

STUDIES ON THE CHEMICAL CONSTITUTION OF SHEEP LIPIDS

By

Y. EL-SHATTORY, T. Z. MORAD,* M. A. ABBASY **
and Z. SHOEB

*Fats and Oils Laboratory, National Research Centre,
Dokki, Cairo, Egypt*

SUMMARY

Physical and Chemical characteristics of neck, sub-skin, tail, kidney and viscera lipids of sheep were determined. The fatty acid constitution of the previous five lipid organs were recorded by gas liquid chromatography (GLC). The total saturated fatty acids of kidney lipids is higher than of the other four lipid samples. Oleic acid for tail lipid (41.56%) is the major fatty acid while lauric acid for sub-skin lipid (0.21%) is the minor fatty acid. The kidney lipid was fractionated on silicic acid column, with subsequent identification of different lipid classes by thin layer chromatography (TLC). Sterol esters, triglycerides, free fatty acids, sterols and phospholipids represent 0.34, 87.73, 0.37, 6.34 and 5.22% respectively of kidney lipid. The total saturated fatty acids of phospholipids are higher than that of kidney lipids, triglycerides, and free fatty acids. Lauric and palmitinoleic acids are absent for phospholipids and triglycerides respectively. Palmitic acid for phospholipids (42.72%) is the major fatty acid while palmitinoleic for kidney lipid (0.93%) is the minor fatty acid.

Introduction

Animal fats are essential sources for humans whether they are used as edible products in various food substances, or non-edible fats to be used in different aspects of industry such as soap, protective coatings, surfactants and other important industries (1, 2, 3, 4, 5).

* Tanta Oil and Soap Company.

** College for Girls, Ain. Shams University.

Cow, buffalo and sheep are the three major producing domestic animals in Egypt.

In the present work, the physical and chemical properties of lipids from neck, sub-skin, tail, kidney and viscera of sheep were investigated. The individual fatty acids of the isolated lipids were elucidated. This help in the identification and evaluation of some important constituents.

Detailed study of quantitative fractionation of kidney lipid on silicic acid column. The fatty acid constitution of the major lipid fractions was also carried out.

Materials and Methods

The lipids of five samples that have been used in the present investigation were brought from the slaughter house from middle aged sheep.

All solvents used were reagent grade. Silicic acid (Mallinckrodt, chromatography, 100 mesh) was heated at 100°C overnight before being used. Silica gel G (according to Stahl E. MERK A.G.) was used for coating TLC plates.

The physical and chemical characteristics including melting point (M.P.), titer, refractive index (R.I.), acid value (A.V.), saponification value (S.V.), iodine value (I.V.), peroxide value (P.V.) and unsaponifiable matter percent (U.S.M.) were determined according to A.O.C.S. methods (6).

EXPERIMENTAL

Column Chromatography of Kidney Lipid :

Chromatographic column (2 × 100 cm.) fitted with a sintered glass disc (medium porosity) and a stopcock on one side, and a Q.F. adaptor on the other side, packed with silicic acid slurry in hexane to give a column height of Ca. 60 cm. A nitrogen pressure of 4 pounds/in² was used for packing and elution.

An exact weight of kidney lipid (2.5636 g.) was placed onto the column. The composition of the eluting solvent is given at the top of the elution curve (figure 1). The number of the fractions collected is given on the X-axis. The weights, in milligrams, of eluted lipids are given on the Y-axis. A total of 320 fractions, 25 ml. each, were collected separately in volumetric flasks. The eluate of each separate fraction was transferred to previously weighed 50 ml. Q.F. round

bottom flask, evaporated under vacuum using a rotary evaporator, then weighed to find the exact weight of eluted material. Shifting from one solvent to another took place when there was no material eluted from the column. Eluted fractions were dissolved in the least volume of chloroform and transferred to 3 ml. screw capped sample bottles and then kept in refrigerator.

Thin Layer Chromatography of Isolated Fractions :

Glass plates (20 x 20 cm.) were coated with silica gel G to give a layer 0.3 mm thick. The plates were dried at room temperature for 30 min. then in the oven at 110°C for 1 hr.

