CXXV. VARIATIONS IN THE COMPONENT FATTY ACIDS OF BUTTER DUE TO CHANGES IN SEASONAL AND FEEDING CONDITIONS.

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Some little time ago a modified form of the ester fractionation process for the analysis of mixed fatty acids, suitable for the case of fats containing appreciable quantities of volatile fatty acids, was worked out and used to determine the composition of three market samples of New Zealand butter [Hilditch and Jones, 1929]. The results then obtained indicated that the analytical procedure was capable of giving more detailed information on the proportions of the various fatty acids present than had formerly been available and held out the possibility that its employment might yield further insight into the much discussed problem of the influence of oleaginous diet on the composition of butter-fat.

The most serious practical difficulty in a programme of this kind is the lengthy nature of the analyses involved, especially in the case of a fat which includes so wide a range of fatty acids as butter; this makes it difficult to deal with more than relatively few test samples in the course of one series of investigations. In illustration of this it may be said that almost two years have been occupied in the detailed study of the five butter-fats which form the main experimental material discussed in this communication, including, in addition to the determination of the fatty acid compositions of the original fats, an examination of their component glyceride structures, with the fatty acid compositions of the fully saturated glycerides present; an account of this part of the work will be presented later. The main part of the investigation was carried out on four samples of butter-fat (about 7–8 lb. each) collected for us from a herd of ten Shorthorn cows at the National Institute for Research in Dairying, Shinfield.

The four samples of butter consisted of (I), butter from the herd at the end of October, 1928, when the cows were still partly pasture-fed but were also receiving winter diet as below; (II) and (III), butter, in each case, from two cows of the herd fed on a general ration with, in addition, either coconut cake or soya bean cake, these butters being from milk collected about the middle of March, 1929, after the cows had been feeding for nearly a month on the respective diets; and (IV), butter from milk from the whole herd of ten cows collected at the end of May, 1929, after the cows had been feeding on early summer pasture for about a month.

It is convenient also to refer to the figures obtained for a sample of New Zealand butter-fat (V) which was kindly supplied to us by Dr F. H. McDowall from a herd at the Dairy Research Institute, Palmerston North, New Zealand, feeding on early summer pasture in December, 1928, since this particular sample brings out certain features of the problem which emphasise its complexity and the difficulty of correlating cause and effect.

As a matter of fact, the information resulting from our analyses of butter-fats I–IV showed that other factors than the fatty diet supplied exerted important influences, and certain subsidiary tests bearing on this aspect were carried out later on samples of butter-fat obtained monthly from the same herd of Shorthorn cows at Shinfield during the winter of 1929–1930 and the following spring, covering the periods when the cows were receiving the same diet as that from which butter-fat I was derived (winter conditions) and when the herd returned to spring pasture.

In view of the complex factors involved, we believe that it will be found most convenient to describe our experimental results in the first place and then to discuss them, both as a whole, and also in their relation to the results obtained by previous workers in this field.

Fatty acid compositions of butter-fats I–V.

The details of the feeding, etc., of the cows may first be recorded.

Dealing first with the butters from Shinfield, these were made in each case from sweet unheated cream (without starter) at the National Institute farm.

Butter-fat I.

This was from milk from the herd of ten Shorthorn cows collected on October 28–30, 1928. The cows were still getting some grass and also 50 lb. kale and 6 lb. clover hay with 3–12 lb. concentrates (according to the milk yield); the latter consisted of dried grains three parts, maize germ meal two parts, soya bean meal extracted one part.

Butter-fat II (coconut cake-fed) and butter-fat III (soya bean cake-fed).

Two cows of the herd received the coconut cake diet, and other two cows were given the soya bean cake diet. The milk from which the butter samples were prepared was collected on March 12–15, 1929, and the details of feeding prior to the preparation of the butter were as follows.

Each cow received daily 30 lb. mangolds, 30 lb. silage and 15 lb. hay.

The cows on coconut cake ration received in addition:

	Per cow daily	
	Coconut cake	Mixture B*
From Feb. 5	3 lb.	13 lb.
Gradually increasing coconut cake to Feb. 25. And continued these quantities to March 20	7 lb.	7 lb.

Each cow was thus receiving 7 lb. of coconut cake and 0·8 lb. of soya cake daily from Feb. 26 to March 20.

The cows on soya cake ration received in addition:

	Per cow daily		
	Soya cake	Crushed oats	Mixture B*
From Feb. 12	2 lb.	3 lb.	9 lb.
Gradually increasing soya cake to Feb. 25	4 lb.	7 lb.	3 lb.

From Feb. 26 to March 20 the two cows each received 13 lb. of a mixture containing two parts soya cake, one part flaked maize, one part crushed oats and one part sharps: each cow was thus receiving 5·2 lb. soya cake daily from Feb. 26 to March 20.

Butter-fat IV.

This was prepared from milk from the whole herd after they had been out on early summer pasture for several weeks; the milk was collected on May 26 and 27, 1929.

Butter-fat V (New Zealand pasture-fed).

The origin of this sample has already been described.

