

killed 26.5 hours later, the acid and lactate portion of the ester found were: respired air 65.5 and 67.4%; liver 4.9 and 2.4%; and carcass 20.7 and 18.9% (Figure 11). There was no outstanding difference between results obtained with the two materials.

Specific Activity of Tissues. Since the amount of activity administered by stomach tube varied among the animals, the specific activity, in terms of disintegrations per milligram of fresh tissue, has been corrected to a standard constant dosage of 100 μ c. for all animals. The data in Table VI represent essentially random distribution, except that in series 1, although the other tissues appear to have attained equilibrium at 24 hours, the specific activity of the liver is ten times higher at 24 than at 48 hours. Data in series 2 indicate that the specific activity of the liver may be a more sensitive indicator than the specific activity in the respired air for measuring equilibrium of lactic acid in the body, since the specific activity of the liver had not yet reached equilibrium (Table VI) whereas the specific activity of the $C^{14}O_2$ in the exhaled air had approached equilibrium (Figure 6). Comparable conclusions may be drawn with respect to

the lactate moiety of the ester (Table VI). Because of the small size of the adrenals and the difficulty in completely and adequately isolating them from all surrounding tissue, the accuracy of their individual determination is less than that obtained on the other individual samples.

The similarity between specific activities under different conditions is shown in Table VII. These values indicated a close similarity between the free and esterified lactate in series 1. The greater variation in series 2 may reflect the lesser activity administered.

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COMPOSITION OF FATS

Fatty Acid Composition of Food Fats

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Gas-liquid chromatography was employed to investigate the nature of the fatty acids present in margarines, spreads, shortenings, and some meat fats. The results obtained from polyester and silicone columns generally agreed, and showed that C_{22} fatty acids were present in 3 of 16 margarines, 6 of 14 spreads, and 1 of 7 shortenings. In these products marine oils appeared to be the main source of the long-chain fatty acids. Fat of animal origin also contained fatty acids of odd-numbered carbons.

AN INTEREST in long-chain fatty acids in foods was aroused when rapeseed oil became a possible constituent of the Canadian diet. Since C_{20} and C_{22} acids are also components of marine oils, it was decided to investigate the nature of the fatty acids present in margarines, spreads, shortenings, and some animal fats. Gas-liquid chromatography was employed as an effective means of obtaining quantitative analyses.

Materials and Methods

One-gram aliquots of margarines and spreads were extracted with diethyl ether; the ether extracts were washed

with water, dried with anhydrous sodium sulfate, and evaporated in the presence of nitrogen. The method of Bligh and Dyer (2) was used to extract the lipid material from meat. By direct transesterification (5, 8), the methyl esters of fatty acids were prepared. Approximately 100 mg. of each margarine and spread fat, of each shortening as purchased, and each meat fat were methylated by refluxing in 10 ml. of methanol and 1 ml. of 7% HCl in methanol for 30 minutes. After removal of the HCl and methanol with the aid of a water bath and a stream of nitrogen, 1 μ l. of the methyl esters was inserted into a Beckman GC-2 gas chromatograph in

which the injector was modified and the gas sampling valve was removed. A 1-mv. recorder was employed.

Methyl esters of fatty acids were chromatographed on a 6-foot, $1/4$ -inch column packed with butanediol succinate (5) of m.p. 97° C. on acid-washed Chromosorb W (1 to 6 parts by weight). The temperatures of the injector and the column were 206° and 232° C., respectively, while the helium flow was 80 ml. per min. For confirmation of the results obtained with the polyester column, the fractions of each chain length were determined with a 2-foot, $1/4$ -inch column of silicone on C_{22} firebrick (1 to 6 parts by weight) operated