Samples eluted from the column were applied by micro-pipettes, 2.5 cm from the bottom of the plate. The developing chambers were prepared 15 min. before insertion of the plates. The chambers were lined on three sides with Whatman 3 MM filter paper wetted with the developing solvent.

For the identification of lipid classes, petroleum ether (30 — 60°C) — diethyl ether-acetic acid (90 : 10 : 1, v/v) was used as developing solvent. After development, the plates were dried and for the visualization of the spots the plates were placed in iodine bath. The identity of the spots was revealed by using authentic standards of characteristic spray.

Percentages of Different Lipids :

Following the TLC identification of the constituents of all the fractions eluted from the silicic acid column, fractions containing similar constituents were collected together. The summation of residue weights of each eluted lipid was calculated.

Fatty Acid Composition :

The component fatty acids of the five lipid samples and also the major lipids isolated from the column were converted to their methyl esters by Luddy et al. procedure (7). Gas liquid chromatographic analysis of the methyl esters was done using a Beckman Gas/chromatograph 2A. A 6 feet copper column, 1/4 in. (i.d.) was packed with 20% diethylene glycol succinate polyester on Gas Chrom. A (80 — 100 mesh, Applied Science Lab. Inc. Product). The column temperature was 182°C and the inlet temperature was 325°C. Nitrogen was used as a carrier gas at a flow rate of 30 lbs/in². The detector current was maintained at 250 m.A.

RESULTS AND DISCUSSION

Physical and Chemical Characteristics.

Physical and chemical characteristics of neck, subskin, tail, kidney and viscera lipids are shown in table 1.

It is found that the melting points (M.P.) of the above five lipid samples range from 33.8 for tail lipid to 51.0 for kidney lipid. Neck and sub-skin lipids show more or less equal values (41.3 and 41.5 respectively).

Concerning the titer (which is the solidifying point), it is found to range from 38.0 for tail lipid to 50.0°C for viscera lipid. These five values are more or less approximate to that of the corresponding melting points.

The refractive indices were measured at 60°C and are recorded to range from 1.4475 for kidney lipid to 1.4525 for tail lipid. Neck and sub-skin lipids have the same value (1.4505).

Regarding the free acidity of the five samples, it is noticed that the corresponding acid values range from 0.33 for neck lipid to 0.80 for viscera lipid. The high free acidity of viscera lipid may be due to the enzymatic activities occurring within the digestive tract «Viscera».

With respect to the saponification values, it can be seen that the tail, kidney, viscera lipids have higher values (197.27, 214.40 and 203.56 respectively) than the neck and sub-skin lipids (186.53 and 185.67 respectively). However, the neck and sub-skin lipids have more or less equal saponification values.

The degree of unsaturation of the lipids as indicated by their iodine values, lies within the range from 33.06 for kidney lipid to 34.09 for viscera lipid. On the other hand neck, sub-skin and tail lipids show higher values (51.3, 44.33 and 45.16 respectively).

A minimum peroxide value of 0.14 is obtained for viscera lipid as compared to the high value 1.10 for neck lipid. On the other side tail and kidney lipids show the same value 0.61 while that of sub-skin lipid is 0.99.

It can be also noticed that the unsaponifiable matter of kidney lipid is the lower value 0.8% and that of viscera lipid is the higher one 1.9%. However neck, sub-skin and tail lipids show middle values 1.7, 1.2 and 1.3% respectively.

*Fatty Acid Composition of Neck, Sub-skin, Tail, Kidney and Viscera
Total Lipids :*

The fatty acid constitution of the five lipid samples is given in table 2.

The investigated lipids are found to contain even and odd numbers fatty acids with chain length from C_{12} to C_{18} .

Lauric acid is found to range from 0.21% for sub-skin lipid to 12.40% for kidney lipid. It composes of the lower percentage of saturated fatty acid for neck, sub-skin, tail and viscera lipids (0.30, 0.21, 0.50 and 0.92 respectively). Lauric acid represents the minor saturated fatty acid for neck lipid.

It can be seen that kidney lipid has the highest percent of myristic acid (6.22) while viscera lipid has the lowest (3.00).

