

## FATTY ACID COMPOSITION OF VARIOUS PARTS OF A POLLED ANGUS STEER\*

S. ADHIKARI

*Fats Research Laboratory, Department of Scientific and Industrial Research, Wellington, New Zealand†*

(Received August 19, 1963)

Fatty acid composition of different parts of a Polled Angus Steer of known age, breed and history was determined by gas-liquid chromatographic analysis. Fatty acids obtained after removal of the unsaponifiable matters from a freshly rendered fat were converted into methyl esters by methanol-acid method. Quantitative estimations of the fatty acids were based on the total area (retention volume) method of analysis and the identifications of fatty acids were done from a study of relative retention time. Standards were used separately. Diethylene glycol adipate was used as a stationary phase in a 8 ft. column packed with graded celite. Details concerning procedures used and the results obtained are presented in this paper.

### Introduction

Analysis of fatty acids by gas liquid chromatography was first introduced by James and Martin in 1952.<sup>1</sup> Since then several developments have been made and the application of gas liquid chromatographic method of fatty acid analysis has become a matter of routine work. The rate of progress of fatty acid analysis by GLC is so rapid and numerous that it has become very difficult to keep abreast of all the works in this field. The method has proved to be more effective than the best of the conventional methods for estimation and identification of the different components in a mixture of fatty acids. Trace components are now identified quickly and confidently.

The distribution of fatty acids in the body of an animal is not uniform. It has been noted by several workers<sup>2-4</sup> that the outer fat of pig, sheep and cow contains a higher proportion of unsaturated fatty acids than the inner fat. In a review of the formation of animal fats, Shorland<sup>5</sup> compared the fatty acid composition of perinephric, intermuscular, intramuscular and subcutaneous fat from an ox but the result was not further specified. In a recent study, Ostrander and Dugan<sup>6</sup> have shown in details the distribution of unsaturated fatty acids in the muscular and covering fats of pork, beef, veal and lamb. Even though the variation of fatty acid composition in the different parts of an animal body was observed before but no complete analysis of the acids was made. In the present investigation the distribution of both saturated and unsaturated fatty acids from the back, cod, caul, anal and kidney of a known animal is shown.

### Experiments and Results

The animal chosen was a two year-old, grass-fed prime Polled Angus steer and was killed at the

Southdown Works of the Auckland Farmers' Freezing Company in February, 1961. Since the animal was a steer it was possible to examine the cod fat which is especially formed in the scrotum of the castrated animals. Other samples are kidney fat (hard adipose tissue surrounding kidneys), back fat (skin fat from back), caul fat (attached to intestinal mesenteries) and anal or channel fat (fat surrounding rectum).

The tissues from the different parts of the animal were rendered in a pressure cooker and the fat obtained was washed and dried separately. Melting points were found out by the capillary tube method and iodine values were determined by Wijs method. Each sample has been analysed for the usual constants and the results are given in Table 1.

After saponification of the fat unsaponifiable matters were removed by extraction with diethyl ether. The fatty acids obtained were methylated with a mixture of methanol-sulphuric acid by refluxing on a water bath. The washed and dried methyl esters were analysed by gas liquid chromatography using an eight-foot column packed with graded celite. The solid support was impregnated with diethylene glycol adipate. Argon carrier gas and strontium 90 detector were used. The column was heated to 207°C. for all runs. Chromatograms of the samples are shown in Figs. 1-5.

The fatty acids were estimated by measuring the retention volume of the individual peaks and were expressed in mole percent. Identification of the methyl esters according to the peaks were done from the retention time comparing with known samples which were used separately under identical conditions. Table 2 shows the fatty acid composition of the different samples.

### Discussion

Whereas the saponification equivalents and hence the mean molecular weights are essentially the same throughout, vast differences between

\* A part of the work was done at the Abels Limited, Auckland.

† Present address: East Regional Laboratories, P.C.S.I.R., Dhanmondi, Dacca.



TABLE I

	Kidney	Anal	Caul	Cod	Back
Unsaponifiable matters	0.52%	0.49%	0.51%	0.57%	0.44%
Melting points	48.0°C.	46.0°C.	48.5°C.	41.0°C.	36.5°C.
Saponification equivalent of fat	285.0	284.0	285.0	283.0	283.5
Average mol. wt. of fatty acids from sap. equivalent	272.3	271.3	272.3	270.3	271.8
Average mol. wt. of fatty acids calculated from GLC analysis	272.3	271.5	274.0	271.9	272.0
Iodine value of fat	36.9	38.1	38.4	48.2	54.3
I.V. of fatty acids calculated from GLC analysis	33.0	35.0	36.3	48.3	55.1