For the purposes of chemical investigation the butters were melted, settled, and the fat decanted and filtered through two layers of thick filter-paper (which had previously been kept in the steam-oven for some time at 100°) in order to remove traces of caseinogen and any residual moisture. Some of the general analytical characteristics of the fats were determined with the following results:

Table I.

Butter-fat	I	II	III	IV	V
Sap. equiv.	253·3	242·7	243·3	248·2	242·2
Iodine value	41·3	31·6	34·8	41·6	34·5
Reichert-Meissl value	29·6	29·3	32·3	25·7	30·3
Polenske value ...	1·9	3·2	2·0	2·2	2·3
Kirschner value ...	23·7	23·7	25·5	20·6	24·7
Refractive index $n_D^{60^\circ}$	1·4477	1·4450	1·4455	1·4473	1·4462

The determination of the fatty acids present in these butters was carried out almost exactly according to the scheme described at length in the former communication [Hilditch and Jones, 1929], and, except for one or two further precautions which it has been found desirable to observe, it is unnecessary here to repeat the full description of the procedure, which is briefly as follows.

* "Mixture B" consisted of three parts dried grains, three parts maize gluten feed, one part crushed oats, one part crushed wheat and one part soya cake.

The fatty acids from about 500 g. of butter-fat are distilled in a current of steam for about 4 or 5 hours and the acids volatile in steam are extracted from the aqueous condensate by ether and fractionated as such, mainly at atmospheric pressure; the extracted aqueous liquors and the recovered distilled ether are titrated with alkali, any acid present being calculated as butyric acid. The acids non-volatile in steam are recovered and weighed and a suitable portion is submitted to the lead salt separation, the resulting groups of acids being converted into methyl esters and fractionated quantitatively in the usual way.

In connection with the separation of the volatile acids, emphasis may be laid on the necessity, in the preliminary saponification of the butter-fat, for employing alcohol which is entirely free from traces of esters; it is advisable to boil the alcohol with excess of potassium hydroxide prior to use in the hydrolysis of the butter-fats, and subsequently to distil it from the potash.

It has also been observed that the residue left after fractionating the extracted volatile acids almost invariably contains a very small amount of unsaturated fatty acid, but no unsaponifiable matter. A correction for the unsaturated material present has been made on the assumption that this is oleic acid; the quantity of unsaturated acid present in the steam-distillate is so small that we have not been able definitely to identify it, but on the other hand, we have established that distillation of oleic acid in steam, under the conditions of time and rate of distillation used in these analyses, results in small amounts of oleic acid being found in the condensate in amount of the same order as those found in the butter analyses.

The composition of the acids non-volatile in steam was determined in exactly the same manner as that described in the former communication [cf. Hilditch and Jones, 1929, pp. 80, 81]: each group of neutral methyl esters (mainly saturated and mainly unsaturated) was fractionally distilled from a Willstätter bulb under high vacuum and the first 25–30 % of the primary distillate was collected in a single fraction, whilst the remainder was collected in relatively small fractions; the first primary fractions were then redistilled and collected in small successive fractions, especially in the earlier stages. Owing to the low boiling-point of methyl caprylate and caprate, it is advisable to distil the lower boiling fractions somewhat slowly and to cool the side-arm and Perkin receiver by means of filter-paper moistened with ice-cold water.

During the refractionation of the lowest-boiling primary fraction of the esters of the "solid" acids, and also (although less prominently) of the esters of the "liquid" acids from butter-fats, slight maxima have almost invariably been observed in the iodine values of fractions which consist mainly of methyl caprate and laurate. Possibly this is due to minute amounts of unsaturated esters of corresponding molecular weight, but more probably to increase in the concentration of methyl oleate in the distillate at these points; that methyl oleate readily forms constant boiling mixtures with saturated esters of a wide range of molecular weight is well known [cf. Crowther and Hynd, 1917], and it may be that the tendency to form such mixtures is greater in the case of specific members of the saturated series.

The method of calculation has also been described at length in the earlier paper and it is perhaps only necessary here to tabulate the final results obtained in the case of each butter-fat; to illustrate the details of the procedure, however, the whole of the experimental data is quoted at length for the analysis of the component fatty acids of butter-fat IV.

Fatty acids of butter-fat IV.

Table II. *Acids volatile in steam.*

No.	g.	B.P. (° C.)	Pressure	Mean equivalent	Acids: Butyric	Caproic	Caprylic	Capric	Oleic
1	In aqueous solution (3750 cc.)			—	0·50				
2	In recovered ether (2760 cc.)			—	0·08				
3	68·41	35–100	Atmospheric	—	3·19				
4	1·64	100–160	,,	—	1·17				
5	2·47	160–165	,,	91·3	2·10	0·37			
6	3·91	165–170	,,	93·3	2·99	0·92			
7	2·04	170–175	,,	94·9	1·43	0·61			
8	2·41	175–177	,,	97·3	1·46	0·95			
9	1·93	93–120	Reduced	105·6	0·60	1·33			
10	1·29	120–132	,,	120·6	—	1·04	0·25		
11	0·85	132–135	,,	133·3	—	0·28	0·57		
12	0·82	Residue (iodine value 14·4)		176·9	—	—	0·14	0·55	0·13
					13·52	5·50	0·96	0·55	0·13

Table III. *Acids non-volatile in steam*, 394 g.

