possibly for Spanish 18, the peanut highest in linoleic acid.

Unsaturated fatty acid analysis by the two procedures shows surprisingly good agreement, Table II, except for Wesson oil, corn oil, and lard. With these samples bought in a local store, both oleic and linolenic acid have shown higher, and linoleic acid lower values, by GLC than by spectral methods. Craig and Murty (6) noted, with oils high in linoleic acid like corn, sunflower, and soybean, a similar lack of agreement in results. Interestingly, the slightly rancid leaf lard fitted into this same pattern. Herb *et al.* (7) demonstrated close agreement for lard by the GLC and UV methods and their analysis fitted our GLC pattern for lard. It is noteworthy that pecan oils (Table II) showed close agreement in linolenic acid content by the two methods while peanut oils did not, even though the linoleic acid ran through a similar range of values in the two series.

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Some Differences in Composition of Covering Fat, Intermuscular Fat, and Intramuscular Fat of Meat Animals[1]

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A method is described for rapid, reproducible extraction of total lipids from adipose and muscle tissues. The distribution of polyunsaturated fatty acids in the depot fats and intramuscular lipids in loin or rib roasts of pork, beef, lamb, and veal has been determined. The phosphatides of the muscle were found to contain a greater amount of polyunsaturated fatty acids than the neutral fats of the intramuscular lipids.

IT HAS long been recognized that the lipids of an animal are not uniform in composition throughout the carcass. Banks and Hilditch (1), Bhattacharya and Hilditch (2), and Dean and Hilditch (6) showed that the inner fat of pigs contained a higher proportion of saturated fatty acids than were found in the outer fats. Hilditch and Zaky (9) studied the body fats of sheep and found a similar relation. They also reviewed other work and noted that a similar distribution occurred in cow fat. Dugan, *et al.* (7) studied the brisket fat of cattle and found that the outer layers were more unsaturated than the inner layers. They showed further that the oleic acid percentage was higher and the saturated acid percentage was lower in brisket fat than in back fats of cattle. Shorland (15) noted that sheep depot fats were higher in stearic acid content and correspondingly lower in long chain polyunsaturated acids than were the muscle fats. Hartman and Shorland (8) reported that the muscle lipids from loin mutton chops contained a somewhat higher proportion of C_{20} unsaturated fatty acids. They found approximately 3% as compared with 1% or less reported by other observers on sheep fats. Callow (4) noted that the iodine value of the fats from fatty tissues of lambs ranged from 43.6 to 56.1 while the iodine values of the fats from muscle ranged from 51.5 to 61.7.

The compositional variations of fat within the carcass have been noted but not systematically studied for all species of meat animals. The observation of Hartman that muscle lipids showed a greater proportion of C_{20} unsaturated fatty acids in one location in one species prompted an investigation of the possible difference between the covering, intermuscular, and intramuscular fat of three species of meat animals.

Methods and Materials

The problem of fat extraction from adipose tissues is fairly commonplace, but the muscle tissues are more difficult to extract due to the lower total lipid, and higher protein and water content. Therefore, the lipid material was extracted from the muscle by a variety of methods. These included:

1. Digestion with sulfuric acid and extraction with petroleum ether b.r. 36–60C (12).
2. Extraction in a Soxhlet extractor using a 50/50 mixture of ethanol (absolute) and petroleum ether.
3. Maceration with hot methanol (absolute) in a Waring Blendor, followed by addition of petroleum ether and blending, then adding a distilled water solution of zinc acetate to precipitate the protein and aid in separation into two phases.
4. A modification of the method of Bligh and Dyer (3) for total lipid from fish.

Since this last method (14) was chosen for subsequent analyses, it is described in detail.

Preparation of the Sample

1. Separate the fat and muscle by dissection. Grind the various samples through a meat grinder with a ⅛-in disk. Grind the adipose tissues two times and the muscle samples three times. Mincing into small pieces with a scalpel serves equally well.
2. Weigh samples for extraction on aluminum foil on an analytical balance. If they are not to be extracted within one day, they are stored at −20C until used.

