TABLE I
Results of Feeding Experiment

Expt. no.	Lot no.	Composition of capsule	Egg (16) discoloration	pH	
				White	Yolk
1	1	125 mg corn oil	0	9.05	6.20
	2	100 mg corn oil 25 mg oleyl ether	0	9.05	6.20
	3	100 mg corn oil 25 mg *Sterculia foetida* oil	5	8.70	8.25
	4	108 mg corn oil 17 mg "sterculyl ether"	5	8.75	8.25
2	5	125 mg corn oil	0	9.05	6.15
	6	75 mg corn oil 50 mg sterculia acid polymer	0	9.00	6.20
	7	75 mg corn oil 50 mg sterculyl ester polymer	0	9.05	6.00
	8	100 mg corn oil 25 mg *Sterculia foetida* esters	5	8.45	8.00
3	9	125 mg corn oil	0	9.10	6.35
	10	17 mg sterculyl alcohol	4	8.70	7.60
	11	18 mg "sterculene"	5	8.60	7.90
	12	100 mg corn oil 25 mg *Sterculia foetida* esters	5	8.60	8.15

diazomethane. Both of the latter methods give some destruction of the cyclopropene ring.

The authenticity of these preparations is clearly shown by examining the infrared spectrophotometric data and comparing with reported standard bands (13,14,15). The large band at 9.92 μ and the weak band at 5.38 μ attributed to the cyclopropene ring, are found in all six sterculyl derivatives and all are of the same magnitude. No decrease in intensity was noted in the 9.92 μ band when the ester, ether, and hydrocarbon were distilled.

In addition to the bands given under the various preparations, bands at 3.47, 3.56, 6.85, and 7.30 μ were found for all five compounds. These bands have been characterized as due to C-H stretching, C-H bending, and symmetrical deformation vibrations of the CH_3 group, which are characteristic of long chain aliphatic material.

The doublet at 7.79, 7.83 μ found in the acid spectrum is due to C-O vibration. When the acid is esterified with a methyl group this doublet is changed into a triplet with bands at 8.07, 8.40, and 8.55 μ. Two bands are characteristic of fatty alcohols, these being 9.38 μ due to the bending motion of the OH group and the 2.8 μ band indicating a normal "free" alcoholic O-H vibration. It was noted that these bands are absent for the methylated alcohol and for "Sterculene." A band at 8.98 μ in the methoxy spectrum indicates a CH_2-O-CH_3 bonding system. The hydrocarbon spectrum is free of any acid, ester, alcohol or ether bands.

Halphen tests run on these derivatives did not produce deviations from the normal behavior of the Halphen reagents on cyclopropene ring containing oils. This indicates that the color of the Halphen test is not affected by the carboxyl group of the fatty acids.

In the biological experiments performed with laying hens *Sterculia foetida* oil, sterculyl ether, *Sterculia foetida* esters, and sterculene all produced pink discoloration in the eggs in about the same degree (Table I). Also the pH changes in the yolks were in the same direction. These facts combined with the absence of pink discoloration of eggs from hens fed sterculic acid polymer and methyl sterculic polymer indicate that the cyclopropene ring but not the COOH group is necessary for discoloration.

