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THE FATTY ACID COMPOSITION OF LIPIDS FROM DIFFERENT LAYERS OF FRESH STEERHIDE*

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ABSTRACT

Columnar and gas-liquid chromatography were employed to determine the fatty acid composition of the principle lipid constituents found in five serial stratigraphic layers of a fresh steerhide. The data indicate that different wax esters, glycerides, and phospholipids predominate in different strata of the hide, and the fatty acid composition of these does not reflect the composition of the free fatty acids present at these locations. The data suggest that the lipid constituents are deposited by specific processes at each location and that there is little physical transport of the lipids from one location to another within the living hide.

INTRODUCTION

The lipids found in hides and skins are usually subdivided into hydrocarbons, waxes (fatty acid esters of aliphatic alcohols or sterols), glycerides, free sterols, free fatty acids, and phospholipids. The waxes, glycerides, and phospholipids may contain a wide variety of fatty acids.

McLaughlin and Theis (1) in 1924 showed that the lipid content of a steerhide was greater in the grain and flesh layers than in the center of the corium. Koppenhoefer and Highberger (2) in 1934 fractionated the lipids from steerhides to determine the differences between the "epidermal" and corium lipids. They concluded that the "epidermal layer" (30% of the total wet weight) contained most of the phospholipid, cholesterol, and waxes and that the corium lipids were to a large extent triglycerides. Later Koppenhoefer (3) studied further the lipids of steerhide and fractionated the fatty acids into the liquid and solid acids. The liquid acids were chiefly oleic acid and the solid acids a mixture of palmitic and stearic acids. A summary of

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the lipid composition of various hides has recently been prepared by Koppenhoefer (4).

The techniques for the separation of lipids and the identification and estimation of the fatty acids have changed considerably in recent years, and a more detailed description of the fatty acids present in the various major lipid constituents is now possible. Two new techniques, column chromatography to separate the major lipid constituents and gas-liquid partition chromatography to identify and determine the fatty acids, have been applied in this study.

Now for the first time the complete fatty acid composition of each of the main lipid constituents in five levels of a steerhide has been determined. The results indicate that the composition of the lipids of steerhides is more complex than previously postulated.

EXPERIMENTAL

Preparation of sample.—A hide from a freshly killed steer was washed, by tumbling in cold water, to remove blood and debris. An area 5″ x 16″ was cut from the middle of the back and shaved with a dry razor to remove the hair as close to the hide as possible without removing any of the hide itself. This piece was frozen in a flat position and split into five approximately equally thick layers parallel to the grain surface on a mechanical splitting machine. The first layer extended from the grain surface to the base of the hair shafts and contained the epidermis and most of the corium minor. The second layer contained some of the lower portion of the corium minor but was mostly the upper portion of the corium major. The third and fourth layers were from the center of the corium. The fifth layer was the lower layer of the corium adjacent to the flesh side. Each layer was cut into 1/8″ squares and extracted four times by grinding with a mortar and pestle in 250 ml. of Bloor's solvent (3 parts 95% alcohol and 1 part ether). All solvents were redistilled to insure freedom from nonvolatile impurities. These four extractions were followed by a single extraction with anhydrous ether. The extracts were combined, clarified by filtering through a bed of filter-aid, and evaporated under reduced pressure until a small volume was reached. The remaining solvent and moisture were removed by blowing with nitrogen at a temperature of 50°C. The dried residue was dissolved in hexane and filtered rapidly through a thin layer of filter-aid to remove suspended solids. The solvent was again evaporated and the lipid weighed. The concentration, purification, and storage of the extracts were done at low temperature and under an atmosphere of nitrogen to minimize oxidative effects.

Fractionation of the lipids.—The fractionation of the lipids into the major class components was done on a silicic acid–filter-aid column according to the procedure of Luddy *et al.* (5). After this preliminary fractionation,

aliquots of each component were treated by the methanolysis procedure of Luddy *et al.* (6) to convert the glycerides or other esters of the fatty acids to their methyl esters. The free fatty acid fraction was converted to methyl esters by esterification (6). These methyl esters were then separated by conventional gas-liquid chromatography using a succinate polyester of ethylene glycol as the stationary phase. The apparatus and conditions of analysis are described by Herb *et al.* (7).

RESULTS AND DISCUSSION

The yield of crude lipids from each of the five layers is shown in Table I. Most of the lipid occurs in the first and fifth slices, and very little occurs in the center slice.

