

Fatty Acid Composition of Meat Tissue Lipids

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SUMMARY

The lipids extracted from beef and pork muscle were fractionated into triglycerides, cephalins, and a mixture of lecithins and sphingomyelins. The fatty acid composition of these fractions was determined, and the possible effect of phospholipids on meat flavor was evaluated.

Cold-water extracts of lean beef and lean pork contain desirable meat-flavor precursors. These extracts do not, however, contain any appreciable proportion of the lipids present in the lean meat. Lipids, particularly the phospholipids, are among the more unstable constituents of lean meat; and Younathan and Watts (1960) have recently suggested that the phospholipids play a major role in accelerating flavor deterioration in cooked meats. This paper is concerned with the effect on flavor of the lipids present in lean meat prior to preparation for consumption. Our studies have therefore been made on aged lean beef and lean pork. The meat tissue lipids have been separated into neutral lipids and phospholipids, the fatty acids present in these fractions determined, and the possible contribution of these fractions to either desirable or undesirable flavor evaluated.

EXPERIMENTAL

Extraction of lipids from muscle. As in previous flavor studies (Hornstein and Crowe, 1960; Hornstein *et al.*, 1960b), fresh meat was aged 10 days at 36–38°F. Several of the muscles were then dissected and stored at 0°F. As needed, samples of meat were thawed, fat was removed, and the trimmed muscle was cut into small sections. The extraction procedure was essentially that of Folch *et al.* (1957). One hundred grams of the diced meat were blended for 5 min with 900 ml of cold 2:1 chloroform-methanol (all solvents are reagent grade and all solvent ratios are v/v) in an Oster blender (mention of trade names

is for identification and implies no endorsement). The slurry was immediately filtered, then mixed with 0.2 its volume of water in a 2-L separatory funnel, and allowed to stand overnight at 3–5°C. The separation into two phases was clean-cut. The lower phase was drained and, without further washing, dried over anhydrous sodium sulfate, concentrated to a small volume on a rotary evaporator at room temperature under partial vacuum, quantitatively transferred with several ml of chloroform to a tared 125-ml Erlenmeyer flask, and dried on the rotary evaporator. Residual solvent was removed under high vacuum. The weighed residue was redissolved in 20 ml of 20:1 chloroform-methanol. Small amounts of undissolved material were removed by centrifugation.

Separation of neutral fat from phospholipids by column chromatography. Fifty grams of silicic acid (Mallinckrodt AR 100-mesh) heated overnight at 130°C were slurried with chloroform and poured into a 2.5 × 90-cm column fitted with a sintered-glass disc. Air bubbles were removed by stirring the mixture with a long glass rod. The silicic acid was allowed to settle and the chloroform drained under slight nitrogen pressure. When the column was compact and with at least 15 cm of liquid above the interface, anhydrous sodium sulfate was added to form a 2.5-cm-thick layer on top of the silicic acid. The column, after washing with 300 ml of chloroform, was ready to use. The entire lipid sample, containing as much as 1 g of phospholipid and 7 g of neutral fat, was added. The column was developed with successive 300-ml portions of chloroform-methanol 20:1, chloroform-methanol 1:1, and methanol. Flow rates of approx 3–5 ml/min were obtained by applying 5 lb of nitrogen pressure to the top of the column. Fractions collected were dried as described for the

total lipid fraction, and the residues were weighed.

