The same reasoning can be applied to produce the simple first order equation:

$$-d(B)/dt = k_2(B) \text{ for species 2.}$$

The removal of the rolled-up films of tristearin that are obtained after a long continuous treatment is very slow and similar to that for species 2. This leads to the speculation that some feature of the rolling-up process is responsible for the formation of species 2.

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Quantitative Gas Liquid Chromatographic Analysis of Butterfat Triglycerides[1]

A. KUKSIS, M. J. McCARTHY, and J. M. R. BEVERIDGE, Department of Biochemistry, Queen's University, Kingston, Ontario, Canada

Abstract

Gas liquid chromatographic triglyceride separation by carbon number, and integration of the area response obtained in the hydrogen flame ionization detector, has permitted the calculation of the following molar proportions for the various triglyceride types in a blended butterfat sample: C_{24} 0.5, C_{25} 0.08, C_{26} 0.57, C_{27} 0.18, C_{28} 0.81, C_{29} 0.17, C_{30} 1.15, C_{31} 0.2, C_{32} 3.1, C_{33} 0.34, C_{34} 5.41, C_{35} 1.0, C_{36} 10.6, C_{37} 1.2, C_{38} 12.8, C_{39} 1.15, C_{40} 10.7, C_{41} 1.0, C_{42} 6.1, C_{43} 0.58, C_{44} 5.15, C_{45} 0.6, C_{46} 5.8, C_{47} 1.0, C_{48} 6.45, C_{49} 1.47, C_{50} 8.5, C_{51} 1.43, C_{52} 6.95, C_{53} 1.05, C_{54} 4.0. The validity of these estimates was verified by similar determinations performed on molecular distillates of butterfat and on butterfats with known amounts of added saturated and unsaturated long chain triglycerides. The fatty acid carbon recoveries estimated on the basis of the observed triglyceride peak proportions were of the order of 95% or better.

A comparison of the experimentally determined triglyceride type distributions with those calculated on the basis of a completely random fatty acid arrangement for the blended and the molecularly distilled samples showed considerable differences, the most apparent of which was the greater proportion of both short and long chain triglycerides consistently predicted for the random population. On the basis of these studies, it is suggested that butterfat possesses a non-random fatty acid distribution which is reflected in its triglyceride type distribution.

Introduction

THE COMPOSITION OF BUTTERFAT has been the subject of many investigations in the past. While the identification and elucidation of the structure of the constituent fatty acids of butterfat has now been almost completed (1,2) virtually nothing is known about the triglyceride structure of this fat.

The large number of fatty acids in butter gives rise to an extremely high number of combinations as glycerides, which differ little in their physical properties when adjacent triglycerides of the series are compared. As a result, quantitative separations of individual triglycerides have proved extremely difficult. Such relatively successful techniques of glyceride fractionation as low temperature crystallization and countercurrent distribution have failed to effect any butterfat separations useful in structural studies (3).

Analyses of the butterfat triglyceride structure by use of pancreatic lipase for cleaving the fatty acids esterified on the 1 and 3 positions of the glycerol have revealed an increased concentration of the C_{10}, C_{12}, and C_{14} saturated acids in the monoglycerides resulting from the action of the enzyme on the intact fat (4,5). On the basis of a mathematical evaluation of the distributions of triglyceride types and isomeric forms in terms of saturation and unsaturation, however, it has been concluded (5), that in butterfat the fatty acyl groups, classified only as saturated and unsaturated, have been brought together in groups of three at random, or nearly so. Accordingly, butterfat has been described as another of the group of fats in which saturated and unsaturated fatty acids are associated as S_3, S_2U, SU_2, and U_3 in proportions which can be specified, at least approximately, by application of the laws of probability operating freely or with some restriction. These conclusions are supported by analyses of the trisaturated glycerides of milk fat by the mercaptoacetic acid method (6).

It is obvious that comparisons of fatty acid positioning on the basis of saturated and unsaturated acids as classes is not an ideal manner of determining triglyceride structure, in that it obscures patterns in the placement of the individual fatty acids. Neither is an enzymatic positional analysis, when performed on a complex mixture of triglycerides. Before such studies become meaningful, an effective preliminary segregation of the fat either on the basis of unsaturation or molecular weight, or both, is obligatory.

[1] Presented at the AOCS meeting in Toronto, Canada, 1962

Successful fractionation of natural triglyceride mixtures by gas chromatography (7) has permitted a new approach to the determination of butterfat triglyceride structure. Though the ultimate analyses will require the collection and fractionation of the triglycerides within each molecular weight group and an identification of their constituent fatty acids, there are a number of important characteristics that are revealed by a simple nonpreparative gas chromatographic segregation of the butterfat triglycerides on the basis of their carbon number and a quantitative evaluation of the individual peak contributions.