TABLE I

YIELD OF CRUDE LIPIDS

Layer No.*	Dry wt.	Lipid Extracted		
	g.	g.	% of dry weight	% of total from all layers
1	25.0	1.88	7.5	42.9
2	15.3	0.28	1.8	6.4
3	29.3	0.12	0.4	2.7
4	33.7	0.32	0.9	7.3
5	23.4	1.78	7.6	40.6
H†	126.7	4.38	3.5	100.0

*Numbered consecutively from the hair side.
†Combined values for over-all thickness of hide.

The results of the fractionation into the major lipid constituents are shown in Tables II and III. In Table II the data are presented on a concentration basis as milligrams per gram of the dry layer. They show that the hydro-

TABLE II

STEERHIDE LIPID CONSTITUENTS

(*mg/g of the Dry Layer*)

Constituent	Layers				
	1	2	3	4	5
Hydrocarbon	0.3	0.2	0.1	0.1	0.1
Wax esters	17.3	2.0	0.3	0.2	0.9
Glycerides	20.2	4.3	1.3	6.6	64.0
Free sterols	4.5	1.7	0.4	0.5	0.4
Free fatty acids	10.1	2.2	0.9	0.6	3.8
Phospholipids	22.6	7.9	1.3	1.6	6.1

carbon constituents are at negligible concentrations in all layers. The wax esters are concentrated chiefly in the first layer, which contains the epidermis and corium minor. The free sterols are slightly more concentrated in the first layer than the others and have concentrations comparable with the wax esters in the last four layers. The glycerides have their highest concentration in the fifth or flesh-side layer, although the concentration in the hair-side layer is also quite high. The free fatty acids have their highest concentration in the first layer. The phospholipids also are most highly concentrated in the first layer.

Table III presents the same data as percent of the total lipids in each layer.

TABLE III

STEERHIDE LIPID CONSTITUENTS

(As Percent of Total in Each Layer)

Constituent	Layers					Total Hide
	1	2	3	4	5	
Hydrocarbon	0.4	1.2	1.9	0.5	0.7	0.8
Wax esters	23.1	11.1	7.9	2.3	1.2	11.5
Glycerides	26.9	23.6	31.5	70.4	84.6	53.3
Free sterols	5.9	9.2	8.9	4.8	0.5	3.9
Free fatty acids	13.7	11.7	20.0	5.5	5.0	9.6
Phospholipids	30.0	43.2	29.8	16.5	8.0	20.9

The first layer has concentrations of wax esters, glycerides, and phospholipids which are quite similar. In the second layer the phospholipids predominate, and their concentration is twice that of the glycerides and four times that of the wax esters and free fatty acids. The third layer has similar concentrations of glycerides, free fatty acids, and phospholipids. The fourth and fifth layers are predominately glycerides. These findings are very similar to those reported by Koppenhoefer (3,4). This indicates that the qualitative aspects of the distribution of these major classifications of lipids in steerhides have been very adequately established.

The methyl esters of the fatty acids obtained from these major lipid classifications are readily fractionated by means of gas-liquid partition chromatography. The results of this fractionation indicate that the fatty acid composition of all the lipid materials in a steerhide is much more complicated than was previously indicated. Saturated and monounsaturated fatty acids containing from 12 to 24 carbons have been found. A more limited range of the more highly unsaturated fatty acids has also been detected. To facilitate comparison of the composition of each lipid component from layer

to layer the data have been grouped into tables for each of the major lipid components. Comparisons of the various lipids in the same layer are also possible.

Composition of lipid fractions

The wax esters.—Table IV shows the fatty acid composition of the wax esters as percent of the particular fatty acid to the total fatty acid present

TABLE IV

FATTY ACID COMPOSITION OF THE WAX ESTERS*

Fatty Acid†	Layers				
	1	2	3	4	5**
12:0 Lauric	0.9	0.6	—	—	—
13:0 Tridecanoic	—	0.1	—	—	—
14:0 Myristic	50.7	35.8	4.4	3.9	—
15:0 Pentadecanoic	1.3	1.9	—	1.1	—
16:0 Palmitic	16.6	15.3	10.1	16.2	—
17:0 Heptadecanoic	1.1	—	0.7	—	—
18:0 Stearic	10.2	6.6	2.4	3.5	—
20:0 Arachidic	1.9	—	1.2	0.9	—
22:0 Behenic	—	1.7	—	—	—
24:0 Lignoceric	—	2.5	—	—	—
14:1 Tetradecenoic‡	—	1.7	1.8	1.5	—
16:1 Hexadecenoic	3.7	4.8	11.0	9.6	—
18:1 Octadecenoic	3.0	4.4	9.2	22.9	—
24:1 Tetracosenoic	—	2.8	34.6	10.3	—
18:2 Octadecadienoic	9.0	12.8	18.1	19.1	
18:3 Octadecatrienoic	0.4	1.6	1.6	1.5	—
20:4 Eicosatetraenoic	—	—	1.6	1.6	—
22:5 Docosapentaenoic	—	0.4	—	—	—
Unknown	1.2	7.0	3.3	7.9	—