Analytical methods. Each fraction was made up to 50 ml with the solvent mixture used for its elution, and aliquots were taken for the determination of phosphorus, total and amino nitrogen, reducing sugars, and fatty acid composition. The infrared spectrum was also recorded. *Phosphorus* was determined after wet oxidation of an aliquot containing 1–10 μg of phosphorus. The phosphate formed was measured by reduction of the phosphomolybdate complex according to the procedure of Chen *et al.* (1956). *Total nitrogen* was determined by the micro-Kjeldahl procedure with mercuric oxide as the catalyst. *Amino nitrogen* was determined by the ninhydrin procedure of Moore and Stein (1948) as modified by Lea and Rhodes (1954). *Reducing sugars* were determined by the anthrone method used by Radin *et al.* (1955) in determination of cerebrosides. *Infrared spectra* were obtained by deposition of a film of the material by solvent evaporation on sodium chloride plates and scanning the region of 2.5–15 μ . The instrument used was a Perkin-Elmer 137 Infracord spectrophotometer. *Fatty acid determinations* were made on aliquots taken to dryness and containing 100–200 mg of material. Twenty-five ml of 0.5*N* alcoholic KOH were added to each residue and the mixture gently refluxed for 6 hr. The contents of the flask were transferred to a 125-ml separatory funnel, 50 ml of water were added, and the solution was made acid to phenolphthalein by dropwise addition of concentrated HCl and then 1 ml excess added. The solution was extracted with three successive 25-ml portions of petroleum ether, and the combined ether extracts were washed three times with 25-ml portions of cold distilled water. At this point, approx 20 mg of *n*-heptadecanoic acid (recrystallized twice from methanol), accurately weighed, were added to the solution to act as an internal standard in gas chromatographic analysis of the fatty acid esters (Hornstein *et al.*, 1960a). The fatty acid methyl esters were prepared as previously described, and dissolved in 1 ml of petroleum ether. An appro-

appropriate aliquot, containing approx 500 μg of total esters, was used for gas chromatography. The gas chromatograph was a Beckman GC-2 equipped with a 1-mv recorder, and the detector was a 4-filament thermal conductivity unit. The column was an 8-ft. \times $\frac{1}{4}$ -in OD coiled Cu tube containing polyvinylacetate on acid and alkali washed Chromosorb R, 15:85 w/w. The operating temperature was 221° and the He flow rate 100 ml/min. Quantitative results were based on recoveries of the C_{17} acid.

RESULTS AND DISCUSSION

Solvent systems. Initial attempts to separate the phospholipids by precipitation with acetone and magnesium chloride left phosphorus in solution. To avoid phospholipid losses, acetone precipitation was discarded and chromatography on silicic acid used (Borgström, 1952). The elution solvents were chloroform-methanol mixtures (Hananan *et al.*, 1957); increasing the percent methanol in chloroform by small increments resulted in smeared bands. The sharpest separations were obtained starting with 20:1 chloroform-methanol (Solvent I), followed by 1:1 chloroform-methanol (Solvent II), and finally eluting with anhydrous methanol (Solvent III). Each of the eluted fractions contained a mixture of lipids in which one class predominated. Solvent I eluted the nonphosphorous-containing lipids. This eluate was collected as two fractions. Fraction Ia contained 85–90% of the neutral lipid fraction; collection was stopped prior to the emergence of a heavily pigmented band. Fraction Ib contained these pigments as well as neutral lipids. Silicic acid and Solvent I had similar refractive indices, and the column was translucent. The progress of Solvent II through the

Table 1. Chemical analysis of fractions obtained by silicic acid chromatography of muscle lipids.

Fraction	Phosphorus (%)	Total nitrogen (%)	Amino nitrogen (%)	Reducing sugars	Infrared absorption at 10.3 μ	Major components
Ia	0.0	0.0	Triglycerides
Ib	0.1	0.0	weak, positive	Triglycerides
II	3.6	1.7	1.7	absent	Cephalins
III	4.0	1.9	0.1	strong	Lecithins and sphingomyelins

column caused the column to become progressively opaque. Just before Solvent II broke through, Fraction II was collected; the last 150 ml of Solvent II eluted only trace amounts of material. Methanol eluted the final fraction. Further elution with methanol, plus 5% water, yielded only traces of material. This same elution pattern was obtained with lipid extracts from both lean beef and lean pork.