Table I. Per Cent Fatty Acids in Margarine Samples A through O

Fatty Acid	Double Bonds	A		B		C		D		E		F		G	
		P.C. ^a	S.C. ^b	P.C.	S.C.	P.C.	S.C.	S.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.
C ₁₂	0	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1
C ₁₄	0	0.3	0.2	0.3	0.2	4.0	3.8	0.4	0.5	0.9	0.8	0.2	0.1	1.3	1.3
C ₁₅	0	0.2	0.4	0.2	0.1
C ₁₆	0	10.9	...	14.2	...	19.1	...	17.9	...	18.0	...	13.4	...	15.2	...
C ₁₆	1	6.4	...	0.1	...	2.3	...	0.5	...	2.5	...
Total C ₁₆		10.9	10.8	14.2	13.8	25.5	24.8	18.0	18.6	20.3	19.8	13.9	13.9	17.7	17.0
C ₁₇	0	0.6
C ₁₇	1	0.4
Total C ₁₇		1.1	0.8
C ₁₈	0	5.9	...	7.5	...	7.4	...	7.9	...	8.3	...	6.4	...	5.9	...
C ₁₈	1	58.3	...	59.4	...	30.9	...	59.9	...	56.9	...	64.5	...	50.3	...
C ₁₈	2	21.1	...	18.6	...	20.1	...	13.7	...	11.5	...	13.9	...	17.5	...
C ₁₈	3	0.8	0.6	...
Total C ₁₈		85.3	85.2	85.5	86.0	58.4	57.6	81.5	80.7	77.5	78.0	84.8	85.2	74.3	74.3
C ₂₀	0	1.4	0.8	0.8	...	0.5	...	2.2	...
C ₂₀	1	1.9	4.2	0.3	...	0.5	...	0.9	...
C ₂₀	2	1.6	0.1	...
C ₂₀	4	0.2	...
Total C ₂₀		3.3	3.6	6.6	7.0	1.1	1.5	1.0	0.8	4.4	4.4
C ₂₂	0	2.3	2.4	...
C ₂₂	1	1.1
C ₂₂	2	0.8
Total C ₂₂		4.2	5.5	2.4	2.4

		H		I		J		K		L		M		N		O	
		P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.
C ₁₂	0	0.1	0.1	1.6	1.9	0.3	0.2
C ₁₄	0	3.5	2.9	0.1	0.1	0.2	0.2	0.2	0.2	1.1	1.0	0.1	0.1	0.3	0.4	0.4	0.5
C ₁₅	0	0.2	0.3
C ₁₆	0	20.2	...	11.8	...	14.5	...	13.9	...	10.9	...	11.6	...	18.2	...	13.7	...
C ₁₆	1	5.9	...	0.3	...	0.4	...	0.3	0.4	0.5	...
Total C ₁₆		26.1	25.5	12.1	12.5	14.9	14.9	14.2	13.9	10.9	10.7	12.0	11.9	18.2	19.0	14.2	13.8
C ₁₇	0	0.8
C ₁₇	1	0.6
Total C ₁₇		1.0	1.0
C ₁₈	0	5.4	...	6.8	...	6.1	...	7.6	...	8.9	...	8.0	...	6.3	...	7.8	...
C ₁₈	1	27.9	...	58.2	...	67.6	...	62.1	...	62.5	...	64.5	...	60.7	...	59.0	...
C ₁₈	2	18.7	...	20.1	...	11.2	...	14.6	...	14.8	...	14.7	...	12.5	...	18.3	...
C ₁₈	3	0.5	0.2
Total C ₁₈		52.5	52.0	85.1	85.4	84.9	84.9	84.5	84.7	86.2	...	87.2	87.1	79.5	78.9	85.1	85.5
C ₂₀	0	6.3	...	1.3	0.8	...	0.1	...	0.7	...	2.0
C ₂₀	1	1.8	...	1.3	0.3	...	0.1
C ₂₀	2	0.6
C ₂₀	4	0.8
Total C ₂₀		9.5	10.5	2.6	1.9	1.1	1.2	0.2	0.4	0.7	0.9	2.0	1.7
C ₂₂	0	6.3
C ₂₂	1	0.4
C ₂₂	2	0.5
Total C ₂₂		7.2	7.8

^a Polyester column.
^b Silicone column.

at 230° C. with an injector temperature of 260° C. and a helium flow rate of 30 ml. per minute. The percentage composition of the methyl esters of fatty acids was calculated from areas obtained by drawing tangents to the curves on the recorder charts, and measuring the height and base of the resultant triangles. Minor fatty acids were tentatively identified from the relationship of their retention times to those of known fatty acids.

Results and Discussion

The fatty acid composition of margarines, spreads, shortenings, and some meat fats is shown in Tables I to IV. In general the total fatty acids of each chain length, as determined with the polyester column, agreed with the results obtained with the short silicone column.