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REFERENCES

1. Masson, J. C., M. G. Vavich, B. W. Heywang, and A. R. Kemmerer, Science, *126*, 751 (1957).
2. Shenstone, F. S., and J. R. Vickery, *Ibid.*, *38*, 1055 (1959).
3. American Oil Chemists' Society, "Official and Tentative Methods," 2nd ed. (1958 revision), Method Cb 1–25.
4. Deutschman, A. J., and I. S. Klaus. Anal. Chem., *32*, 1809 (1960).
5. Faure, P. K. Nature, *178*, 372 (1956).
6. Rinehart, K. L. Jr., S. I. Goldberg, C. L. Tarimv, and T. P. Culbertson, J. Am. Chem. Soc., *83*, 225 (1961).
7. Nunn, J. R. *Ibid.*, *66*, 313 (1952).
8. Nystrom, R. F., and W. G. Brown, *Ibid.*, *69*, 2548 (1947).
9. Smith, C. R., Jr., M. C. Burnett, T. L. Wilson, R. L. Lohmar, and I. A. Wolff, JAOCS, *37*, 320 (1960).
10. Sekera, V. C., and C. S. Marvel, "Organic Synthesis," Coll. Vol. *III*, 366 (1955).
11. Strating, J., and H. J. Backer, Rev. Trav. Chim. *69*, 638 (1950). *C.A.* *44*, 7222d (1950).
12. Masson, J. C., Ph.D. Dissertation, Univ. of Arizona (1959).
13. Markley, K. S., "Fatty Acids," 2nd ed. Interscience Publ., Inc. N.Y. (1960).
14. Colthup, N. B., J. Opt. Soc. Am., *40*, 397 (1950).
15. Shreve, O. D., M. R. Heether, H. B. Knight, and D. Swern, Anal. Chem., *22*, 1498 (1950).
16. Kemmerer, A. R., B. W. Heywang, and M. G. Vavich, Poul. Sci., *40*, 1045 (1961).

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The Determination of Mutton Tallow in Mutton/Beef Tallow Mixtures

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A method is proposed for the determination of the mutton tallow content of mixed beef and mutton tallow samples. The calculation is based on the determination of conjugated diene, *trans* acids, and mono *cis* unsaturated acids in the sample. The analytical findings are employed in a single equation which is weighted to minimize uncertainty due to natural variation. The method is largely independent of the degree of "seeding" of the tallow prior to sampling and is accurate to approximately ± 14%. This accuracy is considered reasonable in view of the natural variations of the two fats and their close analytical similarity.

TALLOW in its various grades and qualities is a raw material of considerable importance to Australian industry, comprising as it does by far the greatest proportion of indigenously produced fats and oils. In the edible fat industry its significance is enhanced on the one hand by cost considerations and on the other by certain legislature requirements.

Under these circumstances, factors affecting quality of crude edible tallows and of products made therefrom have received much attention. In the case of certain grades of "mixed" tallows the problem has been complicated by variability of composition from parcel to parcel with respect to beef and mutton fat contents.

In view of the possible relationship between mutton fat content and shelf-life properties of manufactured products, a means of determining this component was required. The accuracy of the approach described in the present communication is approximately ± 14% which is considered to be probably as good as can be achieved because of the following factors:

1. The general analytical constants of beef and mutton fats are very similar in many respects.
2. Tallows are natural products subject to variations arising from seasonal and other environmental influences.
3. The known variations in fat composition within individual animal bodies depending on the nature of the tissues or organs with which they are associated.
4. Evidence has been obtained (1) that, on occasions, the analytical results have been influenced because the sample has been partially "seeded" prior to sampling.

Experimental

Materials and Apparatus. Samples of "pure" beef and mutton tallow were obtained from abattoirs which were known to kill one animal species only and to be representative of the products obtained by the normal production methods of the particular supplier.

Samples of "mixed" tallows were obtained from abattoirs known to render both beef and mutton tissues. They were known to be representative of the products obtained by normal production methods, but only scant information could be obtained on the likely mutton tallow contents.

Samples of oleo oil and corresponding oleostearin were obtained from a commercial "seeding" installation. Others were produced on a laboratory scale by melting selected tallows, cooling to 35C and maintaining at this temperature for 12 hr. The solid and liquid fractions were separated by centrifugal means.

Ultraviolet spectra were determined on a Unicam Model SP700 double beam recording spectrophotometer.

Infrared spectra were determined on a Perkin-Elmer Model 137 Infracord double beam spectrophotometer. A 1 mm fixed path-length cell was used in the sample beam and a variable path-length cell in the reference beam.

Gas chromatographic data were obtained using a Pye Argon Gas Chromatograph employing a 10 mC radium D ionization detector.