*Fatty acids as percent of the total fatty acids in the particular fraction.
†The fatty acids are designated by two numbers; the first gives the number of carbon atoms and the second, the number of double bonds.
‡The chromatographic column did not differentiate the various isomers possible for the unsaturated acids.
**The fifth layer produced too small an amount of fatty acids to be studied.

in the wax esters of that layer. The wax ester fraction obtained for the fifth layer contained too small an amount of fatty acids to be studied. There are many fatty acids present in small amounts that are fairly uniformly distributed through the layers; also there are a number of fatty acids which are not evenly distributed through the layers. The myristic and stearic acids are much more predominant in the first two layers than in the other layers. On the other hand, palmitic acid makes up about 15% of each layer.

The unsaturated fatty acids, however, are more prominent in the third and fourth layers. These unsaturated fatty acids are chiefly the 16 and 18 monounsaturated and the 18 diunsaturated acids. The 24 carbon monounsaturated acids are found chiefly in the third layer, where they comprise one-third of the total fatty acids. They are entirely lacking in the grain layer of this hide.

The glycerides.—Table V shows the fatty acid composition of the glycerides.

TABLE V

FATTY ACID COMPOSITION OF THE GLYCERIDES*

Fatty Acid†		Layers				
		1	2	3	4	5
12:0	Lauric	1.4	0.4	0.2	0.1	0.1
13:0	Tridecanoic	0.2	0.2	0.1	—	—
14:0	Myristic	39.3	13.9	3.4	1.8	2.0
15:0	Pentadecanoic	0.7	1.1	1.4	1.2	—
16:0	Palmitic	21.4	24.8	30.8	33.3	30.3
17:0	Heptadecanoic	0.4	1.1	0.5	—	—
18:0	Stearic	7.2	10.9	8.1	5.1	5.1
19:0	Nonadecanoic	—	2.6	—	—	—
20:0	Arachidic	0.4	0.6	—	—	—
21:0	Heneicosanoic	—	5.2	—	—	—
22:0	Behenic	—	0.3	—	—	—
23:0	Tricosanoic	—	0.3	—	—	—
14:1	Tetradecenoic	1.2	1.0	1.4	—	1.3
16:1	Hexadecenoic	3.8	6.8	9.7	8.0	10.1
18:1	Octadecenoic	16.0	26.6	40.2	47.1	48.9
20:1	Eicosenoic	—	0.3	—	—	—
18:2	Octadecadienoic	6.6	1.9	2.2	1.6	1.1
18:3	Octadecatrienoic	—	—	0.4	0.6	0.5
Unknown		1.4	2.0	1.6	1.2	0.6

*Fatty acids as percent of the total fatty acids in the particular fraction.
†The fatty acids are designated by two numbers; the first gives the number of carbon atoms and the second, the number of double bonds.

Here again there is a difference in distribution among the layers. The myristic and stearic acids are again prominent in the first two layers. The 18 carbon diunsaturated acids are more prominent in the first layer glycerides than in the remainder of the hide. Palmitic acid and the 16 and 18 carbon monounsaturated acids are more prominent in third, fourth, and fifth layers than in the first two. It is noteworthy that the 24 carbon monounsaturated acids which were prominent in the wax esters of the third layer are completely absent from the glycerides of all layers.

The phospholipids.—Table VI gives the fatty acid composition of the phospholipids in the five layers of the steerhide. Myristic acid and the 18 carbon