The present report describes the molecular weight distribution for butterfat triglycerides and demonstrates the quantitative validity of the estimates. Data are included to show the non-randomness of this distribution in butterfat.

Experimental

The blended butterfat was an authentic sample obtained from Madhu Sahasrabudhe, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada. The following characteristics were also supplied: iodine number 34.8; Reichert-Meissl number 30.1; Polenske number 2.70. This material was shown by gas chromatography to be free of contamination with low molecular weight non-glyceride materials. The molecular butterfat distillates were those described previously (8). Fatty acid analyses were performed on a Beckman GC-2A gas chromatograph equipped with a filament cell (8). The instrument was calibrated with the Metabolism Study Section Standard Mixture A, distributed by the National Institutes of Health, Bethesda, Md. Gas liquid chromatography of the triglycerides was conducted as previously indicated (7). The aged column permitted the elution of the C_{54} peak at a temperature 50C lower than earlier reported. The operating temperatures are recorded in the figure. The areas under the peaks were computed by mechanical integration and/or triangulation as indicated in Figure 1. Only those runs that showed less than 10% variation in the recoveries of the major peaks were used in the calculations.

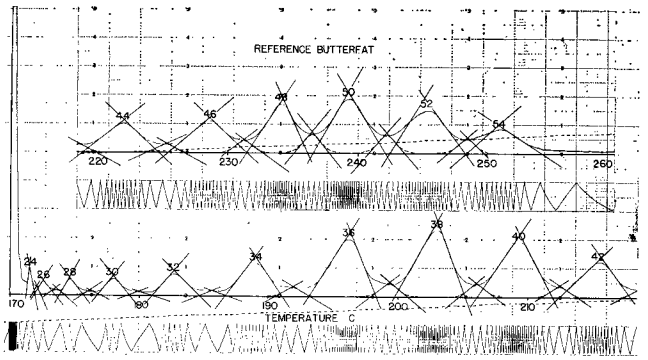

FIG. 1. A low-temperature increment gas-liquid chromatographic elution pattern recorded for blended butterfat sample. Tracings of minor triglyceride peaks completed by hand. Triangulation record superimposed.

Results and Discussion

The conditions for separation of triglycerides by gas liquid chromatography have been discussed before and the general pattern of separations obtained for butterfat has been indicated (7). In these separations, as well as those reported by Huebner (9), butterfat gave the most complex elution pattern of all natural fats, exhibiting a minimum and two maxima in its triglyceride distribution curve. Furthermore, the butterfat triglycerides could not be separated as effectively as triglycerides of comparable chain length

TABLE I
Distribution of Various Triglyceride Types in a Blended Sample of Butterfat

Triglyceride type	Observed % area response for various runs[a]							Observed mole %[b]	Random mole %[c]
	1	2	3	4	5	6	Avg		
C_{56}[d]	....	....	....	....	....	....	.....	.....	0.23
C_{55}	....	....	....	....	....	....	.....	.....	0.25
C_{54}	5.0	5.1	4.2	5.5	5.2	4.9	4.90	4.00	6.24
C_{53}	0.9	1.0	1.0	1.7	1.7	1.3	1.27	1.05	0.90
C_{52}	7.8	8.3	7.5	9.2	8.2	8.5	8.25	6.95	11.43
C_{51}	1.7	1.6	1.0	2.2	2.0	1.5	1.67	1.43	1.65
C_{50}	9.8	9.6	9.0	10.2	10.2	9.9	9.78	8.50	11.82
C_{49}	1.7	1.2	1.3	2.2	1.9	1.6	1.67	1.47	1.52
C_{48}	7.0	7.0	6.9	7.2	7.3	7.0	7.15	6.45	9.07
C_{47}	1.1	0.8	1.1	1.5	0.8	1.2	1.08	0.99	1.02
C_{46}	6.0	5.9	6.2	6.2	6.4	6.3	6.16	5.78	7.32
C_{45}	0.6	0.5	0.8	0.4	0.4	1.1	0.64	0.61	0.65
C_{44}	5.4	5.0	5.4	5.0	5.4	5.5	5.34	5.15	5.86
C_{43}	0.6	0.8	0.8	0.5	0.4	0.5	0.59	0.58	0.55
C_{42}	5.7	6.2	6.5	5.5	6.0	6.5	6.07	6.10	6.07
C_{41}	0.6	0.9	1.0	1.0	1.0	1.4	0.98	1.00	0.46
C_{40}	10.8	10.6	10.7	9.2	10.3	10.4	10.30	10.70	8.39
C_{39}	1.2	1.0	1.0	1.4	0.8	1.0	1.08	1.15	0.70
C_{38}	11.0	12.3	12.0	11.9	11.2	12.0	11.78	12.80	7.58
C_{37}	1.5	1.0	1.2	1.0	0.8	1.0	1.08	1.20	0.64
C_{36}	9.5	9.7	10.0	8.3	9.2	9.3	9.35	10.60	4.98
C_{35}	1.0	1.0	1.5	0.5	0.8	0.5	0.88	1.02	0.33
C_{34}	4.9	4.7	4.2	4.7	4.6	4.3	4.57	5.41	3.02
C_{33}	0.3	0.3	0.4	0.4	0.3	0.2	0.28	0.34	0.15
C_{32}	2.7	2.3	2.6	2.1	2.3	2.6	2.48	3.08	2.24
C_{31}	0.1	0.1	0.2	0.1	0.2	0.2	0.16	0.20	0.08
C_{30}	1.0	1.0	1.0	0.7	0.75	1.0	0.88	1.15	1.62
C_{29}	0.0	0.2	0.2	0.15	0.15	0.1	0.13	0.17	0.07
C_{28}	0.5	0.6	0.6	0.6	0.7	0.5	0.59	0.81	1.60
C_{27}	0.2	0.2	0.15	0.15	0.1	0.0	0.13	0.18	0.05
C_{26}	0.5	0.4	0.35	0.35	0.4	0.4	0.39	0.57	1.50
C_{25}	0.03	0.03	0.1	0.1	0.1	0.0	0.05	0.08	0.05
C_{24}	0.33	0.33	0.33	0.33	0.33	0.29	0.33	0.51	0.80
C_{23}	....	....	....	....	....	....	.....	.....	0.03
C_{22}	....	....	....	....	....	....	.....	.....	0.34
C_{21}	....	....	....	....	....	....	.....	.....	0.00
C_{20}	....	....	....	....	....	....	.....	.....	0.18
C_{19}	....	....	....	....	....	....	.....	.....	0.00
C_{18}	....	....	....	....	....	....	.....	.....	0.50

