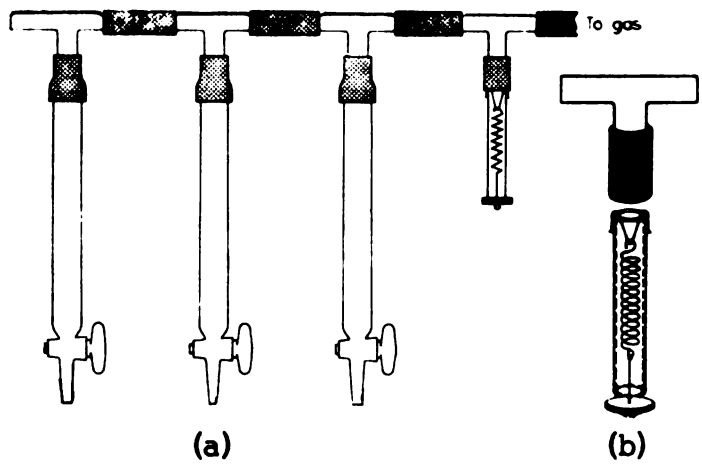

Figure 1. (a) Series of columns with control valve.
(b) Valve detail.