A minimum percent of pentadecanoic acid (0.40) is obtained for viscera lipid as compared to the maximum value (4.21%) for kidney lipid.

Palmitic acid ranges from 19.46% for tail lipid to 11.64% for sub-skin. It constitutes the maximum percent of saturated fatty acids for neck lipid (16.16) and for tail lipid (19.46).

Heptadecanoic acid ranges from 3.22% for sub-skin lipid to 1.40% for kidney.

It can be also recorded that stearic acid ranges from 8.86% for tail lipid to 30.87% for sub-skin lipid. It constitutes of the major saturated fatty acid for sub-skin and kidney lipids.

It can be noticed that kidney and tail lipids have the major percentages of the total saturated fatty acids (61.81 and 37.45) respectively.

Regarding the unsaturated fatty acids of the five lipid samples, the results revealed the following :

1. Palmitoleic acid ranges from 4.77% for viscera lipid to 10.57% for neck lipid.

2. Palmitlinoleic acid is found to range from 0.52% for sub-skin lipid to 4.70% for neck lipid, which is the minor unsaturated fatty acid percent for the five lipid samples.

3. Concerning the oleic acid, it is found to range from 24.05% for kidney lipid to 41.56% for tail lipid. It represents the major unsaturated fatty acid percent for the five lipid samples.

4. Linoleic acid lies within the range from 4.85% for kidney lipid to 13.88% for sub-skin lipid.

5. Palmitoleic acid, linoleic acid percentages for neck lipid are more or less of equal values.

Generally, it can be concluded that the major fatty acid percent (41.56) is oleic acid for tail lipid and the minor fatty acid percent (0.21) is lauric acid for sub-skin lipid.

Column Chromatographic Fractionation of Kidney Lipid :

Figure 1 represents the fractionation of kidney lipid on silicic acid column.

Figure 2 is a tracing of a 20 x 20 cm TLC plate, that illustration the nature of the neutral lipids eluted from the column with solvents starting with n-hexane and ending with methanol. Table 3 gives the amounts of lipids collected from the column after examined by TLC.

Sterol esters were recorded in samples eluted with hexane-benzene (85 : 15 v/v) by spraying TLC plates with antimony trichloride-glacial acetic (8). They constitute of 0.33% of the total lipids introduced and 34% of the total recovered.

Triglycerides were eluted with 5% ether in hexane (85.35% of the total lipids introduced and 87.73% of the total lipids recovered). Only traces of sterol esters were revealed in the first four fractions.

Free fatty acids compose of 0.36% of the total lipids introduced and 0.37% of the total lipids recovered, were eluted with 15% ether in hexane. Only traces of triglycerides were detected in the first three fractions.

Sterols constitute of 6.17 and 6.34% of the total lipids introduced and recovered respectively, were eluted with 50% ether in hexane. They were recorded on TLC plates by spraying with 22% antimony trichloride in chloroform (8).

Phospholipids were eluted with methanol. They constitute 5.08 and 5.22% of the total lipids introduced and recovered respectively.

Generally, it can be notice that the percentage of the total recovered lipids amounts 97.30 of the original amount introduced onto the column. The balance may be lipoprotein material and were not eluted.

Fatty Acid Constitution of Isolated Lipids :

Table 4 gives the fatty acid composition of the kidney lipid, the triglyceride, the free fatty acid and the phospholipid fractions. It is clear that lauric acid is absent for phospholipids. Palmitic acid and pentadecanoic acid constitute the major percent of total fatty acids for phospholipids (42.72) and 27.23 respectively).

With respect to the total saturated fatty acids, it can be seen that the phospholipids have higher value (85.59%) than that of the kidney lipid (61.81%), triglycerides (58.33%) and free fatty acids (59.43%).

Palmit-linoleic acid is absent for triglycerides and represents the minor fatty acid percent (0.93) for kidney lipid. While oleic acid is the major unsaturated fatty acid percent for kidney lipid and triglycerides (24.05 and 22.05% respectively).

On the other side, the total unsaturated fatty acids for triglycerides (41.67%) is considerably higher than that of the kidney lipid (33.19%), free fatty acids (40.57%) and phospholipid (14.41%).