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Sample Source

The samples were selected and prepared as follows for the evaluation of the extraction methods. Three choice grade pork loin roasts were purchased from a local retail store. The muscle was removed and trimmed of all the visible fat, but no attempt was made to dissect out the marbling fat. The sample was ground, mixed, and divided into two portions. One portion was kept in the raw state while the other portion was shaped into a small loaf and cooked in an oven at 350F(176.7C) to an internal temperature of 185F(85C). One-half of the raw sample and one-half of the cooked sample were then lyophilized in a Stokes Tray Lyophilizer.

Samples for the fatty acid analyses were purchased from a retail store. (Data shown in Table II.) Three choice grade loin roasts from pork, 5 choice grade rib roasts from beef, and 4 choice grade loin roasts from lamb and veal were examined. Because these were obtained from a retail store, and in a number of instances were already cut, it was not feasible to compare precise carcass location. However, all samples came from approximately the same carcass location regardless of species.

In order to differentiate between the depot fats, the samples were divided into three portions when possible. These were the subcutaneous depot fats which are referred to here as covering fat, the intermuscular fats, and the muscle lipids derived from the muscle which was trimmed of fat as completely as possible. This trimming did not remove the marbling fat.

Extraction

1. Transfer the sample (2 to 30 g) into a Waring Blendor jar with the aid of one-half facial tissue and 130 ml absolute methanol. (A 50 g sample is the maximum amount which the solvent system can extract satisfactorily.) Wipe any traces of sample from the foil with the facial tissue. Rinse the foil with methanol. Add a 1-in cube of dry ice.
2. Macerate the above for 5 min at medium speed (adjusted by a rheostat for a potential of 70–80 v).
3. Add 65 ml chloroform and blend for 5 min.
4. Add 65 ml chloroform and blend for 30 sec.
5. Add 65 ml distilled water containing 1–1.5 g zinc acetate. Blend for 10 sec. The zinc acetate is used to precipitate the protein.
6. Filter the sample by suction on a No. 3 Buchner funnel using a Whatman No. 1 filter paper. Direct a stream of carbon dioxide from a small laboratory generator onto the sample on the Buchner funnel throughout the filtering process.
7. Return the residue and filter paper to the blendor along with ½ facial tissue used to wipe out the funnel.
8. Macerate for 2½ min with 100 ml chloroform.
9. Refilter as above. Rinse the blendor jar and residue with 50 ml chloroform.
10. Collect the total liquid in a 500 ml graduated cylinder.
11. Determine the total volume of chloroform. Take an aliquot for determination of the percentage fat.
12. Remove the solvents under reduced pressure from the total lipids by means of a rotating flash evaporator.
13. Store the samples under nitrogen in a freezer at 0C or lower.

Analysis for Polyunsaturated Fatty Acids

The polyunsaturated fatty acids are determined by ultraviolet spectrophotometry after alkali isomerization. Features from the AOCS Official Method Cd, 7-58, revised 1959 (13) and that of Holman and Hayes (11) are combined in the method utilized for analysis. The reaction vessels described by Holman and Hayes are employed and prepurified nitrogen is used. The procedure is as follows:

1. Weigh a 2.5 or 0.5 g sample of lipid into a 50 or 25 ml volumetric flask. Add petroleum ether (30–60C b.r.) to dissolve and make to volume.
2. Determine the conjugated polyunsaturated acids as in AOCS Official Method, Cd 7-58 d.
3. Place a 1 ml aliquot of the dissolved sample in each of two reaction vessels for each sample and prepare two blanks by introducing into each vessel 1 ml of the petroleum ether used for dissolving samples.
4. Evaporate the petroleum ether from the vessels by directing a stream of prepurified nitrogen from a tube reaching into the vessel nearly to the surface of the solution. After the petroleum ether has evaporated, add 1 ml of purified ethanol (absolute) to each vessel.
5. Add from a syringe 1 ml of 21% KOH in ethylene glycol.
6. Cover the vessels with the head connected to a manifold for distributing nitrogen. Allow nitrogen to sweep through the vessel for 30 sec. Immerse the vessel in an oil bath at 180C for exactly 20 min while allowing nitrogen to sweep through the reaction vessel.
7. Remove the vessel from the bath, still permitting nitrogen to sweep through the vessel, and cool in a cold water bath. Rinse the nitrogen inlet tube with purified methanol allowing the methanol to flow into the reaction vessel.
8. Make up to volume in the reaction vessels with methanol and use the resulting solution for the ultraviolet spectrophotometric determinations.
9. Calculate polyunsaturated fatty acid values according to the AOCS Official Method Cd 7-58 d.
10. Determine iodine values by the rapid method of Hiscox (10). This method is acceptable unless there are hydroxyl groups which occur naturally in the molecules or are present due to oxidative reactions.
11. Calculate monoenoic acids, as oleic acid, from the iodine value and the polyunsaturated acid content. Subtract the monoenoic and polyunsaturated acid content from the total and report the remainder as total saturated fatty acids.