TABLE VI

FATTY ACID COMPOSITION OF THE PHOSPHOLIPIDS*

Fatty Acid†		Layers				
		1	2	3	4	5
12:0	Lauric	0.3	0.3	0.3	0.5	0.5
13:0	Tridecanoic	—	—	2.4	—	—
14:0	Myristic	6.3	2.3	2.0	1.9	2.5
15:0	Pentadecanoic	0.8	1.4	1.4	—	1.0
16:0	Palmitic	20.0	20.6	18.6	20.2	24.0
17:0	Heptadecanoic	0.9	1.3	1.2	—	—
18:0	Stearic	14.1	13.4	16.9	14.0	8.9
19:0	Nonadecanoic	0.2	—	—	—	—
20:0	Arachidic	2.2	—	—	1.1	0.8
22:0	Behenic	—	—	—	—	0.2
12:1	Dodecenoic	—	—	—	—	0.5
14:1	Tetradecenoic	0.7	4.3	—	1.5	1.0
16:1	Hexadecenoic	3.2	4.3	4.5	5.7	9.4
17:1	Heptadecenoic	2.0	—	—	—	—
18:1	Octadecenoic	19.6	33.9	31.4	32.6	40.7
20:1	Eicosenoic	0.7	1.0	—	—	—
18:2	Octadecadienoic	15.6	7.7	6.1	4.5	2.4
20:2	Eicosadienoic	0.3	—	—	0.2	—
18:3	Octadecatrienoic	0.2	1.0	—	1.5	1.0
20:3	Eicosatrienoic	1.1	0.7	—	1.0	—
22:3	Docosatrienoic	—	1.0	—	—	—
20:4	Eicosatetraenoic	6.5	6.0	8.2	7.9	3.3
22:4	Docosatetraenoic	0.2	—	—	1.4	—
20:5	Eicosapentaenoic	0.9	—	—	—	—
22:5	Docosapentaenoic	1.9	0.8	—	1.5	0.6
Unknown		2.3	0.0	7.0	4.5	2.6

*Fatty acids as percent of the total fatty acids in the particular fraction.

†The fatty acids are designated by two numbers; the first gives the number of carbon atoms and the second, the number of double bonds.

diunsaturated acids are much higher in the first layer than in the remainder of the hide. Stearic acid and the 20 carbon tetraunsaturated acids are quite uniform through the first four layers and drop to lower levels in the fifth layer while the 16 carbon monounsaturated acids remain at fairly even levels through the first four layers and then rise appreciably in the fifth layer. It is remarkable that palmitic acid contributed about 20% to the composition of the phospholipid fatty acids from all layers. The 14 carbon monounsaturated acids appear concentrated only in the second layer, and the 18 carbon

monounsaturated acids have an almost constant level through the second, third, and fourth layers but are reduced in the first layer and reach a higher porportion in the fifth layer. The phospholipids contain a much wider variety of unsaturated fatty acids than either the wax esters or the glycerides.

The free fatty acids.—Table VII shows the composition of the free fatty acids present in each hide layer. These values appear to be more variable

TABLE VII

FATTY ACID COMPOSITION OF THE FREE FATTY ACIDS*

Fatty Acid†	Layers				
	1	2	3	4	5
12:0 Lauric	0.5	1.1	0.8	0.7	—
13:0 Tridecanoic	0.1	—	0.1	—	—
14:0 Myristic	8.6	3.3	3.0	2.1	1.3
15:0 Pentadecanoic	0.7	0.9	2.5	0.9	0.7
16:0 Palmitic	16.2	13.2	22.7	16.4	21.5
17:0 Heptadecanoic	0.5	0.7	1.2	0.3	0.7
18:0 Stearic	6.0	5.9	14.0	6.2	9.6
20:0 Arachidic	0.9	—	0.4	—	0.2
21:0 Heneicosanoic	—	—	—	—	0.2
14:1 Tetradecenoic	0.5	0.4	0.9	1.0	1.0
15:1 Pentadecenoic	—	—	0.6	—	—
16:1 Hexadecenoic	3.6	5.8	5.6	9.5	7.7
17:1 Heptadecenoic	—	—	1.3	—	—
18:1 Octadecenoic	21.7	39.3	26.5	29.8	43.2
19:1 Nonadecenoic	—	—	0.2	—	—
20:1 Eicosenoic	—	—	0.7	—	0.8
24:1 Tetracosenoic	—	0.9	—	1.9	—
18:2 Octadecadienoic	30.1	16.7	8.2	13.5	3.7
20:2 Eicosadienoic	—	—	0.4	—	—
18:3 Octadecatrienoic	1.2	0.9	0.4	—	—
20:3 Eicosatrienoic	2.0	1.2	0.9	1.4	0.6
22:3 Docosatrienoic	—	—	—	—	0.9
20:4 Eicosatetraenoic	5.8	7.2	6.6	14.7	5.0
22:4 Docosatetraenoic	—	—	1.2	—	—
22:5 Docosapentaenoic	—	—	1.4	—	0.5
Unknown	1.6	2.5	0.4	1.6	2.0

*Fatty acids as percent of the total fatty acids in the particular fraction.
†The fatty acids are designated by two numbers; the first gives the number of carbon atoms and the second, the number of double bonds.

than the values obtained for the fatty acids of the wax esters, glycerides, and phospholipids. There is a high value for the palmitic acid in both the third and fifth layers, and the lowest value is in the second layer. The 18 carbon monounsaturated acids show high values in both the second and fifth layers.