References

1. Markley, K.S., «Fatty Acids», 2nd Ed. Part I. Interscience Publishers Inc. (1960).
2. Kirschenauer, H.G., «Fats and Oils», 2nd Ed., Reinhold Publishing Corp. (1960).
3. Patlison, E.S., «Industrial Fatty Acids and their Applications», Reinhold Publishing Corp. (1959).
4. Bailey, A.E. «Industrial Oil and Fat Products», Interscience Publishers (1951).
5. Harwood H. J., J. Amer. Oil Chem. Soc., pp. 432 (1959).
6. «Amer. Oil Chem. Soc. — Official and Tentative Methods» 2nd Ed., Chicago (1957).
7. Luddy, F. E., Barvord R. A. and Reimenschnider R. W., JAOCS 37, 447 (1960).
8. Mangold H. K., from «Thin Layer Chromatography A — Laboratory Handbook», Ed. N. Stahl, Acad. Press (1965).

TABLE 1
 Physical and Chemical Characteristics of Neck, Sub-skin, Tail
 Kidney and Viscera Lipids.

Lipid sample	M. P. °C	Titer °C	R. I. at 60°C	A. V.	S. V.	I. V.	P. V.	U. S. M. %
Neck	41.3	42.0	1.4050	0.33	186.53	51.30	1.10	1.7
Sub-skin	41.5	43.0	1.4505	0.52	185.67	44.33	0.99	1.2
Tail	33.8	38.0	1.4525	0.46	197.27	45.16	0.61	1.3
Kidney	51.0	47.0	1.4475	0.73	214.40	33.06	0.61	0.8
Viscera	47.3	50.0	1.4490	0.80	203.56	34.09	0.14	1.9

TABLE 2

Fatty Acid Composition of Neck, Sub-skin, Tail Kidney
and Viscera Total Lipids

Fatty Acid*	Neck	Sub-skin	Tail	Kidney	Viscera
C _{12:0}	0.30	0.21	0.50	12.40	0.92
C _{14:0}	4.70	3.20	3.88	6.22	3.00
C _{15:0}	0.89	0.88	1.68	4.21	0.40
C _{16:0}	16.16	11.64	19.46	16.35	12.49
C _{17:0}	2.79	3.22	3.07	1.40	2.95
C _{18:0}	14.67	30.87	8.86	21.23	27.19
Total Unsaturated	39.60	50.02	37.45	61.81	46.95
C _{18:1}	10.57	9.80	6.61	8.36	4.77
C _{18:2}	4.70	0.52	2.78	0.93	3.25
C _{18:1}	34.28	26.28	41.56	24.05	39.57
C _{18:2}	10.85	13.38	11.60	4.85	5.46
Total saturated	60.40	49.98	62.55	38.19	53.05

* Relative areas under the curves for the methyl esters of the component fatty acids as estimated for GLC.

TABLE 3

Quantitative Amounts of Kidney Lipids Eluted
from Silicic Acid Column.

Lipid	Weight of Lipid gm	Percentage of total introduced	Lipid of total recovered
Total Lipid Introduced	2.5636		
Sterol Esters	0.0085	0.332	0.341
Triglycerides	2.1881	85.352	87.728
Free Fatty Acids	0.0093	0.362	0.373
Sterols	0.1582	6.171	6.343
Phospholipids	0.1301	5.075	5.216
Total Lipid Recovered	2.4942	97.302	

TABLE 4
 Fatty Acid Composition of Different Kidney Lipid
 Classes Eluted from Silicic Acid Column

Fatty Acid*	Kidney lipid	T. G.	F.F.A.	Phospholipid
C _{12:0}	12.40	10.38	8.81	0.00
C _{14:0}	6.22	5.43	5.03	1.40
C _{15:0}	4.21	2.59	1.38	27.23
C _{16:0}	16.35	18.27	20.15	42.72
C _{17:0}	1.40	5.37	3.49	11.26
C _{18:0}	21.23	16.29	20.57	2.98
Total Saturated	61.81	58.33	59.43	85.59
C _{16:1}	8.36	14.76	9.56	6.17
C _{16:2}	0.93	0.00	7.76	2.80
C _{18:1}	24.05	22.05	19.57	3.97
C _{18:2}	4.85	4.86	3.68	1.47
Total Unsaturated	38.19	41.67	40.57	14.41