Carbon disulphide (Univar A.R. grade), ethylene glycol (B.D.H.) spectroscopic hexane (B.D.H.) and methanol (Merck guaranteed reagent) were found to be suitable for spectroscopic measurements as received.

Analytical Methods. Saponification values were determined by the standard A.O.C.S. method Cd 3-25 and iodine values by the AOCS Wijs method Cd 1-25. AOCS method Cd 7-58 was used for the determination of conjugated and total dienes. The formula for the calculation of nonconjugated dienes was simplified as follows:

$$\text{N.C.D.}\,\% = 1.086\,a'_2 = 1.086\,(a'_{233} - a_2 - 0.03).$$

Where a'_{233} = absorptivity of the isomerized sample a_2 = absorptivity of the preformed conjugated dienes.

This formula does not take into account the absorptivity of unsaturated acids containing more than two double bonds. It is felt that the error introduced in this particular case was small, but the saving in time required to carry out the analysis is considerable. *Trans* unsaturation was estimated by the method of Szonyi *et al.* (2).

The tallow samples were converted to methyl esters for gas chromatographic analysis by the method of Hartman (3). This method was shown to be superior to that whereby the fatty acids are isolated and esterified with diazomethane, in that it gave a quantitative conversion and recovery of low molecular weight fatty acids.

The following conditions were used for the gas chromatographic analyses. Column 120 x 0.4 cm packed with 5% Apiezon L on 100-20# Gas Chrom P[1]; argon flow rate 30 ml/min; temp 200C; detector voltage 1000; sensitivity x 10; chart speed 9 in/hr; sample size 0.05 μl.

Discussion of Results. Twelve samples of beef tallow, 7 of mutton tallow, and 9 mixed tallow samples were analyzed for saponification and iodine values, conjugated and total diene content, *trans* acids content, and gas chromatographic composition of the fatty acid methyl esters. The analytical findings are recorded in Table I.

Gas Chromatographic Data. The main difference between the fatty acid composition of beef and mutton tallows occurred in the shorter chain acids. Mutton tallow was found to contain 0.1% to 0.3% decanoic and lauric acids, while these acids were not present in beef tallow. Beef tallow contained about 0.5% myristoleic acid which was absent from mutton tallow. These differences can be used to distinguish between "pure" beef and "pure" mutton tallows, but are not applicable to the analysis of mixed tallows.

Similarly the stearic acid content of mutton tallow (30.2 to 33.8%) was found to be higher than that of beef tallow (20.7 to 28.3%), but the difference was not sufficient to allow it to be applied to mixed tallow analysis with any degree of confidence. None of the other fatty acids was present in sufficiently characteristic concentrations to allow application to the problem.

Spectrophotometric Data. As a means of highlighting the significance of the various measures of unsaturation (see Table I) they were also expressed in terms of an index, which measures the percentage of the total unsaturation (I.V.) accounted for by the unsaturation of the particular analysis. For example:

$$\text{C.D.I.} = 100\,\text{I.V.}_{\text{c.d.}}/\text{I.V.} \qquad \ldots\ldots\ (1)$$

Where C.D.I. = conjugated diene index.

$\text{I.V.}_{\text{c.d.}}$ = conjugated diene content expressed as an iodine value (assuming dienes are C_{18}).

I.V. = iodine value of sample.

The mono *cis* index[2] was calculated from the other indices as follows:

$$\text{Mono } cis \text{ index} = 100 - (Trans \text{ acids index} + \text{total diene index}) \qquad \ldots\ldots\ (2)$$

Examination of the results shown in Table I shows that there are marked differences in the values determined for conjugated diene, and *trans* acids content and mono-*cis* index. A sample containing only beef or mutton tallow can be identified as beef or mutton from a consideration of these constants. The values

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[1] Obtainable from Applied Science Laboratories Inc., 140 North Barnard Street, State College, Pennsylvania, U.S.A.

[2] The mono-*cis* index is not strictly an accurate measure of the mono-*cis* unsaturated compounds. No attempt has been made to correct for trienes and for non-conjugated *trans,trans-* and *trans,cis-*dienes.