(Sap. equiv. 254·0; iod. val. 45·8.)

Lead salt separation.

	g.	%	Corresponding esters: S.E.	I.V.
'Solid' acids S	173·2	57·8	269·8	19·45
'Liquid' acids L	126·2	42·2	269·0	77·14

Table IV. *Fractionation of methyl esters.*

(i) *Esters of 'solid' acids S.*

Primary fractions: No.	g.	B.P./1 mm.	Sap. eq.	I.V.	Refractionations: No.	g.	B.P./1 mm.	Sap. eq.	I.V
S 1	43·41	66–127°	249·7	6·0	S 11	0·86	64–87°	178·8	8·0
					S 12	1·27	87–102	205·5	5·9
					S 13	3·09	102–110	234·5	3·4
					S 14	10·73	110–117	245·0	3·1
					S 15	4·14	117–126	254·0	3·45
					S 16	12·43	126–130	262·6	5·3
					S 17	6·24	Residue	279·9	16·85
S 2	11·95	127–128	265·5	9·8					
S 3	18·68	128	269·9	12·55					
S 4	7·62	128–133	271·2	14·55					
S 5	9·28	133–135	276·9	17·1					
S 6	21·87	135–137	277·4	23·1					
S 7	8·47	137–143	286·5	31·2					
S 8	21·62	143–156	288·5	37·7					
S 9	20·84	Residue	292·0	37·8					

Table IV (*contd.*).

(ii) *Esters of 'liquid' acids L.*

Primary fractions					Refractionations				
No.	g.	B.P./1 mm.	Sap. eq.	I.V.	No.	g.	B.P./1 mm.	Sap. eq.	I.V.
					L 11	1·27	40–49°	163·9	2·6
					L 12	2·87	49–54	181·9	10·5
					L 13	2·24	54–64	192·9	11·2
L 1	33·18	30–132°	225·4	35·2	L 14	1·36	64–75	207·1	10·5
					L 15	1·50	75–85	218·3	11·9
					L 16	6·25	85–99	227·6	18·85
					L 17	12·45	Residue	274·3	66·85
L 2	7·02	132–137	278·5	85·1					
L 3	12·13	137–140	286·3	85·4					
L 4	12·19	140–142	290·6	93·45					
L 5	21·10	142–143	291·3	95·8					
L 6	20·61	143–148	297·0	96·85					
L 7	11·77	Residue	315·0	114·0					

Table V. *Estimated composition of fatty acids.*

Methyl esters of 'solid' acids S.

	S 11 g.	S 12 g.	S 13 g.	S 14 g.	S 15 g.	S 16 g.	S 17 g.	Total g.	% as esters
Caprylate	0·36	—	—	—	—	—	—	0·36	0·9
Caprate	0·42	0·52	—	—	—	—	—	0·94	2·4
Laurate	—	0·66	0·91	—	—	—	—	1·57	4·1
Myristate	—	—	2·06	9·77	2·39	3·60	—	17·82	46·0
Palmitate	—	—	—	0·57	1·58	8·07	3·87	14·09	36·4
Stearate	—	—	—	—	—	—	1·14	1·14	2·9
Oleate	0·08	0·09	0·12	0·39	0·17	0·76	1·23	2·84	7·3

	S 1 g.	S 2 g.	S 3 g.	S 4 g.	S 5 g.	S 6 g.	S 7 g.	S 8 g.	S 9 g.	Total g.	% as esters	% as fatty acids
Caprylate	0·40	—	—	—	—	—	—	—	—	0·40	0·2	0·2
Caprate	1·05	—	—	—	—	—	—	—	—	1·05	0·6	0·6
Laurate	1·76	—	—	—	—	—	—	—	—	1·76	1·1	1·1
Myristate	19·96	2·90	2·13	0·66	—	—	—	—	—	25·65	15·7	15·6
Palmitate	15·78	7·68	13·82	5·67	6·70	15·18	3·05	6·36	3·46	77·70	47·5	47·5
Stearate	1·28	—	—	—	0·73	0·81	2·34	5·75	8·19	19·10	11·7	11·7
Oleate	3·18	1·37	2·73	1·29	1·85	5·88	3·08	9·51	9·19	38·08	23·2	23·3

Methyl esters of 'liquid' acids L.