Classification of fractions. In order to classify these fractions, extracts from several muscles were analyzed. The data fell into a similar pattern for all muscles; a typical analysis is shown in Table 1. Fractions Ia and Ib, as mentioned, were predominantly triglycerides, and for the purpose of this study were not further characterized. The weak positive tests for phosphorus and reducing sugars suggested the presence of small amounts of phospholipids and cerebrosides in Fraction Ib. Infrared spectra of Fractions II and III gave typical phospholipid spectra (Nelson and Freeman, 1959; Smith and Freeman, 1959). Without pure reference standards, slight variations in spectra cannot be interpreted properly. The major difference between II and III resided in the 10.3- μ region (Fig. 1). The

INFRARED RED SPECTRUM FROM LEAN PORK

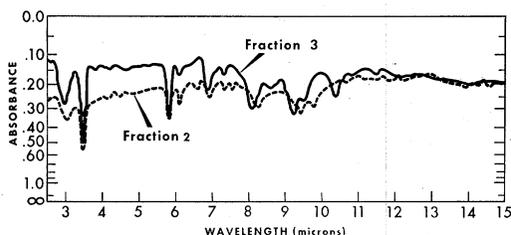


Fig. 1. Infrared spectrum of Fractions 2 and 3 obtained from pork.

absence of absorption at 10.3 μ in Fraction II was indicative of cephalins, and the strong absorption in Fraction III at this wavelength was characteristic of lecithins and sphingomyelins (Nelson and Freeman, 1959; Smith and Freeman, 1959). Fraction II had the expected 1:1:1 molar ratio of amino nitrogen/total nitrogen/phosphorus. However, cephalins accounted for only about

90% of this fraction since these analyses were 10% lower than expected. The very weak test for amino nitrogen in Fraction III excluded cephalins except in trace quantities. Total nitrogen was 1.9% (comparable figures are lecithins 1.7% and sphingomyelins 3.0%). The observed value indicated about 15% sphingomyelins in Fraction III.

The phospholipids in Fractions II and III were associated with protein. Folch and Lees (1951) described a class of lipoproteins that had the solubility characteristics of lipids; when dried, even under the mildest conditions, these lipoproteins yielded an insoluble protein fraction. The cephalin fraction we obtained contained about 20% of this "proteolipid" material. For example, a typical Fraction II, weight 331.8 mg, was dried and then redissolved in Solvent II, and 50.3 mg of residue remained. Successive dryings yielded 8.2 mg and 4.0 mg of residue. Analysis of this residue showed no phosphorus and 6.1% nitrogen. Fraction III typically contained about 2% of this proteinaceous material. This protein denaturation is minimized in the presence of fat since the total extract, when taken to dryness, did not exhibit this phenomenon to as great an extent. Our analytical data are reported on the basis of protein-free phospholipids.

Fatty acid determinations. Table 2 tabulates the lipid fractions extracted from typical samples of beef and pork. In general, beef muscle contained 2.0–4.0% of nonphosphorous-containing lipids, and pork muscle contained 5.0–7.0%. The phospholipid content was 0.8–1.0% in beef and 0.7–0.9% in pork. The approximate composition of the phospholipid fraction for both beef and pork was cephalin 40–45%, lecithin 40–45%, sphingomyelin 10–15%, and protein 5–10%. Table 3 shows the fatty acid composition of the fractions isolated from these samples. The absolute recoveries of fatty acids in Fractions Ia and Ib, based on 90% fatty acid content, were about 95%. Recoveries based on 75% fatty acid content for protein-free Fractions II and III were respectively approx 50 and 80%. In part, these low recoveries can be attributed to the presence of plasmalogens as evidenced

Table 2. Weight in grams of lipid fractions isolated from 100 g of muscle by chloroform-methanol extraction, followed by silicic acid chromatography.

	Beef	Pork
Total lipids	<u>4.5699</u>	<u>6.6098</u>
Non-phosphorus lipids	<u>3.5539</u>	<u>5.8615</u>
Fraction Ia (triglycerides)	2.8962	5.4473
Fraction Ib (triglycerides)	0.6577	0.4142
Phospholipids	<u>1.0040</u>	<u>0.7462</u>
Fraction II (cephalins)	0.4330	0.3155
Fraction III (lecithins and sphingomyelins)	0.4990	0.3737
Denatured proteins	0.0720	0.0570
Total recovery	4.5489 (99.5%)	6.6077 (99.9%)

by the formation of 2, 4-dinitrophenylhydrazones of long-chain carbonyl compounds after mild acid hydrolysis of these fractions, and to the formation of oxidized phospholipids as shown by the characteristic ultraviolet absorption at approx 235 $m\mu$ and 275 $m\mu$ (Lea, 1957).