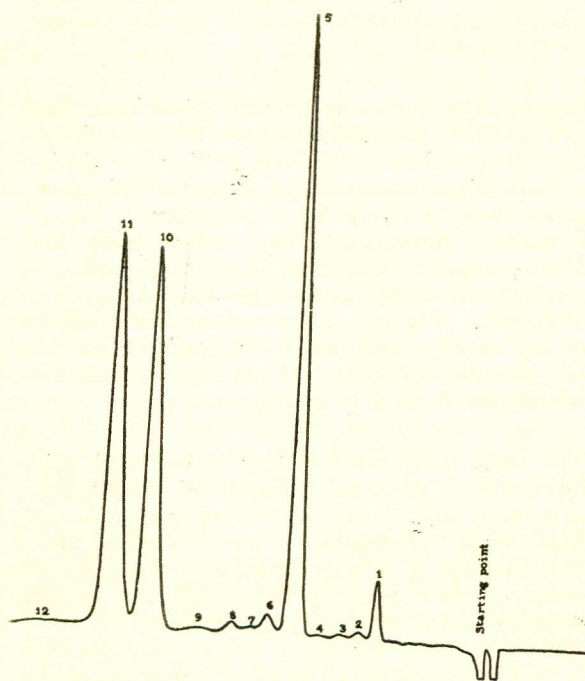


Fig. 1.—Gas-liquid chromatogram of methyl ester of kidney fat.

the iodine values and melting points are apparent (Table 1). The percentage of unsaponifiable matters are once again nearly the same for all samples. Fat from the back skin has the highest

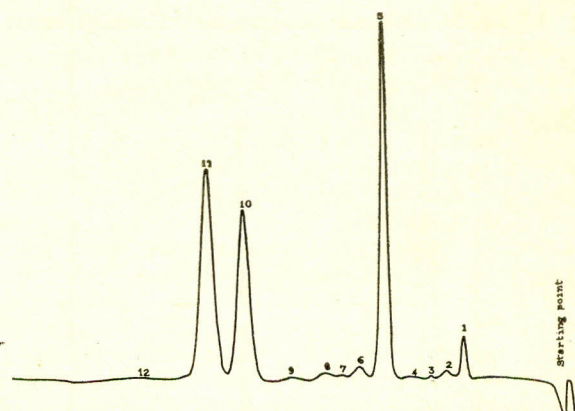


Fig. 2.—Gas-liquid chromatogram of methyl esters of anal fat.

iodine value (54.3), whereas inner kidney fat has the lowest unsaturation (I.V. 36.9). Though the average molecular weight of mixed fatty acids obtained from saponification equivalent agrees with the results calculated from the chromatographic analysis, the iodine values found experimentally show a small variation from that of the calculated results of the GLC analysis.

Altogether twelve peaks were found to appear on each of the chromatograms of the different samples of fat. The peak Nos. 1, 2, 5, 6, 10, 11 and 12 have the retention times corresponding to known methyl esters of myristic, myristoleic, palmitic, palmitoleic, stearic, oleic and linoleic acids.



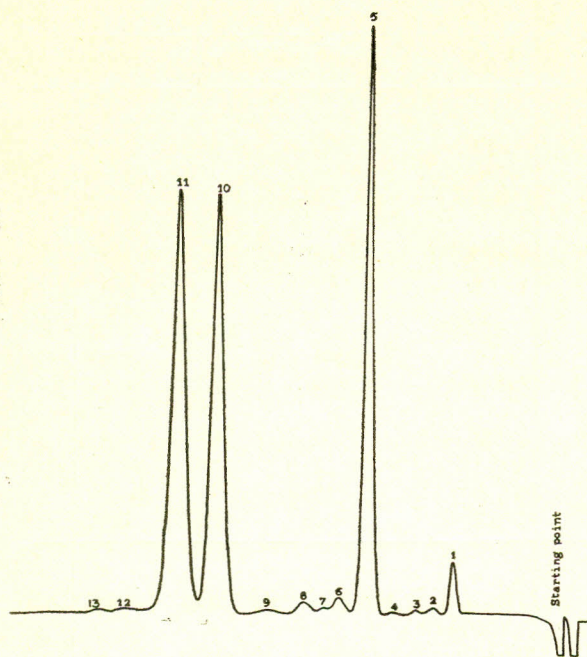


Fig. 3.—Gas-liquid chromatogram of methyl esters of caul fat.

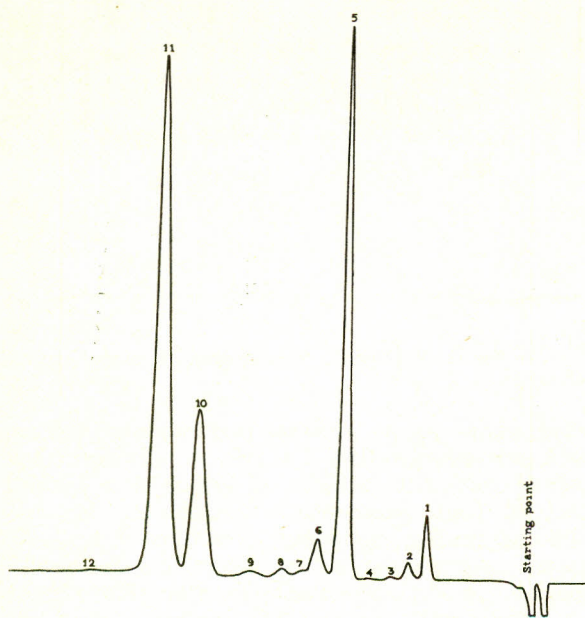


Fig. 4.—Gas-liquid chromatogram of methyl esters of cod fat.