Separation of Phosphatides

The phosphatides are separated from the neutral fats using the procedure reported by Choudhury and Arnold (5).

1. Place 5 g of liquid lipid on 25 g powdered silicic acid in a 125 ml Erlenmeyer flask.
2. Add 50 ml of chloroform.
3. Agitate in a mechanical shaker for 10 min.
4. Blanket all operations under a layer of carbon dioxide.
5. Filter the chloroform off by suction through a sintered glass funnel.
6. Wash the silicic acid with six 50 ml portions of chloroform.
7. Collect the phosphatide fraction in another flask by

TABLE I

Fat Obtained from Pork Muscle Using Four Methods of Extraction

Sample	Acid digestion	Soxhlet (EtOH-Pet E)	Waring Blendor					
			Hot MeOH-PE			MeOH-CHCl3		
	%	%	%	n'	s*	%	n'	s*
Raw	36.0	31.7	31.4	15	1.11	36.2	7	0.49
Raw-lyophilized	36.3	35.7	33.1	5	0.47	36.1	5	1.19
Cooked	35.4	34.0	37.8	9	1.25	37.6	9	0.41
Cooked-lyophilized	36.1	36.4	30.0	5	0.93	36.3	5	0.79

n' = number of analyses, one operator. s* = standard deviation.

TABLE II

Fatty Acids and Iodine Values of Samples of Pork, Beef, Veal, and Lamb

Sample	Fatty Acids						Iodine values
	Oleic	Linoleic	Linolenic	Arachidonic	Pentaenoic	Total saturated	
	$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$
Pork							
Intramuscular fat	45.2 ± 1.06	4.2 ± 0.29	0.4 ± 0.04	0.4 ± 0.06	0.1 ± 0.02	44.8 ± 0.91	53.3 ± 1.04
Intermuscular fat	44.9 ± 0.50	6.8 ± 0.00	0.4 ± 0.05	0.4 ± 0.00	0.0 ± 0.02	43.1 ± 0.40	54.5 ± 0.17
Covering fat	43.3 ± 0.48	7.4 ± 0.22	0.4 ± 0.02	0.2 ± 0.01	0.1 ± 0.01	44.0 ± 0.40	55.8 ± 0.72
Beef							
Intramuscular fat	42.6 ± 3.86	1.1 ± 0.09	0.3 ± 0.03	0.2 ± 0.03	0.1 ± 0.02	59.3 ± 3.94	51.0 ± 4.19
Intermuscular fat	41.9 ± 3.24	1.2 ± 0.12	0.2 ± 0.05	0.0 ± 0.01	0.0 ± 0.01	52.1 ± 3.40	47.6 ± 3.23
Covering fat	44.9 ± 2.05	1.1 ± 0.09	0.2 ± 0.04	0.0 ± 0.01	0.0 ± 0.00	48.8 ± 2.18	51.8 ± 3.40
Veal							
Intramuscular fat	34.1 ± 1.40	4.2 ± 0.30	2.0 ± 0.22	2.1 ± 0.42	1.3 ± 0.35	51.0 ± 1.80	59.4 ± 3.70
Intermuscular fat	43.3 ± 1.26	1.3 ± 0.08	0.6 ± 0.07	0.2 ± 0.04	0.1 ± 0.03	49.7 ± 1.33	44.3 ± 1.21
Covering fat	48.0 ± 1.68	1.5 ± 0.12	0.6 ± 0.09	0.2 ± 0.03	0.1 ± 0.02	41.1 ± 2.14	46.7 ± 2.58
Lamb							
Intramuscular fat	41.6 ± 1.44	2.7 ± 0.66	1.5 ± 0.23	0.8 ± 0.24	0.7 ± 0.11	46.7 ± 1.24	55.5 ± 1.20
Intermuscular fat	33.2 ± 1.70	1.5 ± 0.58	0.8 ± 0.15	0.1 ± 0.00	0.1 ± 0.02	58.9 ± 1.67	37.1 ± 1.48
Covering fat	34.8 ± 1.90	1.7 ± 0.57	0.7 ± 0.15	0.1 ± 0.01	0.1 ± 0.01	57.1 ± 1.96	38.7 ± 2.48