TABLE I

Tallow species	Beef[a]			Mutton[b]			Mixed[c]
	Range	Mean	S.D.	Range	Mean	S.D.	Range
Saponification value	195	195	......	195–197	196	......	194 – 195
Iodine value	36.0 –47.1	41.1	3.1	39.2 –42.8	40.9	1.4	35.9 –48.6
Conj. diene %	0.69– 0.95	0.79	0.08	1.29– 1.68	1.46	0.17	0.75– 1.44
Conj. diene index	2.92– 3.87	3.51	0.33	5.81– 7.40	6.47	0.67	3.78– 5.57
Total diene %	1.71– 2.88	1.96	0.32	1.85– 4.34	3.38	0.99	2.10– 4.36
Total diene index	7.47–11.64	8.78	1.06	8.43–18.60	14.91	4.18	9.43–16.86
Trans acid %	5.29– 8.09	6.79	0.93	10.52–13.59	12.12	1.16	7.42– 9.98
Trans acid index	10.9 –18.9	14.4	2.5	21.5 –29.4	25.5	2.8	13.2 –19.9
Mono-*cis* index	72.32–80.19	76.88	2.87	56.22–62.90	59.59	2.58	68.09–78.49
Fatty acid analysis							
C10 saturated	......	......	......	0.1	0.1	......	0.0 – 0.1
C12 saturated	0 – 0.1[d]	......	......	0.1 – 0.4	0.23	0.095	9.9 – 0.2
C14 branched	0.0 – 0.1	0.04	0.05	0.0 – 0.1[d]	......	......	......
C14 one double bond	0.2 – 0.5	0.35	0.10	......	......	......	0.2 – 0.6
C14 saturated	2.6 – 4.3	3.14	0.46	3.2 – 4.4	4.03	0.54	2.2 – 4.3
C15 branched	0.4 – 0.9	0.65	0.17	0.5 – 0.9	0.69	0.12	0.5 – 0.9
C15 saturated	0.3 – 0.7	0.54	0.13	0.5 – 0.7	0.64	0.08	0.5 – 0.9
C16 one double bond	1.9 – 3.3	2.46	0.48	1.4 – 2.1	1.66	0.25	1.9 – 3.3
C16 saturated	26.0 –35.2	29.5	2.5	24.8 –27.3	25.9	0.8	26.3 –31.1
C17 branched	1.3 – 1.8	1.56	0.12	1.2 – 1.8	1.50	0.26	1.5 – 2.4
C17 saturated	0.9 – 1.4	1.13	0.17	1.1 – 1.8	1.53	0.31	1.0 – 1.9
C18 one double bond	31.4 –43.1	36.1	3.3	30.1 –32.6	31.7	1.0	30.8 –44.4
C18 saturated	20.7 –28.3	24.5	2.8	30.2 –33.9	32.0	1.5	19.4 –31.7

[a] 12 Samples. [b] 7 Samples. [c] 9 Samples. [d] 1 Sample.