	L 11 g.	L 12 g.	L 13 g.	L 14 g.	L 15 g.	L 16 g.	L 17 g.	Total g.	% as esters
Caprylate	1·01	1·02	0·09	—	—	—	—	2·12	7·6
Caprate	0·23	1·54	1·89	0·58	0·11	—	—	4·35	15·6
Laurate	—	—	—	0·63	1·21	4·77	0·76	7·37	26·4
Myristate	—	—	—	—	—	0·26	3·10	3·36	12·0
C_{18} unsat.	0·03	0·31	0·26	0·15	0·18	1·22	8·59	10·74	38·4

	L 1 g.	L 2 g.	L 3 g.	L 4 g.	L 5 g.	L 6 g.	L 7 g.	Total g.	% as esters	% as fatty acids
Caprylate	2·52	—	—	—	—	—	—	2·52	2·1	2·1
Caprate	5·16	—	—	—	—	—	—	5·16	4·4	4·25
Laurate	8·75	0·85	0·54	—	—	—	—	10·14	8·6	8·45
Myristate	3·99	—	0·90	0·43	0·22	—	—	5·54	4·7	4·7
Oleate Linoleate	12·76	6·17	10·69	11·76	20·88	20·61	11·07	82·01 11·93	69·5 10·1	69·8 10·1
Unsaponifiable	—	—	—	—	—	—	0·70	0·70	0·6	0·6

Table VI. *Summarised experimental data for fatty acids of butters I–V.*

Acid	Volatile acids g.	Acids non-volatile in steam: Solid acids S g.	Liquid acids L g.	Total	% (excluding unsaponifiable matter)
		Butter-fat I.			
	9·46	81·17	79·83	170·46	—
Butyric	5·27	—	—	5·27	3·1
Caproic	2·92	—	—	2·92	1·7
Caprylic	1·27	—	1·45	2·72	1·6
Capric	—	—	3·55	3·55	2·1
Lauric	—	0·08	5·75	5·83	3·4
Myristic	—	8·25	3·45	11·70	6·9
Palmitic	—	49·29	—	49·29	29·0
Stearic	—	12·90	—	12·90	7·6
Arachidic	—	1·54	—	1·54	0·9
Oleic	—	9·04	59·06	68·10	40·1
Linoleic	—	—	6·21	6·21	3·6
Unsaponifiable	—	0·07	0·36	0·43	—
		Butter-fat II.			
	34·48	298·85	232·15	565·48	—
Butyric	19·43	—	—	19·43	3·4
Caproic	10·97	—	—	10·97	2·0
Caprylic	1·94	—	4·18	6·12	1·1
Capric	1·62	2·39	13·93	17·94	3·2
Lauric	0·24	15·54	25·08	40·86	7·3
Myristic	—	79·50	16·72	96·22	17·1
Palmitic	—	141·94	9·75	151·69	27·0
Stearic	—	27·20	—	27·20	4·8
Oleic	0·28	31·68	146·47	178·43	31·7
Linoleic	—	—	13·23	13·23	2·4
Unsaponifiable	—	0·60	2·79	3·39	—
		Butter-fat III.			
	32·74	288·0	242·0	562·74	—
Butyric	20·35	—	—	20·35	3·6
Caproic	8·42	—	—	8·42	1·5
Caprylic	1·77	0·29	7·50	9·56	1·7
Capric	1·82	2·02	17·42	21·26	3·8
Lauric	—	4·61	31·94	36·55	6·5
Myristic	—	54·44	4·84	59·28	10·6
Palmitic	—	147·16	—	147·16	26·3
Stearic	—	46·37	—	46·37	8·3
Arachidic	—	6·91	—	6·91	1·2
Oleic	0·38	26·20	157·57	184·15	32·9
Linoleic	—	—	20·33	20·33	3·6
Unsaponifiable	—	—	2·40	2·40	—
		Butter-fat IV.			
	20·66	227·9	166·1	414·66	—
Butyric	13·52	—	—	13·52	3·3
Caproic	5·50	—	—	5·50	1·3
Caprylic	0·96	0·53	3·41	4·90	1·2
Capric	0·55	1·42	7·08	9·05	2·2
Lauric	—	2·41	14·06	16·47	4·0
Myristic	—	35·47	7·74	43·21	10·4
Palmitic	—	108·10	—	108·10	26·1
Stearic	—	26·72	—	26·72	6·5
Oleic	0·13	53·25	115·91	169·29	40·9
Linoleic	—	—	16·86	16·86	4·1
Unsaponifiable	—	—	1·04	1·04	—

Table VI (*contd.*).

Acid	Volatile acids g.	Acids non-volatile in steam: Solid acids S g.	Acids non-volatile in steam: Liquid acids L g.	Total	% (excluding unsaponifiable matter)
		Butter-fat V.			
	27·24	246·3	196·7	470·24	—
Butyric	16·30	—	—	16·30	3·5
Caproic	7·89	—	—	7·89	1·7
Caprylic	1·14	—	5·10	6·24	1·3
Capric	1·59	—	12·83	14·42	3·1
Lauric	—	4·15	14·90	19·05	4·1
Myristic	—	29·48	22·77	52·25	11·1
Palmitic	—	127·80	—	127·80	27·3
Stearic	—	53·85	—	53·85	11·5
Arachidic	—	3·01	—	3·01	0·6
Oleic	0·32	27·78	118·80	146·90	31·3
Linoleic	—	—	20·95	20·95	4·5
Unsaponifiable	—	0·23	1·35	1·58	—

Discussion.