$\bar{x} \pm s_{\bar{x}}$ = The mean ± the standard error of the mean.

washing the silicic acid with four 50 ml portions of methanol.

Results

The effectiveness of the various methods used for extraction of total lipid is shown by the data in Table I. The percentages of fat represent average values based on the dry weight.

The differences in fatty acid composition of the total lipids from covering fat, intermuscular fat, and intramuscular fat in the same carcass location of an animal are shown in Table II. The values for pork represent 10 separate analyses and those for beef represent 9 separate analyses. The values for lamb and veal represent 4 analyses each.

It should be noted that the linoleic content of pork depot fats was much higher than that of the depot fats of the other species examined. The linoleic content of veal intramuscular fat equalled that of the pork samples examined. The linolenic content of pork and beef were low and no difference existed between the depot and intramuscular fats. Both veal and lamb exhibited a higher level of linolenic acid in intramuscular fat than in depot fats.

The distribution pattern of arachidonic and pentaenoic acids resembled that for linolenic acid among the animals investigated.

The polyunsaturated fatty acid contents of the neutral fats and of the phosphatides from a single sample from each type of intramuscular fat noted above are shown in Table III.

The linoleic acid content varied from a ten-fold excess in beef muscle phosphatides over those in the associated neutral fats to essentially equal quantities in each class of lipid from pork intramuscular lipids. The linolenic acid content did not vary widely between the two lipid types. Arachidonic and pentaenoic acid are higher in the phosphatides than in the neutral fats.

Discussion

The main reasons for rejecting the first three methods of lipid extraction are as follows:

1. The acid digestion method gives undesirable breakdown products for fatty acid determinations by alkaline isomerization.
2. The Soxhlet extraction method is too time consuming.
3. The hot methanol-petroleum ether method forms viscous gels which make quantitation difficult.

The fourth method, methanol-chloroform, has the following desirable characteristics: reproducibility, complete lipid extraction, 15 min extraction time, ease of manipulation, suitable sample condition for alkaline isomerization. It also extracts total lipids equally well from a variety of tissues and tissue states.

There were few clearly defined features in the fatty acid composition. Of the essential fatty acids, the linoleic acid content was greatest in muscle fat of veal and lamb, and was about equally distributed in the beef fats; but in pork the linoleic content of the intramuscular fat was lower than it was in the depot fats. The oleic acid content was higher only in pork and lamb muscle as compared to the depot fats.

Since the intramuscular lipids were markedly different in composition from depot fats in various samples, it was apparent that the difference must be in the presence of lipids which would be more or less unique to the muscle tissues. It was concluded that these most probably would be phospholipids; there-

TABLE III

Polyunsaturated Fatty Acids in the Neutral Fat and Phosphatides of Muscle Fat from Lamb, Pork, Veal, and Beef

Sample	Linoleic	Linolenic	Arachidonic	Pentaenoic
	%	%	%	%
Lamb				
Neutral fat	1.1	0.8	0.2	0.2
Phosphatides	2.1	0.7	1.6	1.1
Pork				
Neutral fat	3.2	0.4	0.2	0.1
Phosphatides	3.8	0.8	2.5	0.6
Veal				
Neutral fat	3.9	1.7	0.7	0.8
Phosphatides	0.1	1.8	1.4	1.0
Beef				
Neutral fat	1.0	0.3	0.0	0.0
Phosphatides	11.3	1.3	3.7	3.9

fore, neutral fats and phospholipids were separated and the fatty acid composition of each determined.

The method of separation provided only a gross phospholipid fraction. This fraction contains all the muscle lipids which are not washed from silicic acid by chloroform. The values reported will be diluted by the proportion of the nonphospholipid moieties in each sample.