The rest of the peaks were tentatively identified from the results obtained earlier by using the same column. No capric or lauric acids were found. Szonyi and et al.<sup>7</sup> obtained 0.0–0.4%  $C_{14}$  branched

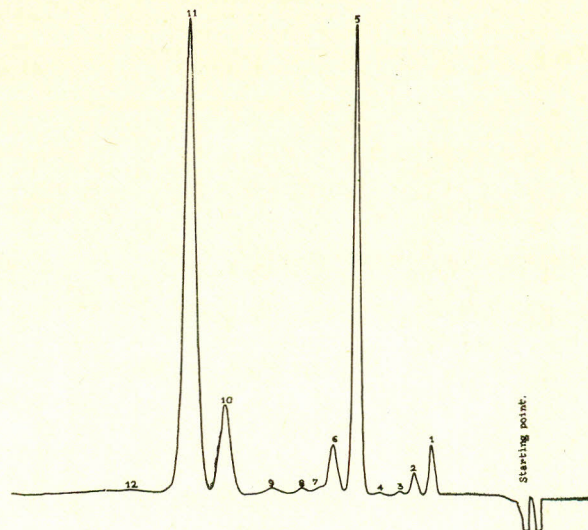


Fig. 5.—Gas-liquid chromatogram of methyl esters of back fat.

chain fatty acid which appeared to be completely missing in the present samples. On the other hand the samples were found to contain linoleic (0.1–0.4%) acid.

Significantly the mole percent of myristic and palmitic acids are almost constant throughout, with average values 2.5% and 30% respectively. The bulk of the unsaturation occurs as oleic acid, and the sum of  $C_{18}$  acids is once again almost the same throughout. The main acids are palmitic, stearic, oleic and their total amounts in back and in kidney fats are 90.7% and 93.6% respectively. Though these results show fairly good agreement with those obtained by Szonyi et al. mentioned earlier, yet smaller fractions are doubtful due to lack of known standards.

The most remarkable feature of the fatty acid composition is the wide variation of palmitoleic, stearic and oleic acids in various parts of the animal body. Palmitoleic acid varied from 1.3% (caul fat) to 4.5% (back fat). The highest amount of oleic acid (52.8%) and the lowest amount of stearic acid (9.5%) are found in back fat and on the other hand kidney fat contains only 32.8% of oleic acid and 28.4% of stearic acid. The proportions of these three acids are mainly responsible for different iodine values of the samples. The amount of the essential fatty acid is nearly the same for all samples except cod fat. Very close to this acid in caul fat there appeared another peak (No. 13) whose retention time closely corresponds to that of linolenic acid. No trace of arachidonic acid was found.



TABLE 2.—FATTY ACID COMPOSITION IN MOLE %.

Peak No.	Acid	Kidney	Anal	Caul	Cod	Back
1.	C <sub>14</sub>	2.5	3.0	2.1	3.0	2.1
2.	C <sub>14</sub> <sup>Δ</sup>	0.5	0.9	0.4	1.1	1.2
3.	C <sub>15</sub> Br	0.2	0.1	0.1	0.1	0.1
4.	C <sub>16</sub>	0.1	0.2	traces	traces	traces
5.	C <sub>16</sub>	32.4	33.1	27.4	29.1	28.4
6.	C <sub>16</sub> <sup>Δ</sup> Br	1.4	1.5	1.3	3.0	4.5
7.	C <sub>17</sub>	0.3	0.3	0.2	0.3	0.3
8.	C <sub>17</sub> Br	0.8	1.2	0.9	0.6	0.4
9.	C <sub>18</sub>	0.3	0.2	0.2	0.4	0.4
10.	C <sub>18</sub>	28.4	24.8	31.1	14.9	9.5
11.	C <sub>18</sub> <sup>Δ</sup>	32.8	34.4	35.9	47.4	52.8
12.	C <sub>18</sub> <sup>Δ</sup> <sup>2</sup>	0.3	0.3	0.4	0.1	0.3

**Acknowledgement.**—The author wishes to thank Mr. J.G. Colebrook for assistance, Mr. S.G. Brooker, Abels Limited, Auckland, and Dr. F.B. Shorland, Fats Research Laboratory, Wellington, for giving the facilities to carry out the work and Mr. Rands, Auckland Farmers' Freezing Company for supplying the samples of adipose tissues.

#### References

1. James and Martin, *Biochem. J.*, **50**, 679 (1952).
2. Dean and Hilditch, *Biochem. J.*, **27**, 1950 (1933).
3. Hilditch and Zaky, *Biochem. J.*, **35**, 940 (1941).
4. Dugan et al., *J. Am. Oil Chemists' Soc.*, **29**, 298 (1952).
5. Shorland, *Progress in the Chemistry of Fats and other Lipids*, **3** (1955).
6. Ostrander and Dugan, *J. Am. Oil Chemists' Soc.*, **39**, 178 (1962).
7. Szonyi et al., *J. Am. Oil Chemists' Soc.*, **39**, 185 (1962).