The experimental data for the four butter-fats from the Shinfield herd and those for the New Zealand sample V may usefully be considered in conjunction with the results obtained formerly from market samples of New Zealand butter.

Table VII *a*. *Summary of fatty acid analyses (weight percentages) of butter-fats.*

Butters from Shinfield, Reading.

Acid	I (Autumn-fed, 1928) %	II (Coconut cake, 1929) %	III (Soya cake, 1929) %	IV (Early summer pasture, 1929) %
Butyric	3·1	3·4	3·6	3·3
Caproic	1·7	2·0	1·5	1·3
Caprylic	1·6	1·1	1·7	1·2
Capric	2·1	3·2	3·8	2·2
Lauric	3·4	7·3	6·5	4·0
Myristic	6·9	17·1	10·6	10·4
Palmitic	29·0	27·0	26·3	26·1
Stearic	7·6	4·8	8·3	6·5
Arachidic	0·9	—	1·2	—
Oleic	40·1	31·7	32·9	40·9
Linoleic	3·6	2·4	3·6	4·1

New Zealand butters.

	(Pasture-fed, December, 1928) V %	Market samples [Hilditch and Jones, 1929] "A" %	"B" %	"C" %
Butyric	3·5	3·4	3·1	3·2
Caproic	1·7	1·8	1·9	1·7
Caprylic	1·3	0·9	0·8	0·8
Capric	3·1	1·9	2·0	2·3
Lauric	4·1	3·1	3·9	4·3
Myristic	11·1	9·7	10·6	10·8
Palmitic	27·3	27·6	28·1	28·4
Stearic	11·5	12·2	8·5	9·4
Arachidic	0·6	0·7	1·0	0·5
Oleic	31·3	34·3	36·4	33·1
Linoleic	4·5	4·4	3·7	5·4

Table VII *b*. *Summary of fatty acid analyses (molar percentages) of butter-fats.*

Butters from Shinfield, Reading.

Acid	I (Autumn-fed, 1928) %	II (Coconut cake, 1929) %	III (Soya cake, 1929) %	IV (Early summer pasture, 1929) %
Butyric	8·4	9·0	9·6	8·9
Caproic	3·5	3·9	3·0	2·7
Caprylic	2·7	1·7	2·8	2·0
Capric	2·9	4·3	5·1	3·0
Lauric	4·1	8·3	7·5	4·7
Myristic	7·2	17·2	10·7	10·9
Palmitic	27·1	24·1	23·7	24·3
Stearic	6·4	3·9	6·7	5·4
Arachidic	0·7	—	0·9	—
Oleic	33·9	25·7	27·0	34·6
Linoleic	3·1	1·9	3·0	3·5

New Zealand butters.

	(Pasture-fed, December, 1928) V	Market samples [Hilditch and Jones, 1929] "A"	"B"	"C"
	%	%	%	%
Butyric	9·2	9·2	8·4	8·7
Caproic	3·4	3·7	3·9	3·4
Caprylic	2·2	1·4	1·3	1·4
Capric	4·2	2·7	2·8	3·1
Lauric	4·7	3·7	4·6	5·1
Myristic	11·5	10·2	11·0	11·2
Palmitic	25·0	25·7	26·2	26·3
Stearic	9·5	10·2	7·1	7·8
Arachidic	0·5	0·5	0·8	0·6
Oleic	26·1	28·9	30·8	27·9
Linoleic	3·7	3·8	3·1	4·5

The percentage compositions of the fatty acids in these eight samples are collected in Tables VII *a* and VII *b*; in Table VII *a* will be found the percentages by weight as determined in the analyses, whilst, since a much clearer view of some aspects of the variations in composition is obtained by considering the relative numbers of molecules of the different acids present, the corresponding molar percentages for the eight samples are given in Table VII *b*.

If the Shinfield butters are first of all considered separately, either as regards their component fatty acids (Table VII) or their general analytical characteristics (Table I), it will be seen that sample I (obtained in late autumn, 1928, with the herd on diet consisting mainly of normal winter rations with some grass) resembles butter-fat IV (from the cows on pasture in early summer, 1929) much more closely than do samples II and III (in which special fats were included in the diet). The most noticeable difference between fats II and III and fats I and IV is a quite marked diminution in the proportion of unsaturated acids present. It had originally been hoped that the autumn-fed 1928 butter (I) might serve as a starting-point for comparison of the effects of added fat in the diet, but whilst conceivably a coconut cake diet might lower the proportion of unsaturated acids, such a result would not be anticipated in the case of a diet including soya cake. Moreover, these data are quite at variance with the general results (as measured by the iodine values of the butter-fats) obtained in a similar systematic examination of the influence of fatty diet on milk-fat made by Holland and Buckley [1918, 1923].