[a] The % area distributions determined and recorded here approximate those for the weight distribution of simple triglycerides in the hydrogen flame ionization detector.
[b] The mole % distributions were calculated from the weight % distributions by dividing by molecular weight and adjusting for 100.
[c] The random distribution was calculated as described in the text from the fatty acid composition indicated in Table III.
[d] The triglyceride type is indicated by the total number of fatty acid carbons.

from coconut oil. In contrast to analytical runs on the coconut oil, the elution patterns obtained with butterfats were characterized by an incomplete return of the recorder pen to the base line between any two neighbouring peaks representing triglycerides with even carbon numbers. Chromatographic runs of butterfat samples at lower temperatures and with smaller or irregular temperature increments have now demonstrated that this incomplete return to base line is actually due to the occurrence in butterfat of triglycerides of an odd carbon number, producing minor peaks between any two adjacent major triglyceride peaks. The presence of such peaks was to be expected in view of the demonstration of detectable amounts of odd carbon number fatty acids in this fat. Incorporation of such acids into triglycerides together with even carbon number fatty acids would retain the odd carbon number and would facilitate their determination by increasing the total carbon mass associated with them.

Figure 1 represents a slow temperature-increment run, in which the even carbon number triglyceride peaks have been separated far enough to permit a complete resolution, as demonstrated with triglyceride mixtures containing no odd carbon number triglycerides. In this chromatogram the locations of the minor peaks have been indicated by completing their tracings by hand. Inspection of numerous similar chromatograms of the same sample with the tracings completed for all peaks revealed an opportunity for making a quantitative estimation of the various triglyceride types. With the type of triangulation indicated in Figure 1, and with frequent reference to the integrator record for the total area available for individual peaks and their combinations, it was possible to obtain the results presented in Table I. The individual estimates are reasonably close and seldom vary by more than 10% of the average peak area. This variation appears to be due primarily to the difficulty of completing correctly the tracings and the error in triangulating the rather flat peaks. It should be mentioned that some of these estimates for the minor triglycerides may be in error also because of incorrect carbon number assignments. Close inspection of several butterfat chromatograms made under a variety of conditions has suggested that there may be triglyceride peaks occurring between some adjacent odd and even carbon number triglycerides. These could be due to triglycerides containing branched chain fatty acids (1,2). In the present quantitative evaluation their contributions have been ignored, and the entire area between adjacent even carbon number peaks has been assigned to the corresponding odd carbon number peak. Furthermore, during linear temperature programming, the distances between adjacent triglycerides progressively decrease, resulting in a closer spacing of the peaks for the last few members of the homologous series. This may have resulted in an overestimation of the higher molecular weight odd carbon number triglycerides.

