The Accuracy of Ester Fractionation Analysis of Butterfat

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THE investigations of Cox and McDowall (1) established that the properties of New Zealand butterfat as measured by the iodine values, saponification values, Reichert values, and softening points, showed periodic variations. The trend of variations of any one property was further established to be remarkably uniform, both for different factories in one season and for any one factory in the four seasons reviewed. In order to correlate these variations with fundamental changes in chemical composition, it was required that a corresponding survey be made of the fatty acid composition of butterfat.

The purpose of this paper is to show that the ester fractionation method of analysis of butterfat, under appropriate conditions, can be employed to give results which are reproducible within sufficiently small limits of error to render practicable the determination of seasonal variations in the fatty acid composition of butterfat.

No technique has been devised to determine the absolute accuracy of butterfat analyses. As pointed out by Hilditch (2), concordant results obtained by comparing the fatty acid compositions of different specimens of the same kind of fat do not necessarily imply their accuracy, but when supported by agreement between results of analyses of the corresponding hydrogenated fat, the total evidence may be accepted as a guide to absolute accuracy. Hilditch (3) considers that a more rigorous check on accuracy than formerly, now exists in comparing the summed-up analyses of acids in glyceride fractions of a fat (from 5 to 10 fractions in some cases) with the total component fatty acid analyses.

Referring to the ester fractionation method of analysis, the same author (4) states ". . . . a final accuracy in the higher complex unsaturated acids of not more than ± 2 units percent may be reached. In the numerous natural fats with simpler mixtures of component acids however the final figures should be within ± 0.5 per cent of the true values."

In this present investigation, which is part of a project undertaken to determine seasonal variations in New Zealand butterfat, no confirmation of absolute accuracy has been attempted. A consistent error in the ester fractionation method itself, in relation to absolute accuracy, will be common to all analyses. As a means of measuring the degree of reproducibility of results however, all analyses have been done in triplicate and the standard deviation and standard error determined statistically.

Preliminary Investigations

Investigation of the methods used for fatty acid analyses of butterfat [cf. Hilditch (4), Smith and Dastur (5)] suggested that without modification these methods were not sufficiently refined to detect small seasonal changes in so complex a mixture of fatty acids as that derived from butterfat. When experimenting with different methods and with weights varying between 100 grams and 300 grams, results were obtained which displayed appreciable lack of agreement. The results of five analyses of one sample for instance, were calculated to have a standard deviation of ± 2.0 and ± 2.5 for palmitic and palmitoleic acids, respectively. Thus it was found impossible to determine whether differences in corresponding acids were due to variations in composition or to experimental error. Furthermore it was concluded that the practice of relying on the results of single ester fractionation analyses assumed both in fractionation and in the determination of constants a greater degree of precision than was normally attainable.

Experimental

The procedure finally adopted incorporated the following modifications to the conventional ester fractionation method of analysis described by Hilditch (4):

a) The quantity of butterfat analyzed was increased to 750 grams.

b) The steam non-volatile acids were separated into "solids" and "liquids" by three crystallizations in 15 volumes of acetone at −30°C. [cf. (6)] instead of by the customary lead-salt-alcohol process.

c) Meticulous care was exercised in determining the chemical constants of the fractions distilled. This was effected by the inclusion of reference standards in the form of peanut

TABLE I

Fatty Acid Composition of New Zealand Butterfat Sample B/1[b]
Saponification equivalent—246.0
Iodine value— 39.6
Triplicate analyses expressed in mols. %

Sample	Component Acids—Mols. %													
	Saturated								Unsaturated					
	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	$C_{18}+C_{20}$	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}[a]	C_{20}
B/1, 9	10.0	4.2	1.7	3.3	3.6	10.3	23.7	11.3	0.3	0.3	1.4	2.3	26.1	1.5
B/1, 12	10.8	3.6	1.8	3.3	4.0	9.5	22.6	11.9	0.3	0.3	1.2	1.7	27.2	1.8
B/1, 25	10.8	3.8	2.0	3.3	3.7	9.5	23.6	11.5	0.3	0.3	1.1	1.7	27.0	1.4
Mean	10.5	3.9	1.8	3.3	3.8	9.8	23.3	11.6	0.3	0.3	1.2	1.9	26.8	1.6
Standard deviation (7)	0.46	0.30	0.16	0.0	0.21	0.46	0.61	0.30	0.0	0.0	0.16	0.35	0.59	0.22
Standard error (8)[c]	0.27	0.17	0.09	0.0	0.12	0.27	0.35	0.17	0.0	0.0	0.09	0.20	0.34	0.13

[a] Mean unsaturation of C_{18} equivalent to −2.2 H. Mean unsaturation of C_{20} equivalent to −3.8 H.

[b] Butterfat sample B/1 was supplied by the Dairy Research Institute and was derived from an April 1945 churning of the butter from a mixed herd at Massey Agricultural College, Palmerston, North, New Zealand.