The identities of the saturated acids and oleic, linoleic, linolenic, and arachidonic acids

were established by direct comparison of retention volumes of the methyl esters with those of authentic specimens. The other assignments were based on plots of the log of retention volumes versus the number of carbon atoms. Saturated fatty acid methyl esters fall along a straight line—those containing one double bond along a parallel line, those with two double bonds along a second parallel line, etc. (Hornstein *et al.*, 1960a). As the number of double bonds in the molecule increases, the distance between these parallel lines tends to decrease; and it is possible, for example, that the “envelope” assigned to arachidonic acid may contain higher unsaturated C_{20} acids. The assignment made for tetradecenoic, tetradecadienoic, palmitoleic, eicosatrienoic, and docosadienoic should be considered rendered highly probable rather than absolute, since they were not based on direct comparison with authentic samples. Table 4 shows the fatty acid composition of the combined triglyceride fractions and of the combined phospholipid fractions. Unsaturated acids containing two or more double bonds make up 10% of the triglyceride fraction and about 50% of the phospholipid fraction; however, the actual milligrams of these acids contributed by the two fractions are similar since the ratio of triglycerides to phospholipids is about 4:1 in beef and about 8:1 in

Table 3. Fatty acid composition of lipid fractions (% of total fatty acids) obtained from lean beef and pork. Fractions Ia and Ib triglycerides, Fraction II cephalins, Fraction III lecithins and sphingomyelins.

Acid	Fraction Ia		Fraction Ib		Fraction II		Fraction III	
	Beef	Pork	Beef	Pork	Beef	Pork	Beef	Pork
Capric	1.1	2.7
Lauric	0.2	0.6	0.4
Myristic	2.1	1.1	2.8	2.0	1.4	3.6	3.6	1.0
Tetradecenoic	1.0	1.5	0.6	0.1	1.1	0.3
Tetradecadienoic	0.6	0.6	0.6	0.8	1.3	0.5
Palmitic	28.3	24.0	19.8	22.5	2.8	4.1	21.0	30.5
Palmitoleic	4.6	7.2	5.9	9.8	1.8	2.4	2.4	2.4
Stearic	17.0	12.1	16.2	7.0	27.4	20.6	6.6	4.6
Oleic	42.5	45.8	30.6	39.4	13.8	14.3	26.9	17.4
Linoleic	3.0	7.9	17.2	15.3	16.2	15.2	23.6	36.3
Linolenic	0.9	1.7	2.5	1.7	2.1	0.4	1.7	1.4
Eicosatrienoic	4.0	3.2
Arachidonic	1.2	1.2	33.3	32.4	8.6	5.6
Docosadienoic	2.1
Weight of fraction (g)	2.8962	5.4473	0.6577	0.4142	0.4330	0.3155	0.4900	0.3737

Table 4. Fatty acid composition of combined triglyceride fractions and of combined phospholipid fractions from Table 3 (% of total fatty acids).

Acid	Triglycerides		Phospholipids	
	Beef	Pork	Beef	Pork
Capric	0.1	0.1
Lauric	0.1	0.2
Myristic	2.2	1.2	2.6	2.0
Tetradecenoic	1.0	0.9	0.2
Tetradecadienoic	0.6	1.3	0.6
Palmitic	27.5	23.9	13.2	20.0
Palmitoleic	4.7	7.4	2.2	2.3
Stearic	16.9	11.6	15.6	11.0
Oleic	41.3	45.2	21.2	16.2
Linoleic	4.4	8.7	20.2	27.9
Linolenic	1.1	1.6	1.8	1.0
Eicosatrienoic	1.8	1.6
Arachidonic	0.1	0.1	19.2	16.3
Docosadienoic	0.9
Total saturated acids	46.7	37.0	31.4	33.0
Total monounsaturated acids	47.1	52.6	24.3	18.8
Total dienoic acids	5.0	8.7	21.5	29.3
Total trienoic acids	1.1	1.6	3.7	2.6
Total tetraenoic acids	0.1	0.1	19.1	16.3

pork. The major quantitative difference is the large amount of arachidonic acid (possible maximum 150 mg per 100 g of muscle in beef and 100 mg/100 g of muscle in pork) contributed by the phospholipid fraction that has no counterpart in the triglyceride fraction (Table 3).