The 16 carbon monounsaturated acids have a low value in the first layer and a high value in the fourth layer. The 18 carbon diunsaturated acids are very high in the first layer and have a low value in the fifth layer with variable intermediate values. For the various layers, distribution of the fatty acids in the free fatty acids is, therefore, much more variable than in the combined fatty acids.

Comparison between layers.—In general there is a marked difference between the proportion of fatty acids present in the first and subsequent layers. In most cases any fatty acid which was present in good proportion in any layer was also present to some extent in the other layers. The fatty acids which were present in very small proportion were quite erratic in their distribution.

One of the most noticeable differences is the myristic acid which is prominent in the first layer for each of the four components and except for its high values in the wax esters and glycerides of the second layer is only a trace fatty acid in the remaining layers.

Palmitic and stearic acids made significant contributions to most layers and most major components, although there are some large fluctuations in the degree of contribution.

The 16 and 18 carbon monounsaturated acids also make significant contributions to all layers and all components, although there is a definite trend for these acids to be concentrated in the center and flesh side layers.

The 18 carbon diunsaturated acids are present in most layers in significant proportion with a tendency to be higher in the grain side layers. The contribution of this acid to the glyceride fraction is notably low except in the first or grain layer.

The values in Tables IV through VII are presented as ratios of the total fatty acid content of each layer and, therefore, cannot be added directly to obtain the quantity of each fatty acid present in the over-all specimen of hide. The average yield of free fatty acids from the various lipid components was: wax esters, 40%; glycerides, 95%; and phospholipids, 35%. These average values were used to calculate the actual weight concentration of the various fatty acids in each layer, and these concentrations times the weight of the layer were summed and divided by the total weight to obtain the value for the over-all specimen of hide. The values for acids that were present to the extent of at least 1 mg. per 100 g. are presented in Table VIII. The bottom of the table presents summary values for the saturated and unsaturated fatty acids. It is obvious that the saturated acids are more prevalent in the grain or hair side of the hide (layer 1) and the unsaturated acids are more prevalent in the flesh side of the hide. In fact, for this particular hide the ratio of saturated to unsaturated acid is exactly reversed in the two layers. For the hide used in this study the value of 1 to 2 for the ratio of

TABLE VIII

APPROXIMATE TOTAL CONCENTRATION OF INDIVIDUAL FATTY ACIDS IN EACH LAYER AND THE FULL HIDE

(mg/100g Dry Weight)

Fatty Acid	Layers					Full Hide
	1	2	3	4	5	
12:0 Lauric	40	5	1	1	7	10
13:0 Tridecanoic	5	1	1	—	1	2
14:0 Myristic	1243	99	8	14	131	286
15:0 Pentadecanoic	35	12	5	8	5	13
16:0 Palmitic	848	200	68	231	1974	627
18:0 Stearic	381	100	31	44	366	173
19:0 Nonadecanoic	2	11	—	—	—	2
20:0 Arachidic	48	3	—	1	3	11
21:0 Heneicosanoic	—	21	—	—	1	3
24:0 Lignoceric	—	2	—	—	—	—
14:1 Tetradecenoic	28	18	3	1	85	24
16:1 Hexadecenoic	161	56	20	66	664	181
17:1 Heptadecenoic	16	—	1	—	—	3
18:1 Octadecenoic	702	293	89	333	3224	868
24:1 Tetracosenoic	—	4	4	2	1	2
18:2 Octadecadienoic	616	76	15	22	86	156
20:3 Eicosatrienoic	29	5	1	1	2	7
20:4 Eicosatetraenoic	110	32	10	13	26	36
Total Saturated	2602	454	114	299	2488	1127
Total Unsaturated	1662	484	143	438	4088	1277
Total Fatty Acids	4264	938	257	737	6576	2404
% Saturated	61	48	44	41	38	47

solid to liquid (saturated to unsaturated) fatty acids in the fifth layer is almost identical with the ratio given by Koppenhoefer (3) for the corium of steerhide. For the over-all thickness of hide the ratio of solid to liquid (saturated to unsaturated) fatty acids is almost 1.

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