The validity of the estimates for the even carbon number triglycerides was verified by determining the proportionality of recoveries following co-chromatography of butterfat with known amounts of added saturated and unsaturated long chain triglycerides, and by analyzing molecular distillates of butter oil. The data obtained from butterfats with known amounts of added fat are presented elsewhere (10) in connection with studies on butterfat adulteration. Essentially correct proportional recoveries were found. The results with the molecular distillates are recorded in Table II. Summation of the corresponding triglyc-

TABLE II

Distribution of Triglyceride Types by Carbon Number in Butter Oil and Its Molecular Distillates[a]

Triglyceride type	Distribution of triglyceride types in distillate or oil (Moles, %)[b]													
	R-1		R-2		R-3		R-4		D-2		D-3		Original oil[c]	
	Found	Random	Found	Random	Found	Random	Found	Random	Found	Random	Found	Random	Found	Random
C_{54}	.....	0.05	.....	0.05	.....	0.43	.....	1.37	.....	1.31	5.67	6.99	4.14	4.07
C_{52}	.....	0.35	.....	0.47	.....	2.59	.....	6.65	1.34	5.48	13.31	15.89	8.58	10.39
C_{51}	.....	0.02	.....	.....	.....	0.12	.....	0.24	.....	0.20	.....	0.51	.....	0.35
C_{50}	.....	1.18	.....	1.81	.....	6.72	.....	12.37	1.48	10.32	19.84	19.26	9.30	14.31
C_{49}	.....	0.11	.....	0.00	.....	0.48	.....	0.78	.....	0.56	.....	0.77	.....	0.64
C_{48}	.....	2.43	.....	3.98	.....	10.13	.....	11.69	1.48	11.80	15.20	15.73	7.19	13.29
C_{47}	.....	0.23	.....	.....	.....	0.76	.....	0.82	.....	0.66	.....	0.64	.....	0.62
C_{46}	.....	3.43	.....	5.48	.....	10.45	1.26	7.91	1.88	10.08	11.81	11.10	5.81	10.26
C_{45}	.....	0.29	.....	.....	.....	0.66	.....	0.38	.....	0.46	.....	0.35	.....	0.39
C_{44}	.....	4.88	.....	6.00	.....	8.88	2.73	6.50	3.15	8.07	9.45	7.57	5.31	7.68
C_{43}	.....	0.29	.....	.....	.....	0.43	.....	0.26	.....	0.29	.....	0.21	.....	0.25
C_{42}	.....	5.56	.....	6.24	2.08	7.76	5.72	6.69	6.58	7.40	8.27	5.75	7.70	6.50
C_{41}	.....	0.31	.....	.....	.....	0.32	.....	0.24	.....	0.23	.....	0.13	.....	0.18
C_{40}	1.14	6.76	2.23	7.22	5.56	8.11	16.36	8.73	15.84	8.35	7.72	5.04	12.35	6.63
C_{39}	.....	0.34	.....	.....	.....	0.32	.....	0.29	.....	0.24	.....	0.11	.....	0.17
C_{38}	3.88	8.25	8.84	8.55	19.08	9.25	33.31	10.11	28.99	9.09	6.06	3.98	14.83	6.53
C_{37}	.....	0.32	.....	.....	.....	0.41	.....	0.43	.....	0.32	.....	0.11	.....	0.22
C_{36}	10.49	9.38	20.34	10.44	31.96	8.89	31.38	7.35	26.04	7.56	2.68	2.51	12.14	5.18
C_{35}	.....	0.45	.....	.....	.....	0.39	.....	0.32	.....	0.26	.....	0.06	.....	0.16
C_{34}	16.53	9.24	25.81	9.89	25.90	6.52	9.24	4.00	10.20	4.76	.....	1.35	5.98	3.50
C_{33}	.....	0.33	.....	.....	.....	0.20	.....	0.10	.....	0.11	.....	0.02	.....	0.07
C_{32}	18.98	8.43	20.88	7.85	11.31	4.27	.....	2.92	2.55	3.23	.....	0.77	2.98	2.27
C_{31}	.....	0.21	.....	.....	.....	0.08	.....	0.06	.....	0.05	.....	0.01	.....	0.03
C_{30}	18.53	7.79	12.52	6.62	3.41	3.14	.....	2.50	0.42	2.47	.....	0.46	1.60	1.72
C_{29}	.....	0.19	.....	.....	.....	0.06	.....	0.05	.....	0.04	.....	0.00	.....	0.03
C_{28}	16.93	7.10	6.20	5.91	0.69	2.65	.....	2.43	.....	2.16	.....	0.30	0.80	1.46
C_{27}	.....	0.13	.....	.....	.....	0.05	.....	0.04	.....	0.03	.....	0.00	.....	0.02
C_{26}	10.09	6.40	1.93	5.66	.....	2.35	.....	2.19	.....	1.85	.....	0.20	0.87	1.36
C_{25}	.....	0.12	.....	.....	.....	0.05	.....	0.05	.....	0.03	.....	0.00	.....	0.02
C_{24}	3.42	5.35	1.26	5.47	.....	1.66	.....	1.19	.....	1.16	.....	0.10	0.44	0.85
C_{23}	.....	0.09	.....	.....	.....	0.03	.....	0.02	.....	0.02	.....	0.00	.....	0.01
C_{22}	.....	3.60	.....	3.33	.....	0.80	.....	0.45	.....	0.66	.....	0.04	.....	0.48
C_{20}	.....	2.36	.....	1.79	.....	0.39	.....	0.29	.....	0.27	.....	0.02	.....	0.24
C_{18}	.....	1.77	.....	1.43	.....	0.26	.....	0.22	.....	0.18	.....	0.01	.....	0.16
C_{16}	.....	1.12	.....	0.84	.....	0.18	.....	0.17	.....	0.13	.....	0.00	.....	0.09
C_{14}	.....	0.78	.....	0.84	.....	0.14	.....	0.13	.....	0.10	.....	0.00	.....	0.09
C_{12}	.....	0.39	.....	0.59	.....	0.06	.....	0.04	.....	0.03	.....	0.00	.....	0.05