TABLE II

Sample	Expected mutton content	Mutton Contents Calculated from:						
		Conj. diene, %	Conj. diene index	Total diene, %	Total diene index	*Trans* acid, %	*Trans* acid index	Mono-*cis* index
New Zealand No. 1	0	−4	−20	+7	−14	−4	−21	−18
New Zealand No. 2	0	+15	+7	+1	−8	+10	0	−2
New Zealand No. 3	0	−15	−18	−16	−21	−2	−6	−11
New Zealand No. 4	0	−12	−8	−7	−4	−18	−15	−11
Queensland No. 1	0	−10	−1	−16	−6	+16	+29	+17
Queensland No. 2	0	−4	+10	−16	−1	+8	+26	+17
Queensland No. 3	0	0	+12	−11	+1	+24	+41	+26
New South Wales No. 1	0	+12	+9	+8	+8	+6	+2	+5
New South Wales No. 2	0	−4	−7	−11	−15	−19	−22	−19
New South Wales No. 3	0	+6	+2	−18	+2	−28	−32	−19
West Australia No. 1	0	+24	+11	+65	+46	+19	+5	+20
West Australia No. 2	0	−1	0	+5	+6	−12	−11	−4
New South Wales (Gunnedah)	100	96	86	168	156	73	64	97
New South Wales (Homebush)	100	133	111	108	98	114	103	109
New South Wales (Riverstone)	100	133	120	130	130	114	113	119
New South Wales (Wagga)	100	113	118	151	160	95	97	120
New South Wales (Aberdeen No. 1)	100	75	71	−8	−6	128	135	85
New South Wales (Aberdeen No. 2)	100	78	86	+8	+16	106	116	81
New South Wales (Aberdeen No. 3)	100	69	78	142	146	70	71	98
Maximum + ve deviation	......	33	20	68	60	28	41	26
Maximum — ve deviation	......	31	29	108	106	30	36	19
Standard deviation	......	18	14	44	42	18	23	16

TABLE III

Tallow source	Iodine value	Apparent mutton tallow % based on			
		Conj. diene %	*Trans* acid %	Conj. diene index	*Trans* acid index
Barnsley	48.4	50	12	24	−3
G. O'Riordan	46.8	96	26	70	8
Goulburn	48.6	45	15	19	0
Gunnedah	38.3	21	43	30	54
Ipswich	35.9	−8	23	10	44

TABLE IV

Sample	T1[a]	S1[b]	O1[c]	T2	S2	O2	T3	S3	O3	TWA	SWA	OWA	TM	SM	OM
Parameter															
I.V.	43.5	31.2	48.0	44.3	31.9	49.8	44.9	30.9	49.6	45.9	36.4	53.1	45.8	39.0	52.0
Yield, %	......	26.8	73.2	......	30.7	69.3	......	25.1	74.9	......	43.1	56.9	......	47.7	52.3
Conj. diene, %	1.01	0.75	1.03	1.01	0.78	1.23	1.03	0.84	1.12	1.13	0.86	1.31	1.33	1.13	1.60
Conj. diene index (C.D.I.)	4.20	4.35	3.88	4.13	4.43	4.47	4.15	4.92	4.09	4.46	4.28	4.47	5.26	5.24	5.57
Total diene, %	2.33	1.72	2.80	3.02	1.67	3.25	2.73	2.16	3.34	2.65	1.76	2.77	2.76	2.28	3.20
Total diene index (T.D.I.)	9.7	10.0	10.6	12.3	9.5	11.8	11.0	12.7	12.2	10.5	8.8	9.4	10.9	10.6	11.1
Trans acid, %	6.98	6.71	7.24	6.89	6.79	7.62	7.40	6.98	8.03	7.04	7.39	7.08	9.00	9.13	9.26
Trans acid index (TrI)	13.8	18.5	13.0	13.4	19.0	13.2	14.2	19.4	13.9	13.2	17.5	11.5	16.9	20.1	15.3
Mono-*cis* index (M.C.I.)	76.5	71.5	76.4	74.3	71.5	75.0	74.8	67.9	73.9	76.3	73.7	79.1	72.2	69.3	73.6

[a] T = Tallow. [b] = Stearin. [c] O = Oleo oil.

obtained for total diene did not vary to such an extent and as the ranges overlapped slightly the identification would be made with less certainty when using this characteristic alone. In Table II the apparent mutton tallow contents have been determined for the 12 beef and 7 mutton tallow samples using each of the spectrophotometric analytical determinations.

However, application of these analytical techniques to the estimation of mutton tallow content of mixed tallows gave rise to considerable uncertainty, due to natural variations and to the possibility that some of the samples were rich in the oleo oil or the stearin fraction of tallow. Typical anomalous results are shown in Table III.

A comparison of the differential infrared spectra of beef and mutton tallows indicated no significant differences other than that discussed above due to the different contents of *trans* unsaturated acids.