These workers employed a herd of four cows (comparatively fresh in lactation) and maintained two of the cows on a daily ration of 24 lb. of hay and 10 lb. of a grain mixture throughout; the diet of the other two cows was augmented for about a month at a time by ¾ lb. daily of different fatty oils, coconut, ground-nut, maize and soya bean oil being administered during successive periods. The iodine values of butter-fats obtained from the individual cows, and the dates on which the milk samples were taken, were as follows:

	Cow no.	1	2		3	4
		Normal rations only		Corresponding period with oils		
	Daily ration					
Nov. 18–20, 27–29, 1919	24 lb. hay + 10 lb. grain mixture	27·3	28·0	No oils	27·7	27·6
Dec. 11–13, 25–27, 1919	24 lb. hay + ¾ lb. coconut fat	28·8	27·3	C.N.O.	25·3	26·5
Jan. 8–10, 22–24, 1920	24 lb. hay + ¾ lb. ground-nut oil	27·6	27·7	G.N.O.	36·2	38·0
Feb. 5–7, 19–21, 1920	24 lb. hay + ¾ lb. maize oil	27·9	28·3	M.O.	39·9	40·4
Mar. 4–6, 18–20, 1920	24 lb. hay + ¾ lb. soya bean oil	28·3	28·6	S.B.O.	41·1	41·2

Holland and Buckley state that whilst coconut oil decreased the content of butyric-capric acids and also of oleic acid and increased the proportion of lauric and myristic acids, the other three oils caused an increase in the amount of butyric, stearic and oleic acids and a progressively increasing fall in the proportions of the acids from caproic to palmitic; and they conclude that feeding with different oils and fats definitely modifies the chemical composition of butter-fat.

It is further noticeable in Holland and Buckley's experiments (i) that the cows fed only on normal rations yielded butter-fat of approximately the same (and abnormally low) iodine value all the time, and (ii) that the daily amount of additional fat given was of the same order as that present in the coconut cake and soya bean cake feed of the cows for our samples II and III. In the experience of these workers, coconut oil in the diet caused a slight but definite fall in the iodine value of the butter-fat and the presence of the other three oils in the diet caused marked increases in the corresponding iodine values, these increases being moreover roughly proportionate to the iodine values of the oils administered.

It seemed probable, on the contrary, that our own results were influenced by some condition or conditions producing a greater effect than the mere presence of specific fats in the diet, and, since the same group of ten Shorthorn cows was to be kept during the winter of 1929–1930 on the same normal winter ration (without added oil cake) as during the previous winter, it was decided to conduct a brief examination of the fat from milk collected at approximately monthly intervals during this winter season and after the cows had returned to pasture in the spring of 1930. The results of this further study are collected in Table VIII.

Table VIII. *Butter-fats from herd of ten Shorthorn cows, Shinfield, November, 1929–June, 1930.*

Milk collected	Conditions	Iod. value	Sap. eq.	R.M. value	Polenske value	Kirschner value
Nov. 20–21, 1929	Indoor winter feed	42·2	248·1	27·0	1·8	21·3
Dec. 18–19, 1929	,,	38·3	247·6	29·3	1·7	22·6
Feb. 4–5, 1930	,,	38·9	247·9	27·3	1·6	21·4
Feb. 25–26, 1930	,,	36·5	244·6	29·7	2·0	23·8
Mar. 16–17, 1930	,,	37·3	246·8	30·6	1·8	24·8
Apr. 26–27, 1930	Spring pasture	43·3	244·6	28·4	1·9	23·5
June 2–3, 1930	,,	44·2	249·5	24·6	1·8	20·6

The iodine values in Table VIII show that the November, 1929 sample was of practically the same degree of unsaturation as butter-fat I (end of October, 1928), and that successive samples showed a drop in iodine value which was at a maximum at about the end of February, 1930; on returning to spring pasture the iodine value was at once restored to a figure somewhat in excess of the November sample, the parallelism with the 1928–1929 observations being complete in this respect, and the restoration of the iodine value after return to pasture being extremely well defined.

It is reasonable, in view of these figures, to infer that a similar progressive variation was in progress during the 1928–1929 season and, in the light of Holland and Buckley's observation that added coconut oil in the diet does not seriously diminish the iodine value of the fat, it may also be concluded that the iodine value of butter-fat II (31·6) is not greatly below the minimum iodine value of butter-fat from this group of cows fed on the normal ration at the corresponding period of the 1928–1929 winter. If this be the case it follows that the observed content of oleic and linoleic acids in the soya cake-fed butter III represents a slight increase over the normal figure.

It is also apparent that the decrease in iodine value during the 1928–1929 season was considerably greater than the corresponding decrease in 1929–1930. Further, the minimum iodine value of the 1929–1930 series was observed towards the end of February, 1930, and butter-fats II and III were prepared from milk collected about the middle of March, 1929; thus the minimum iodine value observed in the 1929–1930 series coincided with the coolest period of a particularly mild winter, whilst samples II and III of the 1928–1929 season were obtained immediately following the intense cold of February, 1929. These facts, particularly the exceptionally low iodine values of the March, 1929 butters, suggest that an important factor in the composition of butter-fat may be the atmospheric temperature, at all events during the winter months; we believe that the results now presented indicate a general change in the composition of the butter-fat, defined more particularly by alteration in the content of oleic acid, and due mainly to "winter conditions" (that is, either the change from outdoor to indoor conditions or from grass to indoor rations or both) and probably also to seasonal changes of temperature. In the case of butter-fats I–IV there is little doubt that causes of this description have exerted far more influence on the composition of the fat than any addition of fatty oil to the diet.