[a] The butter oil distillates have been previously described (8). They consisted of the first most volatile 2.5% cut (R-1), the second most volatile 2.5% cut (R-2), the third most volatile 2.5% cut (R-3), the fourth most volatile 2.5% cut (R-4), the next most volatile 40% cut (D-2), and the 50% residue (D-3).

[b] The mole % distributions were calculated from the weight % distributions by dividing by molecular weight and adjusting for 100.

[c] The fatty acid and triglyceride compositions of the original oil were calculated on the basis of the % area response and the % weight distribution of the distillates.

TABLE III
Fatty Acid Composition of Blended and Molecularly Distilled Butterfats[a]

	Composition of fatty acids (Moles, %)[b]														
	4:0[c]	6:0	8:0	10:0	12:0	14:0	14:1	15:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2	19:0	20:0
Blended butterfat	7.8	4.7	1.8	4.7	3.6	10.1		1.5	24.5		1.3			39.0		0.5	0.5
Distillate R-1	15.6	10.5	7.9	11.5	8.5	17.9		1.3	19.1		....			7.7		....	....
Distillate R-2	18.1	8.6	4.5	9.6	7.0	19.8		....	24.4		....			8.0		....	....
Distillate R-3	8.3	6.6	3.6	5.6	6.1	19.5		1.5	32.5		....			16.3		....	....
Distillate R-4	7.5	7.5	2.9	4.6	4.2	9.2		1.4	38.8		....			23.9		....	....
Distillate D-2	7.0	6.5	2.7	5.6	4.4	16.2		1.2	32.8		....			23.6		....	....
Distillate D-3	2.0	2.0	1.4	3.6	3.4	14.2		1.0	31.2		....			41.2		....	....

[a] See footnote a to Table II.
[b] The molar fatty acid concentrations were calculated from the data previously presented (8) and were based on measurements in the filament cell detector.
[c] First figure refers to number of carbons; second to number of double bonds. Carbon number was obtained by semi-log plots of retention time vs. chain length.

eride types occuring in various distillates, and correction for the individual distillate contributions to the total fat, gives a triglyceride type distribution for the original oil that differs little from that recorded for the blended sample in Table I. The differences in the estimates of the individual peaks differ little when corrected for the area contributions made by the minor peaks in the blended sample, and can be accounted for by the discrepancy in the fatty acid composition of the two butterfats. (The contributions of the minor peaks were estimated only in a few runs, as it became obvious that they did not significantly affect the results but greatly complicated the calculations.) If there had been any selective losses in the recovery of the higher molecular weight triglycerides, the relative contributions of the lower molecular weight triglycerides would have been overestimated. As a further indicator of the completeness of the triglyceride recoveries, the fatty acid carbon recoveries were calculated. The method of computation is indicated in Table IV, and is based on the molar proportions by carbon number of both the fatty acids (Table III) and the triglyceride types (Table I). The figure computed for the illustrative sample is 96.21%. Similar calculations on the averages of other series of runs have shown fatty acid carbon recoveries of 95% or better. The accuracy of this determination depends on the correctness of the estimates of the molar ratios of the fatty acids and the glycerides, which in this case may not have been completely comparable, since the fatty acids were analyzed in a filament detector and the triglycerides in a hydrogen flame ionization detector. These results indicate that the triglyceride distribution found for the blended butterfat sample is essentially correct.

It is of interest to compare this distribution with that calculated for a completely random arrangement of the fatty acids. The method of calculation of the random distribution was that described by Bailey (11), except that the per cent estimates of the individual triglycerides were grouped by carbon number. An illustrative example is presented in Table V. The random triglyceride distribution for the fatty acid complement of the blended butterfat sample is given in Table I. Although both distributions have the same general pattern, exhibiting a minimum and two maxima, they differ significantly in the magnitude of the contributions of the various triglyceride types making up these maxima. The calculated distribution predicts considerably more of both the shorter and the longer chain triglycerides. There are differences in detail. The experimentally determined distribution has its first maximum at C_{38} and second maximum at C_{50}, while the calculated distribution has its first maximum at C_{40} and the second maximum at C_{50}-C_{52}. Both distributions have their minima at C_{44}.