In the fatty acids of the margarines analyzed, Table I, palmitic constituted 10.9 to 20.2%; stearic, 5.4 to 8.9%;

oleic, 27.9 to 67.6%; linoleic, 11.2 to 21.1%. Other work involving earlier samples of margarine and older methods of analysis showed that margarine fatty acids were comprised of 42 to 79% oleic acid and 3 to 13% linoleic acid (7), or 2 to 8% linoleic acid (7). In the products studied here, vegetable oils were the source of most of the margarine fats. The presence of marine oils, which contain a higher ratio of C₁₆ to C₁₈ acids than vegetable oils and appreci-

Table II. Per Cent Fatty Acids in Spread Samples A through N

Fatty Acid	Double Bonds	A		B		C		D		E		F	
		P.C. ^a	S.C. ^a	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.
C ₁₂	0	2.5	2.4	0.2	0.2	0.1	0.1	0.1	0.1
C ₁₄	0	1.8	1.6	4.2	4.4	0.1	0.1	1.9	1.8	2.6	2.7	0.2	0.2
C ₁₅	0	0.3	0.3	0.1	0.1	0.2	0.3
C ₁₆	0	15.9	...	20.0	...	11.0	11.2	23.8	...	22.1	...	16.7	...
C ₁₆	1	0.4	...	9.0	3.2	...	6.1	...	0.1	...
Total C ₁₆		16.3	17.2	29.0	29.8	11.0	11.2	27.0	26.5	28.2	28.6	16.8	17.2
C ₁₇	0	1.3	0.3	...	0.6
C ₁₇	1	0.3	0.2	...	0.6
Total C ₁₇		1.6	0.6	0.5	0.9	1.2	0.4
C ₁₈	0	6.6	...	8.3	...	8.3	...	13.4	...	10.3	...	7.0	...
C ₁₈	1	60.6	...	31.7	...	67.5	...	44.2	...	34.3	...	61.9	...
C ₁₈	2	11.7	...	5.2	...	11.5	...	9.1	...	7.1	...	13.6	...
C ₁₈	3	0.6	...	0.3	...	0.3	...	2.4
C ₁₈	4
Total C ₁₈		78.9	78.2	45.8	44.8	87.6	87.3	67.0	66.1	54.1	54.7	82.5	82.1
C ₂₀	0	0.5	...	6.8	...	0.5	...	1.6	...	6.1	...	0.5	...
C ₂₀	1	3.4	...	0.4	...	0.7	...	2.6
C ₂₀	2	1.2	0.1	...	0.6
C ₂₀	3	0.2	0.3
C ₂₀	4	0.5	0.5
Total C ₂₀		0.5	0.6	12.1	12.5	0.9	1.1	2.4	3.2	10.1	9.1	0.5	0.5
C ₂₂	0	5.5	...	0.4	...	1.0	...	3.5
C ₂₂	1	1.3
Total C ₂₂		6.8	7.5	0.4	0.3	1.0	1.3	3.5	4.1

		G		H		I		J		K		L		M		N	
		P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.
C ₁₂	0	0.1	0.1	0.2	0.1	1.2	1.4	0.1	0.1	0.2	0.1	0.1	0.1
C ₁₄	0	0.5	0.4	0.5	0.3	1.0	0.9	0.2	0.1	0.2	0.2	2.3	1.6	0.8	0.7	2.6	2.3
C ₁₅	0	0.3	0.1	0.2	0.2
C ₁₆	0	17.4	...	12.1	...	10.8	...	13.0	...	13.7	...	22.1	...	16.0	...	23.9	...
C ₁₆	1	0.2	...	0.2	...	0.2	...	0.2	...	0.5	...	5.0	3.9	...
Total C ₁₆		17.6	17.1	12.3	12.4	11.0	10.8	13.2	13.9	14.2	14.2	27.1	27.1	16.0	15.9	27.8	27.1
C ₁₇	0	0.2	...	0.3	0.2	0.7	...	0.1	...	0.5	...
C ₁₇	1	0.1	...	0.3	0.3	0.3	...	0.1
Total C ₁₇		0.3	0.1	0.6	0.2	0.5	0.1	1.0	0.6	0.2	0.1	0.5	0.7
C ₁₈	0	7.4	...	7.8	...	8.3	...	7.3	...	7.3	...	10.5	...	8.1	...	14.3	...
C ₁₈	1	60.5	...	61.1	...	63.8	...	65.8	...	65.3	...	40.9	...	67.2	...	39.9	...
C ₁₈	2	13.0	...	14.8	...	13.9	...	12.3	...	12.4	...	5.5	...	5.7	...	6.8	...
C ₁₈	3	0.2	...	0.3	...	0.2	...	0.3	...	0.2	...	0.4	0.1	...
C ₁₈	4	0.5
Total C ₁₈		81.1	81.9	84.5	85.1	86.2	86.6	85.7	85.5	85.2	85.2	57.3	56.6	81.0	81.0	61.1	61.6
C ₂₀	0	0.5	...	0.8	...	0.5	...	0.4	...	0.3	...	7.3	...	0.6	...	3.3	...
C ₂₀	1	1.2	1.1	...	1.2	...	1.0	...
C ₂₀	2	1.1	...	1.2	...	1.0	...
C ₂₀	3	0.3	0.8	...
C ₂₀	4
Total C ₂₀		0.5	0.4	2.0	1.9	0.5	0.3	0.4	0.4	0.3	0.3	9.0	9.9	1.8	2.2	5.8	6.7
C ₂₂	0	2.6	1.7	...
C ₂₂	1	0.5
Total C ₂₂		3.1	4.1	1.7	1.3