The data in Table III indicate that the phosphatides are a rich source of polyunsaturated fatty acids in the muscle lipids. Arachidonic acid, calculated as C_{18} and C_{20} tetraenoic acids, is found consistently in a greater quantity in the phosphatides than in the neutral fats. The distribution pattern was then found to be reversed in veal lipids where the linoleic acid content of the neutral fats exceeded that of the phosphatides by a manyfold factor. This was completely unexpected and no explanation can be made until further data have been obtained.

If the marbling fat is similar in composition to the depot fats, then the intramuscular fats of fatter animals would be expected to be more nearly like the depot fats. For more complete knowledge of the muscle lipid composition a study of leaner animals would seem desirable.

The above data on fatty acid composition are only a preliminary study in an attempt to detect trends. A more thorough investigation is in progress.

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Metal Soaps of Wool Wax Acids as Stabilizers for Plasticized Polyvinyl Chloride[1]

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Copper, magnesium, lead, nickel, cobalt, manganese, iron, chromium, cadmium, and barium soaps of the wool wax acid fraction have been tested as stabilizers for plasticized polyvinyl chloride polymers. Barium, cadmium, and lead soaps performed well in the light stability evaluations. In the heat aging tests barium, magnesium, lead, and nickel soaps were superior while cadmium, and manganese soaps performed poorly. The copper, cobalt, iron, and chromium soaps were ineffective.

Two tests for stability were employed: an accelerated light aging test and an accelerated heat aging test. Combinations of barium and cadmium soaps were tested for synergistic effects and found to be more effective as stabilizers than the individual soaps. Soaps made from fractions of acids by partitioning the whole wool wax acid fraction were also tested. Neither the hydroxy nor the non-hydroxy acid fraction soaps had as good stabilizing properties for PVC as those of the whole acid fraction.

A VERY LARGE NUMBER of substances, including a variety of metal soaps, have been used to stabilize plasticized polyvinyl chloride films against breakdown by heat and light.

This laboratory has been interested for some time in wool wax and its fractionation products. The unusual combination of acids (normal, iso, ante-iso, and hydroxy) comprising the wool wax acid fraction suggested investigating the use of their metal soaps as stabilizers for polyvinyl chloride. The only prior work on the use of wool wax acid metal soaps in this regard is that revealed in a patent by de Nie (1). The disclosure is concerned primarily with salts made from a methyl alcohol soluble fraction of wool wax acids and metals from the silver group (Analytical Group I—silver, mercury, and lead). The soaps of these metals presumably react with any photochemically or thermochemically released halide atoms to form insoluble halides and inhibit further deterioration of the polymer.

The present paper describes investigations of the use of wool wax acid metal soaps as stabilizers for plasticized polyvinyl chloride films. Soaps of the whole acid fraction as well as soaps of partitioned acid fractions were studied. The following soaps of the whole acid fraction were tested: Copper, magnesium, lead, nickel, cobalt, manganese, iron, chromium, cadmium, and barium. In addition to the soaps mentioned above, barium and cadmium soaps of the hydroxy and nonhydroxy acid fractions obtained by partitioning the whole wool wax acids fraction (2) were also evaluated. The preparation and properties of these soaps are described in an accompanying paper (3). Included in this study are comparisons of stabilizing properties of the wool wax acid metal soaps with those of barium and cadmium stearates and laurates. Accelerated light and heat aging tests were used to determine stability of the films. The comparisons of the relative stabilizing ability of the different systems were made by visual inspection and by light transmission measurements on the test samples when mounted on a white paper background.

Preparation of Test Films. Test films were prepared from: polyvinyl chloride (Geon 101) (65%); plasticizer (34 to 32%), and stabilizer (1 to 3%). Either di-2-ethylhexyl phthalate (DOP) or tricresyl phosphate (TCP) was used as the plasticizer. The Geon 101, plasticizer, and stabilizer were first dry mixed in a beaker, then milled on a heated two roll rubber compounding mill at 160C for 8 min. The fused sheet was then molded in a form (3 in x 6 in x 0.07 in) maintained at 150C and 1000 psi for 20 min and then allowed to cool at room temperature.

Light and Heat Stability. An Atlas Weather-

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