These differences could be accounted for in part if the fatty acid composition used in the calculation of the random distribution did not match that for the experimental butter sample. It does not appear, however, that the fatty acid values presented in Table III for this butter sample could have been significantly in error, as they compare favourably with those reported by other workers (1,2,12). In addition, studies with a great variety of butterfats have this far failed to equal or even approximate the considerable proportions of the short chain triglycerides predicted by the random distribution. The only exception to this has been the triglyceride distributions determined for a rearranged butter sample, which also showed a high proportion of the short chain triglycerides, although there were other differences (13).

In Table II the experimentally determined triglyceride distributions of the distillates are contrasted with the triglyceride distributions calculated for a random fatty acid distribution. In the last column of this table summations are presented for both the triglycerides found in the distillates and those predicted on a random basis. The differences between these two distributions are essentially those seen in Table I. The experimentally determined distributions for both butterfats differ from the corresponding random distributions and point towards a specific fatty acid and triglyceride distribution for this fat.

A degree of non-randomness in the intra-glyceride distribution of butterfat fatty acids has been sug-

TABLE IV
Calculation of Fatty Acid Carbon Recoveries

Theoretical fatty acid carbon number (TCN)	Fatty acid carbon number for calculated random triglyceride distribution (TCN)	Fatty acid carbon number for experimentally determined triglyceride distribution (ECN)
Formula: $TCN = 3\Sigma_k[(\text{Mole \% FA}) \times X]$ X (number of carbon atoms per FA residue) k is the number of FA Recoveries for the blended butterfat sample (Table III): $TCN = 3\Sigma_{12}(7.8 \times 4) + (4.7 \times 6) + \ldots$ $\ldots + (0.5 \times 20) = 439050$	$TCN = \Sigma_m[(\text{Mole \% triglyc. type}) \times X]$ X (number of FA carbon atoms per triglyc. residue) m is the number of triglyc. types Recoveries for the random distribution (Table I): $TCN = \Sigma_{39}(0.23 \times 56) + (0.25 \times 55) + \ldots$ $\ldots + (0.50 \times 18) = 438412$	$ECN = \Sigma_n[(\text{Mole \% triglyc. type}) \times X]$ X (number of FA carbon atoms per triglyc. residue) n is the number of triglyc. types Recoveries for the experimental distribution (Table I): $ECN = \Sigma_{31}(4.00 \times 54) + (1.05 \times 53) + \ldots$ $\ldots + (0.51 \times 24) = 421811$

$$\% \text{ recovery of theoretical carbon yield} = \frac{ECN \times 100}{TCN} = \frac{421811 \times 100}{438412} = 96.21\%$$

TABLE V

Calculation of Random Distirbution of Triglyceride Types by Carbon Number for a Hypothetical Fat[a]

Individual triglycerides[b]		Triglycerides by carbon number	
Type	%	Type	%
Myristic-myristic-myristic (20 × 20 × 20) 1/10,000 =	0.8	C_{54} Stearic-stearic-stearic	12.5
Palmitic-palmitic-palmitic (30 × 30 × 30) 1/10,000 =	2.7	C_{52} Stearic-stearic-palmitic	22.5
Stearic-stearic-stearic (50 × 50 × 50) 1/10,000 =	12.5	C_{50} Palmitic-palmitic-stearic + Stearic-stearic-myristic	28.5
Myristic-myristic-palmitic (20 × 20 × 30) 3/10,000 =	3.6	C_{48} Myristic-palmitic-stearic + Palmitic-palmitic-palmitic	20.7
Myristic-myristic-stearic (20 × 20 × 50) 3/10,000 =	6.0	C_{46} Myristic-myristic-stearic + Myristic-palmitic-palmitic	11.4
Palmitic-palmitic-myristic (30 × 30 × 20) 3/10,000 =	5.4	C_{44} Myristic-myristic-palmitic	3.6
Palmitic-palmitic-stearic (30 × 30 × 50) 3/10,000 =	13.5	C_{42} Myristic-myristic-myristic	0.8
Stearic-stearic-myristic (50 × 50 × 20) 3/10,000 =	15.0	Total	100.0
Stearic-stearic-palmitic (50 × 50 × 30) 3/10,000 =	22.5		
Myristic-palmitic-stearic (20 × 30 × 50) 6/10,000 =	18.0		
Total	100.0		

[a] The hypothetical fat was assumed to have the following molar fatty acid composition: myristic acid 20%, palmitic acid 30%, and stearic acid 50%.