^a See Table I.

able amounts of C₂₀ and C₂₂ acids, was indicated in margarines C, G, and H.

In the spreads, Table II, the major fatty acids were: palmitic, 11.0 to 23.9%; stearic, 6.6 to 14.3%; oleic, 31.7 to 67.5%; linoleic, 5.2 to 14.8%. Products B, D, E, L, and N appeared to contain marine oil.

Sample D of the shortenings, Table III, was labeled as "pure vegetable shortening" and contained the highest proportion of C₁₈ acids. It was evident from the fatty acid composition of sample

F that some marine oil was present. The major fatty acids in shortening were: palmitic, 14.1 to 25.4%; stearic, 14.1 to 18.5%; oleic, 34.8 to 56.2%; linoleic, 7.1 to 18.2%.

Hartman (6) reported that odd-numbered, straight-chain acids and branched chain acids appeared in ox, sheep, and butterfat; Chisholm and Hopkins (3) found that heptadecanoic and heptadecenoic acids occurred on musk-ox fat. From the analyses of the materials studied here, fatty acids of odd-numbered car-

bons were apparent in products containing marine oil or other animal fat and in most of the meat fats shown in Table IV.

Samples of meat fat were obtained from available supplies without knowledge of the dietary history of the animal involved. The fatty acid composition of each fat could be expected to vary, for pork fat was reported to contain 2 to 6% linoleic acid (4). In haddock fat, the sample which provided the least agreement between the polyester and

Table III. Per Cent Fatty Acids in Shortening Samples A through G

Fatty Acid	Double Bonds	A		B		C		D		E		F		G	
		P.C. ^a	S.C. ^a	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.
C ₁₂	0	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
C ₁₄	0	1.3	...	1.9	...	1.2	...	0.2	...	1.6	...	2.1	...	1.8	...
C ₁₄	1	0.4	...	0.3	...	0.1	0.3	...	0.2	...	0.3	...
Total C ₁₄		1.7	1.2	2.2	2.0	1.3	1.0	0.2	0.3	1.9	1.2	2.3	1.9	2.1	1.8
C ₁₅	0	0.3	0.2	0.2	0.4	0.1	0.1	0.3	0.4	0.2	0.2	0.3	0.1
C ₁₆	0	17.9	...	25.4	...	20.1	...	14.1	...	21.9	...	23.2	...	23.9	...
C ₁₆	1	1.4	...	2.8	...	1.6	...	0.3	...	2.3	...	3.6	...	4.2	...
Total C ₁₆		19.3	18.7	28.2	27.8	21.7	22.0	14.4	14.5	24.2	24.4	26.8	26.1	28.1	27.3
C ₁₇	0	0.6	...	0.7	...	0.5	1.0	...	0.8	...	0.8	...
C ₁₇	1	0.2	...	0.3	...	0.2	0.5	...	0.2	...	0.1	...
Total C ₁₇		0.8	1.3	1.0	1.4	0.7	0.3	1.5	1.3	1.0	0.8	0.9	1.0
C ₁₈	0	15.3	...	17.4	...	14.8	...	14.1	...	18.5	...	17.1	...	15.6	...
C ₁₈	1	42.2	...	39.6	...	41.1	...	56.2	...	38.1	...	34.8	...	41.3	...
C ₁₈	2	18.2	...	9.9	...	18.2	...	13.9	...	13.1	...	7.1	...	9.9	...
C ₁₈	3	1.4	...	0.3	...	0.6	...	0.4	...	0.8	...	0.2	...	0.5	...
C ₁₈	4	1.3
Total C ₁₈		77.1	77.6	67.2	67.0	74.7	75.1	84.6	84.3	70.5	71.3	60.5	60.5	67.3	68.1
C ₂₀	0	0.3	...	0.7	...	0.2	...	0.5	...	0.7	...	3.6	...	0.2	...
C ₂₀	1	0.3	...	0.4	...	1.0	...	0.1	...	0.8	...	0.8	...	0.9	...
C ₂₀	2	0.1	0.7	...	0.1	...
C ₂₀	3	0.5
C ₂₀	4	0.2
Total C ₂₀		0.6	0.9	1.1	1.3	1.3	1.3	0.6	0.8	1.5	1.3	5.8	6.7	1.2	1.6
C ₂₂	0	2.8
C ₂₂	1	0.5
Total C ₂₂		3.3	3.7