We may add that the appearance of a more saturated fatty acid mixture in butter under cold conditions is, of course, in harmony with accepted views, in so far that the body reserves of fat may be more drawn upon under cold conditions, and presumably the more unsaturated portions will be more readily consumed.

The fact that, on a similar diet, the Shinfield cows exhibited change in character of the butter-fat during the winter season, whilst the cows observed by Holland and Buckley yielded, over a similar period, milk-fats of almost identical (but extremely low) iodine value, is in our opinion a further argument in support of the contention that the chemical composition of a butter-fat is mainly determined by a variety of factors, the cumulative effect of which is certainly much greater than that produced by direct ingestion of fatty oils.

The consistently low iodine values of the butter-fats from the cows fed on the "normal" ration, in the experiments of Holland and Buckley, may be connected with the fact that the cows in question were comparatively early in lactation. Other figures given by these authors go to show that the amount of unsaturated acids in butter increases with the time that the cows have been in lactation. The Shinfield herd, from which our samples of butter were derived, was made up of cows which were at an intermediate or comparatively late stage in lactation.

It may also be pointed out that the "normal" ration given by Holland and Buckley consisted of dried hay and grain mixture, whereas that for the Shinfield cows included a substantial proportion of "roots"[1] with some hay and grain mixture; this difference in the basal diet may further have affected the composition of the resulting butters.

In this connection, and further to illustrate the difficulty of distinguishing between the various determining factors, it is worthy of note that the total amount of unsaturated components in Holland and Buckley's butter-fat from cows fed on soya bean cake, expressed in terms of iodine value (41·1), is no greater than that which we found for the "normal" butter-fats I (winter-fed, iodine value 41·3) and IV (spring pasture, iodine value 41·6) or for the corresponding fats obtained in the following season, namely, November, 1929 (iodine value 42·2) and April or May, 1930 (iodine values 43·3 and 44·2).

[1] It is worth recording that the butters from cows receiving cabbage (35 lb.) or kale (50 lb.) with 10 lb. hay (October 1928 and November 1929) had respective iodine values 41·3 and 42·2, and that those produced from December 1929—March 1930 (iodine values 36·5–38·9) were from a diet of mangolds (40 lb.) and hay (14 lb.).

In the next place, attention may be drawn to the corresponding details for the New Zealand butters. All of these possess lower iodine values than the pasture-fed English butter IV and, notably, the New Zealand December pasture-fed butter-fat V (which may be supposed, *ceteris paribus*, to be a close analogue of butter-fat IV) has a much smaller proportion of unsaturated acids and, in fact, is curiously analogous in composition to the English winter butter-fat III. Although the total amount of unsaturated acids is thus lower in the New Zealand butters studied than in butters I–IV, the proportion of linoleic acid in the unsaturated acids is consistently higher in the New Zealand samples (10–14 % of the total unsaturated acids) than in the four Shinfield butters (7–10 %). Apart from this and from the not unusual variation in stearic acid contents, the experimental values for all four New Zealand butters are of much the same order, the differences in some cases being almost within the error of experiment. The point to which we desire to draw attention is the general difference (especially in the unsaturated acid content)[1] between pasture-fed New Zealand and Shinfield butters, as disclosed in these analyses, and emphasis is laid on the fact that such differences may be as great as those observed under the most extreme variation in feeding and other conditions of the same herd throughout an annual cycle. Our results, indeed, emphasise the complexity of a problem such as the present, in which the composition of a milk-fat probably depends on indoor or outdoor life, climate, feeding and similar conditions and, possibly above all, on the individual reaction of different animals to any or all of these influences.

On the other hand, although we have felt it necessary to stress the foregoing aspects of the experimental evidence, several points of detail show quite clearly the definite though minor influence of the fatty oils which were administered to the cows in the case of butters II and III.

The chief variations in molar percentages of fatty acids (apart from butter-fats II and III) are observed in myristic and stearic acids on the one hand and in oleic acid on the other, the maximum difference even in the latter case, however, not exceeding 9 mols. %. In the whole series of eight butters, it will be noticed that within broad limits the proportion of palmitic acid does not vary very widely, the extreme values in molar percentages being 23·7 to 27·1; it was mentioned in the former paper [Hilditch and Jones, 1929, p. 86] that this proportion is, moreover, not far removed from that which obtains in most beef tallows. Again, as was also suggested in the previous paper, butyric and myristic acids are present in something approaching equimolecular proportions in all cases, with the exception of butter-fat II (where the diet had included coconut cake).

[1] If the suggestion is correct that low iodine values of the Shinfield butter-fats may be correlated with low temperature conditions, there may perhaps be a similar connection between the somewhat greater variation in day and night temperatures in New Zealand than in England on the one hand and the relatively lower unsaturation of the New Zealand pasture-fed butters on the other.