[c] Standard error $= \frac{\sigma}{\sqrt{n}}$ where σ = Standard deviation. n = Number of variates.

TABLE II

Comparison of May and November Butterfat Samples [b]

(C/18) May sample: Saponification equivalent—250.1 Iodine value— 41.9

(B/36) November sample: Saponification equivalent—239.1 Iodine value— 33.8

	Component Acids—Mols. %													
	Saturated								Unsaturated					
	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	$C_{18}+C_{20}$	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}
C/18 May														
Standard deviation	10.2 (0.17)	3.8 (0.25)	1.3 (0.17)	2.7 (0.10)	3.4 (0.21)	9.4 (0.17)	21.6 (0.25)	12.4 (0.15)	0.3 (0.06)	0.3 (0.06)	1.0 (0.06)	1.9 (0.15)	29.6 (0.15)	2.1 (0.0)
Standard error (E_A)	(0.10)	(0.14)	(0.10)	(0.06)	(0.12)	(0.10)	(0.14)	(0.09)	(0.03)	(0.03)	(0.03)	(0.09)	(0.09)	(0.0)
B/36 November														
Standard deviation	10.9 (0.39)	4.8 (0.26)	2.2 (0.0)	4.3 (0.22)	5.1 (0.31)	12.2 (0.40)	22.8 (0.20)	10.9 (0.40)	0.3 (0.0)	0.3 (0.0)	1.0 (0.07)	1.6 (0.17)	22.1 (0.29)	1.5 (0.37)
Standard error (E_B)	(0.22)	(0.15)	(0.00)	(0.13)	(0.18)	(0.23)	(0.12)	(0.24)	(0.0)	(0.0)	(0.04)	(0.10)	(0.17)	(0.29)
Difference (D) C/18-B/36	−0.7	−1.0	−0.9	−1.6	−1.7	−2.8	−1.2	+1.5	0.0	0.0	0.0	+0.3	+7.5	+0.6
$\frac{D}{E_D}$ [c] (9)	2.9	4.8	9.0	11.4	7.3	11.2	6.5	5.8	....	....	....	2.3	39.5	3.0
	(S)[a]	(HS)	(HS)	(HS)	(HS)	(HS)	(HS)	(HS)	....	....	....	(N)	(HS)	(S)

[a] S = Significant ($P \leqslant 0.05$), HS = Highly significant ($P \leqslant 0.01$), N = Non-significant ($P > 0.05$).

[b] Butterfat sample with laboratory numbers B/36 and C/18 were derived from butter supplied by the Rangitaiki Plains Dairy Co., Whakatane, New Zealand. The dates of churning were November 1946 for B/36 and May 1947 for C/18.

[c] D = Difference between two means (9)

Standard error of the difference $E_D = \sqrt{E_A^2 + E_B^2}$ (9)

oil and methyl palmitate, respectively, in all batches of iodine value and saponification equivalent determinations.

d) Improved and efficient electrically heated packed fractionating columns were employed.

Results

As illustrative of the degree of reproducibility of ester fractionation analyses of butterfat, triplicate analyses of one of the three samples studied is presented in Table I.

Two other butterfat samples (whose compositions are compared in Table II) were analyzed in triplicate. By statistical procedure the standard deviation for the total component acids within the three triplicated analyses is calculated from an analysis of variance (10) to be ± 0.26. For the saturated constituents alone the standard deviation within analyses is ± 0.29 while for the unsaturated it is ± 0.22.

As shown in Table II, statistical evaluation of the corresponding fatty acids in the two butterfats compared reveals that the differences in eight of the constituent acid groups (viz., the saturated acids C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, $C_{18} + C_{20}$, and the unsaturated acid C_{18}) are significant at the 1% level ($P = 0.01$). These differences are interpreted as being highly significant. In both the C_4 saturated and the C_{20} unsaturated components, the variations are significant at the 5% level ($P = 0.05$). When comparing the values for the C_{16} unsaturated component however, the difference is calculated to be non-significant. Mean values for C_{10}, C_{12}, and C_{14} unsaturated acids were identical in the two analyses compared.

Summary

The ester fractionation method under the conditions described, has been shown to yield for triplicate fatty acid analyses of three different butterfat samples, results which are reproducible within an over-all standard deviation of ± 0.26.

Statistical interpretation of the differences between fatty acid analyses of two samples shows that the method used in this work is sufficiently accurate to detect seasonal variations in butterfat.

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8. Ibid. p. 12.
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10. Ibid. pp. 31-69.
11. Ibid. p. 30, p. 69.

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Correction

In reference to the paper on "Properties of Some Newly Developed Nonionic Detergents" by Vaughn, Suter, Lunsted, and Kramer, J.A.O.C.S., **28**, 294-299, July 1951, in the table on page 299, the carbon soil removal value for 0.1% Pluronic L62 + 0.01% Carbose in hard water should have been 43 instead of 159. This information has been supplied by M. G. Kramer of Wyandotte Chemicals Corp., Wyandotte, Mich.