* Relative areas under the curves for the methyl esters of the component fatty acids as estimated from GLC

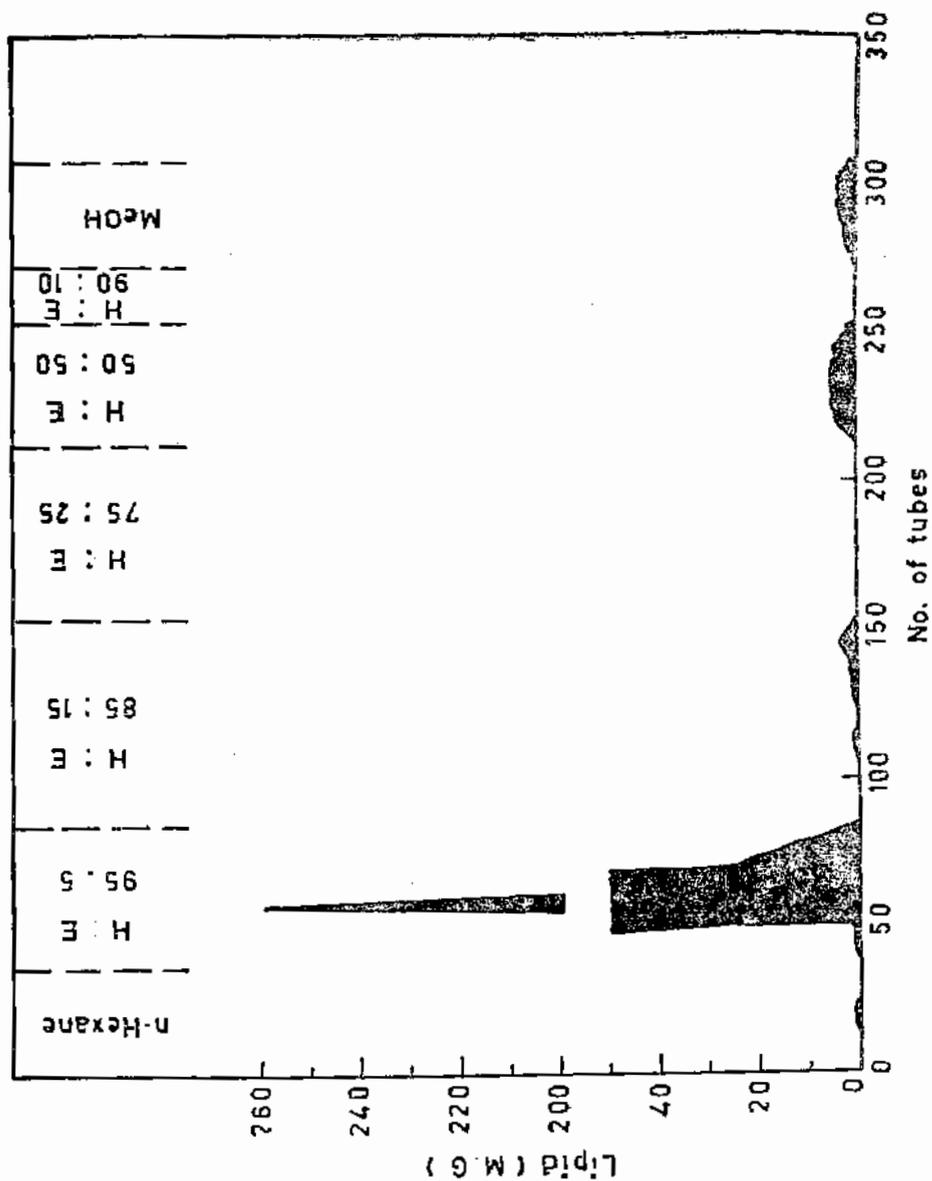


Fig. 2 — Illustration of the nature of the lipid eluted from silicic acid column.