Seeding of Tallows. In order to determine the influence of "seeding" on the analytical results obtained above, a number of tallows were seeded (two

TABLE V

Parameter	Deviation expressed in % of values obtained on tallow		
	Max.+ ve deviation	Max.— ve deviation	Standard deviation
Trans acid %	+10.6	−5.7	5.6
Conj. diene index	+18.6	−7.6	8.1
Mono-*cis* index	+ 3.7	−9.2	4.6

TABLE VI

Calculation of Mutton Tallow Contents Using Equation 3

Pure Tallows			
Sample	Mutton content expected	Mutton content found	Deviation from expected value
New Zealand No. 1	0	−12	−12
New Zealand No. 2	0	+ 7	+ 7
New Zealand No. 3	0	− 9	− 9
New Zealand No. 4	0	−14	−14
Queensland No. 1	0	+11	+11
Queensland No. 2	0	+10	+10
Queensland No. 3	0	+20	+20
New South Wales No. 1	0	+ 7	+ 7
New South Wales No. 2	0	−15	−15
New South Wales No. 3	0	−16	−16
West Australian No. 1	0	+16	+16
West Australian No. 2	0	− 7	− 7
New South Wales (Gunnedah)	100	81	−19
New South Wales (Homebush)	100	112	+12
New South Wales (Riverstone)	100	117	+17
New South Wales (Wagga)	100	107	+ 7
New South Wales (Aberdeen No. 1)	100	102	+ 2
New South Wales (Aberdeen No. 2)	100	97	− 3
New South Wales (Aberdeen No. 3)	100	77	−23
Standard deviation			14
Maximum + deviation			20
Maximum − deviation			23

Mixed Tallows	
Sample	Mutton content found
1	23
2	7
3	52
4	45
5	15
6	39
7	21
8	9
9	14
Tallow 1	11
Oleo 1	9
Stearin 1	13
Tallow 2	10
Oleo 2	20
Stearin 2	16
Tallow 3	15
Oleo 3	21
Stearin 3	27
Melbourne tallow	45
Melbourne oleo	50
Melbourne stearin	49
W. A. tallow	14
W. A. oleo	11
W. A. stearin	17

on the laboratory scale and three on a commercial scale) and the characteristics of the separated fractions compared with those of the original tallow. Results are shown in Table IV.

The parameters least affected by seeding are the *trans* acid %, the conjugated diene index, and the mono-*cis* index. Changes due to seeding in these values were converted into per cent changes of the original tallow values, and the standard deviations obtained on seeding were calculated (see Table V).

Mutton contents should be most accurately determined using the above parameters, because the effect of separation due to seeding is small. On the other hand, it was considered advisable to use all three for the estimation of mutton contents, in order to compensate random variations which affect the parameters in different ways.

Comparing the beef and mutton averages, we find that a 100% change in mutton content involves a change of 78.5% in *trans* acid content, 84.3% in conjugated diene index and 22.5% in mono-*cis* index. Hence the standard deviations of changes obtained on seeding correspond to an error of 7.1% mutton content when based on the *trans* acid % determination, 9.6% in case of conjugated diene index and 20.4% in case of mono-*cis* index determinations. Mutton contents were therefore calculated as a weighted average in the following manner:

Let M_{Tr}, M_{CDI}, and M_{MCI} designate the mutton content in percentages calculated from the *trans* acids %, the conjugated diene index, and the mono-*cis* index of the sample, respectively. The mutton content of the sample may then be calculated from:

$$\text{Mutton}\ \% = \frac{3\,M_{Tr} + 2M_{CDI} + M_{MCI}}{6} \quad \ldots\ldots\ldots(3)$$

When applied for the 12 beef and 7 mutton samples, this correlation gave a standard deviation of ± 13.6. Individual results are shown in Table VI.

REFERENCES

1. Szonyi, C., R. S. Tait, and J. D. Craske. Unpublished results.
2. Szonyi, C., R. S. Tait, and J. D. Craske. Submitted for publication.
3. Hartman, L., JAOCS, *34*, 165 (1957).

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