[b] The following formulae were used in the calculations (Bailey):

% glyceride aaa = (A.A.A.) 1/10,000
% glyceride aab = (A.A.B.) 3/10,000
% glyceride abc = (A.B.C.) 6/10,000

where A, B, and C are the mole percentages of fatty acids a, b, and c.

gested before (4,5), but this specificity was supposed to have been lost when considering the overall or inter-glyceride fatty acid distribution as indicated by analyses for saturated and unsaturated fatty acids and triglycerides as classes (5,6) In view of the present results, it would appear that there exists in butterfat, in addition to the intra-glyceride non-randomness, also a degree of non-randomness in the inter-glyceride fatty acid distribution; or that the former is reflected in the latter. It would also appear that a simple segregation of fatty acids or triglycerides into classes, on the basis of unsaturation alone, may not always be sufficient to demonstrate randomness or its absence.

Figure 2 illustrates graphically the differences observed in the determined and calculated triglyceride distributions presented in Table II for the molecular distillates of butter oil. The random distributions were computed from the fatty acid ratios in Table III. Except for distillate D-3, which was the distillation residue, the discrepancies between the experimental and random distributions are dramatic. Of course, there may be no reason to anticipate a random, or nearly random, distribution for any of these distillates, as a molecular distillation may have been simply a means of biased sampling of an essentially random population. However, the observation that distillate D-3 represents 50% of the original oil, and exhibits an essentially random triglyceride distribution, while the rest of the distillates, comprising fractions from 2.5–40% of the original oil, deviate greatly from random distribution, may be taken as an indication of the underlying heterogeneity in the distribution of the constituent triglyceride populations. The studies with these distillates indicate that the non-randomly distributed triglyceride population of butterfat may be separated by molecular distillation into two 50% portions one of which possesses a nearly random distribution the other an exaggerated non-random distribution.

Whether or not these results apply to milk fat cannot be said with certainty, as both commercial butter churning and butter oil manufacture may have served as a means for non-random sampling of a random population. It should also be remembered that butter is made from milk pooled from several herds of many cows. Hence it is possible that true milk fat from individual cows might actually possess a completely random triglyceride distribution. Despite this possibility, selective losses of large proportions of individual triglyceride types on the basis of carbon number are unlikely, and the demonstrated non-random distribution of butterfat triglycerides is probably also true for milk fat.

This non-randomness in the fatty acid and triglyceride distribution in butterfat would mean that certain fatty acids tend to associate with certain others on some basis other than molar concentration, or that