The points which appear to be of outstanding interest in the butter-fats II and III (from cows fed on an oil cake diet) are as follows.

(1) In the case of coconut cake feed the amount of lauric acid is definitely increased, but in proportionately less degree than the increase in myristic acid content; the latter rises from about 7 to 17 mols. %, whereas the lauric acid content is only increased from 4 to 8 mols. %. This is somewhat surprising in view of the fact that coconut oil contains nearly 50 % of lauric acid and only about 17 % of myristic acid; it is perhaps an indication that lauric acid is more readily transformed to other products during digestive processes than is myristic acid, and the same may be said of caprylic and capric acids, both of which occur in fair quantity in coconut oil, whilst the butter-fat II does not contain an abnormal amount of either of these components.

(2) In the case of soya bean cake feed the detailed composition is even more interesting because, although the oleic acid content is slightly above that of the corresponding coconut cake-fed butter, the linoleic acid figure is of the usual relative order, or at all events the proportion of linoleic to oleic acid is not increased by more than about 10 %. In soya bean oil, on the other hand, the relative proportions of linoleic and oleic acids are something like 2 to 1, so that practically none of the extra linoleic acid ingested by the animal has appeared unchanged in the milk. The only other marked difference between the soya cake-fed fat and either of the corresponding more normal butters I and IV (other, of course, than the general drop in unsaturation which we ascribe to extraneous conditions) is the presence of a definitely larger proportion of lauric acid than usual, which, curiously enough, approaches that found in the butter from coconut cake-fed cattle, although there is, of course, no lauric acid present in soya bean oil.

The results of Holland and Buckley have been compared with the present data in detail since the experimental material is of the same type in each case and the investigators in question carried out an exceptionally large number of systematic feeding and control tests. Reference should also be made, however, to the work of Cranfield [1916], who examined the butter-fats from cows fed during the spring of 1916 on a ration of 56 lb. mangolds, 19 lb. hay, 1 lb. of bran and ½ lb. of dried yeast, with 4 lb. of either decorticated ground-nut or decorticated cotton seed cake; the resulting butter-fats were in each case of much the same composition (Reichert-Meissl values 29·3–32·3, Polenske values 1·87–1·96, Kirschner values 22·0–24·2, iodine values 38·4–43·8), and it will be noticed that these values are of the same general types as those for the Shinfield autumn butters of 1928 and 1929. Cranfield considered that the amount of unsaturated acids was rather greater, on the whole, after ground-nut cake than after cotton-seed cake diet; ground-nut oil fatty acids contain about 55–65 % oleic and 20–25 % linoleic acid, whereas in cotton-seed oil fatty acids the respective proportions are about 25 % oleic and 45–50 % linoleic acid.

An observation by Iyer [1919], to the effect that rice polish used with a ration of linseed cake for cows has the result of increasing the volatile acids and diminishing the unsaturated acids in the butter-fat (ghee), may have some bearing on the influence of bran and grain fed to cattle in "grain mixtures," and interesting results might be obtained by investigating the influence of other components of the diet than oil-cake on the fatty acid composition of butters.

The comprehensive paper by Channon, Drummond and Golding [1924] on the effect of certain oils in the diet of cows upon various characteristics of butter-fat has special reference to cod-liver oil, but the effects of ground nut and coconut oils were also studied with results very similar to those observed by us in the respective cases of soya bean and coconut oils. Moreover, the iodine values of the butters throughout the winter and spring seasons of 1922–1923 at Shinfield show the same general trend of variations as in the present work.

Summary.

Detailed analyses have been made of the fatty acids present in four English butter-fats obtained from members of the same herd of cows fed on various diets, and in a New Zealand pasture-fed (December) butter-fat, with the object of tracing any effects due to the ingestion of fatty oil. The results show that the influence of added fat in the diet, although definite, is of a minor order compared with that due to other causes.

Much more profound changes in fatty acid composition appear to be caused by conditions such as the change from outdoor to indoor life, the general character of the diet (*e.g.* pasture, "roots," hay, etc.), and seasonal changes in the temperature. Additional information on the incidence of these factors was obtained from a survey of the characteristics of butters obtained monthly from the same herd of cows when receiving a standard diet (without oil-cake).

The greater differences which are believed to be associated with such factors as the type of diet, the indoor or outdoor life, or the seasonal temperature, lie mainly in variation of the amount of unsaturated acids present, and in the amount of the lower saturated acids (butyric to lauric); palmitic acid appears relatively to be the most constant in proportion of any of the components (24–27 %), whilst the stearic acid content varies somewhat erratically from one butter to another.

Taken as a whole, all the butter-fats which have been analysed in detail in this and in a preceding investigation [Hilditch and Jones, 1929] are characterised by amounts of butyric to myristic acids which are more or less specific for cow milk-fat, and by amounts of palmitic, oleic and linoleic acids which, although equally characteristic of most butter-fats, are also not widely dissimilar from the proportions of the same acids which are found in the body- or storage-fats of the same class of animal.

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