Flavor characteristics. We attempted to evaluate the aromas developed by heating freshly isolated lipid fractions in air. The odors of the beef and pork triglyceride fractions were respectively reminiscent of "fried-fat" and "bacon." The cephalin fractions from both beef and pork produced strong "fishy" odors, probably attributable to the high arachidonic acid content of these fractions. The heated lecithin-sphingomyelin fractions from both beef and pork were also alike in odor, the "fishy" smell less pronounced than in the cephalin fraction and superimposed on an aroma suggestive of liver. Total lipid and phospholipid extracts of pork and beef were exposed to air, and their odors noted at 24-hr intervals. Rancid

odors developed more quickly in these samples than in neutral fat. At the end of one week, the samples were all highly rancid. The odor of the pork total lipid extract was the least objectionable; the large amount of triglyceride present either dissolved the potent odor compounds produced, thus lowering their vapor pressure, or mechanically inhibited the oxidation of the phospholipids by limiting their surface exposed to air. It was concluded that phospholipids did not contribute to desirable meat flavor and that the possibility existed that in excessively lean meat they could contribute to poor flavor. The triglyceride contribution was similar to that previously described (Hornstein and Crowe, 1960). Changes in color were also noted in these fractions. The phospholipids darkened rapidly, the total lipid fraction changed color more slowly, and the neutral lipids showed the least color change.

REFERENCES

- Borgström, B. 1952. Investigation of lipid separation methods. Separation of phospholipids from neutral fat and fatty acids. *Acta Physiol. Scand.* **25**, 101.
- Chen, P. S. Jr., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. *Anal. Chem.* **28**, 1756.
- Folch, J., and M. Lees. 1951. Proteolipids, a new type of tissue lipoproteins. *J. Biol. Chem.* **191**, 807.
- Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**, 497.
- Hanahan, D. J., J. C. Dittmer, and E. Warashina. 1957. A column chromatographic separation of classes of phospholipides. *J. Biol. Chem.* **228**, 685.
- Hornstein, I., and P. F. Crowe. 1960. Flavor studies on beef and pork. *J. Agr. Food Chem.* **8**, 494.
- Hornstein, I., J. A. Alford, L. E. Elliott, and P. F. Crowe. 1960a. Determination of free fatty acids in fat. *Anal. Chem.* **32**, 540.
- Hornstein, I., P. F. Crowe, and W. L. Sulzbacher. 1960b. Constituents of meat flavor: Beef. *J. Agr. Food Chem.* **8**, 65.
- Lea, C. H. 1957. Deteriorative reactions involving phospholipids and lipoproteins. *J. Sci. Food Agr.* **8**, 1.

- Lea, C. H., and D. N. Rhodes. 1954. Phospholipids. 2. Estimation of amino nitrogen in intact phospholipids. *Biochem. J.* **56**, 613.
- Moore, S., and W. H. Stein. 1948. Photometric ninhydrin method for use in the chromatography of amino acids. *J. Biol. Chem.* **176**, 367.
- Nelson, G. J., and N. K. Freeman. 1959. Serum phospholipide analysis by chromatography and infrared spectrophotometry. *J. Biol. Chem.* **234**, 1375.
- Radin, N. S., F. B. Lavin, and J. R. Brown. 1955. Determination of cerebroside. *J. Biol. Chem.* **217**, 789.
- Smith, L. M., and N. K. Freeman. 1959. Analysis of milk phospholipids by chromatography and infrared spectrophotometry. *J. Dairy Sci.* **42**, 1450.
- Younathan, M. T., and B. M. Watts. 1960. Oxidation of tissue lipids in cooked pork. *Food Research* **25**, 538.