^a See Table I.

Table IV. Per Cent Fatty Acids in Various Samples

Fatty Acids	Double Bonds	Pork		Beef		Mutton		Lamb		Horse		Chicken		Haddock	
		P.C. ^a	S.C. ^a	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.	P.C.	S.C.
C ₁₂	0	0.1	0.1	0.1	0.1	0.8	0.8	0.3	0.2
C ₁₄	0	1.8	...	4.3	...	2.2	...	6.7	...	5.3	...	0.5	...	1.1	...
C ₁₄	1	1.4	...	1.2	...	1.2	...	0.4
Total C ₁₄		1.8	1.6	5.7	5.2	3.4	2.5	7.9	8.0	5.7	5.6	0.5	1.3	1.1	1.1
C ₁₅	0	0.3	...	0.7	...	1.0	...	0.9	...	0.4	0.5	...
C ₁₅	1	0.4	...	0.6	...	0.4
Total C ₁₅		0.3	0.2	0.7	0.6	1.4	1.8	1.5	1.3	0.8	0.3	0.5	0.7
C ₁₆	0	25.9	...	27.1	...	21.0	...	21.0	...	26.7	...	24.5	...	22.7	...
C ₁₆	1	3.4	...	5.3	...	3.0	...	3.5	...	7.8	...	3.2	...	4.7	...
C ₁₆	2	0.9	...	0.8	0.3
Total C ₁₆		29.3	28.9	32.4	31.8	24.9	24.2	25.3	25.6	34.5	35.0	28.0	28.0	27.4	24.6
C ₁₇	0	0.8	...	1.6	...	2.1	...	1.6	...	0.8	1.5	...
C ₁₇	1	0.4	...	1.4	...	0.7	...	1.0	...	1.1	0.5	...
Total C ₁₇		1.2	0.6	3.0	3.2	2.8	3.3	2.6	2.6	1.9	1.0	2.0	3.7
C ₁₈	0	14.8	...	15.7	...	27.1	...	17.5	...	4.4	...	9.6	...	5.3	...
C ₁₈	1	43.7	...	38.6	...	34.5	...	37.2	...	27.5	...	39.1	...	15.1	...
C ₁₈	2	6.3	...	3.4	...	3.0	...	3.8	...	9.9	...	20.8	...	2.5	...
C ₁₈	3	1.1	2.9	...	2.9	...	14.2	...	1.0	...	0.3	...
Total C ₁₈		65.9	66.3	57.7	58.8	67.5	68.2	61.4	60.8	56.0	56.9	70.5	69.5	23.2	22.1
C ₂₀	0	0.4	0.5	...	0.8	...	0.6	...	0.3	...
C ₂₀	1	1.4	0.2	...	3.5	...
C ₂₀	2	0.3	...	0.4	...
C ₂₀	4	1.8	...
C ₂₀	5	14.3	...
Total C ₂₀		1.4	2.3	0.4	0.2	0.5	0.6	0.8	1.0	1.1	2.2	20.3	20.1
C ₂₂	4	0.4	...
C ₂₂	5	0.7	...
C ₂₂	6	24.3	...
Total C ₂₂		25.4	27.8

^a See Table I.

silicone columns, the many unsaturated C₂₀ and C₂₂ acids constituted a large portion of the total fatty acids and the long-chain fatty acids were as prevalent as in many rapeseed oils. Fats of marine origin are a likely source of dietary long-chain fatty acids.

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