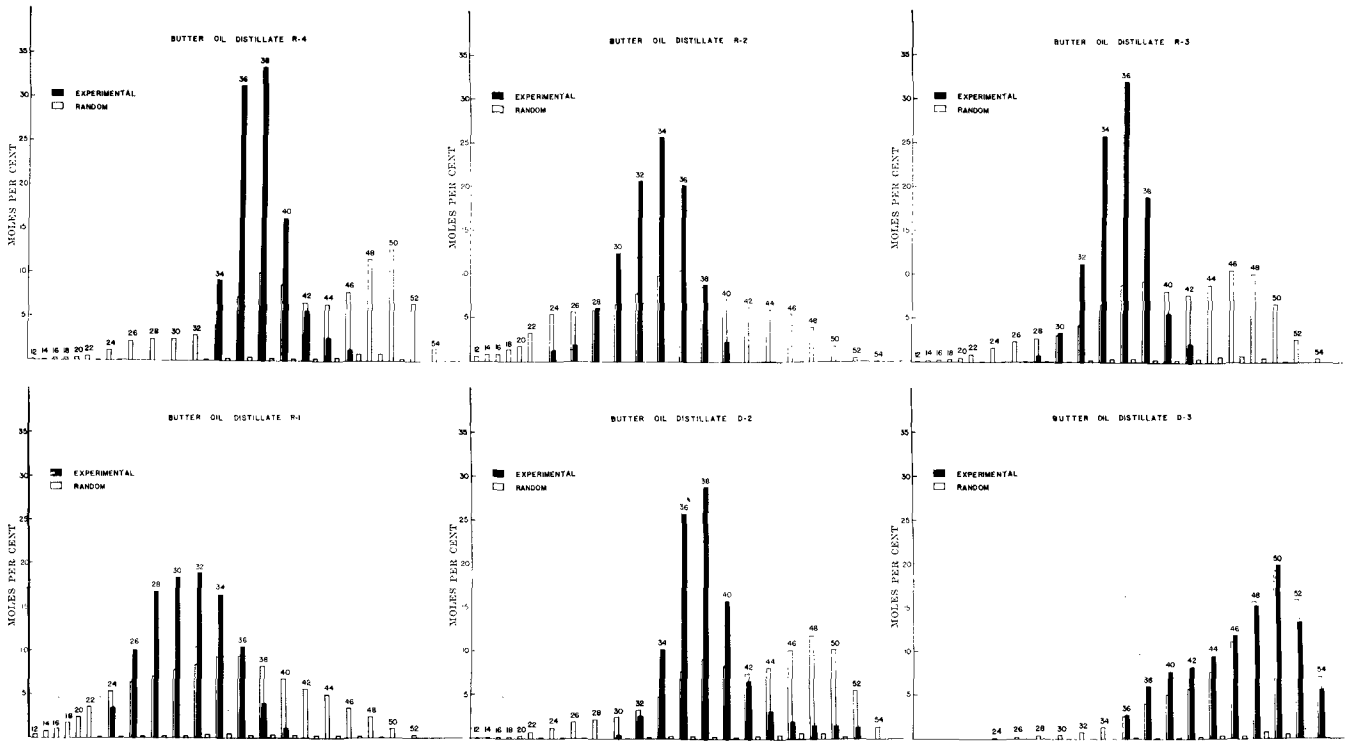

FIG. 2. Comparison of experimental and random distributions for molecular distillates of butterfat triglycerides.

the butterfat triglycerides represent pooled contributions of triglycerides from two or more distinct triglyceride populations, each of which may possess a random distribution for its fatty acids. It has already been postulated that both blood and the mammary glands contribute triglycerides to butterfat (4). In the lactating woman, milk fat triglycerides have been shown (14) to be derived from dietary and depot fat, as well as from fat synthesized in the mammary glands.

Although the fatty acids from the individual triglyceride peaks have not yet been isolated and identified, mathematical evaluations of the peak composition based on the peak proportions indicate that such short chain fatty acids as butyric and caproic, which occur to a significant extent in this fat, are found exclusively in combination with medium and long chain fatty acids, as there are only traces of triglycerides found of carbon number lower than 26. Also, this would mean that these fatty acids occur rarely in combinations of two per given triglyceride molecule. These observations are supported by the analyses of the molecular distillates of butter oil, all of which have been shown to contain about the same fatty acids despite considerable differences in the carbon number of the constituent triglycerides. Even with short chain triglyceride enrichments approximating 20–25 fold, there were no indications (8) obtained of the occurrence of any tributyrin, tricaproin, or even any significant amounts of the dibutyro- or dihexano-glycerides of medium chain length fatty acids Support for such a distribution for butyric acid residues is also suggested by the observation that pancreatic lipase is capable of releasing practically all of the butyric acid by hydrolyzing the alpha-, alpha'-linkages of the glycerides (15).

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A Comparison of the Cup Refining Loss and Neutral Oil Determinations for Evaluating Crude Soybean Oil[1]

T. J. POTTS, Ralston Purina Co., St. Louis, Missouri

Abstract

Data from 833 non-degummed and degummed soybean oil samples, which were analyzed by both the neutral oil loss and cup loss methods, were examined, and it was found that the total premiums paid under the cup loss method and the proposed National Soybean Processors Association Technical Committee's neutral oil analysis were the same. However, better quality oils would have received a higher premium, while poorer oils would have been penalized more heavily under the new procedure.

Introduction

APPROXIMATELY 42 years ago a group of cottonseed crushers and oil refiners operating through an association of the Interstate Cottonseed Crushers set a series of specifications for crude cottonseed oil. At that time the average kettle refining loss was 9.0%. Oils having a loss of 9% or less were considered prime. Other specifications were included such as odor, taste, and color. Penalties were assessed for oil having more than 9.0% loss at the rate of three-quarters of 1% of the purchase price for each percent in excess of 9.0%. This led the Chemists' Committee of the Association (1) to develop what is commonly referred to as the cup loss determination (2) for trading.

It has been reported (1) that some mill managers established the practice of adding cottonseed meal to oil containing less than 9.0% cup loss because the crusher could then sell his meal at oil prices. The practice spread and in 1927 the refiners agreed to pay a premium for oils having a settlement loss under 9.0%, at the same rate as the penalty. The Interstate Cottonseed Crushers Association was succeeded by the National Cottonseed Products Association and since that time the cup loss has served very well for controlling the quality of crude oil. About 1936 soybean oil began to appear on the vegetable oil market in appreciable quantity. However, no means of trading on quality existed. In World War II the OPA froze the vegetable oil prices and the premium system for cottonseed oil put soybean oil at a definite disadvantage. This led to the establishment of the National Soybean Processors Association. It was not until after the war when price controls were removed that the premium system using the cup loss method was organized. Prime oil was set at 7.0% cup loss and the same premium rate as cottonseed oil.

For many years the cup loss test has served the refineries as a means of measuring plant efficiency, and the method worked very well during the period when open kettle refining was paramount. Later technical and mechanical improvements in refining methods reached the point where plant losses were generally lower than the laboratory estimates. For

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