SOCIETY OF CHEMICAL INDUSTRY

INSTRUCTIONS TO AUTHORS

Submission of inadequately prepared typescripts will cause unnecessary delay. Authors should therefore conform closely to the instructions given below.

I. Introduction

The Society publishes:

(*a*) The *Journal of Applied Chemistry* (formerly known as the *Journal of the Society of Chemical Industry*), which appears monthly and contains papers (except those concerning food and agriculture) describing original investigations which have not been published elsewhere.

(*b*) The *Journal of the Science of Food and Agriculture*, which appears monthly and contains papers describing original investigations on food and agriculture which have not been published elsewhere, and an invited review article.

(*c*) *Chemistry and Industry*, the Society's weekly news journal, containing review articles on some aspects of chemical industry, articles dealing with current plant practice, descriptions of new apparatus, historical articles and, occasionally, original work not suitable for inclusion in (*a*) or (*b*), news items, etc.

All papers and correspondence relating to them are to be sent to the Editor of the appropriate Journal, 56 Victoria Street, London, S.W.1.

II. General

(*a*) Papers offered for publication will be brought before the Publications Committee and subsequently submitted to a referee.

(*b*) Papers may not be offered for publication elsewhere while under consideration by the Publications Committee.

(*c*) Papers accepted by the Society for publication may not be published elsewhere without permission of the Publications Committee, except as provided in VII below.

III. Style and Layout of Papers

(*a*) Papers must be written in clear and concise English.

(*b*) Although unnecessary standardization is not desired, and due allowance for the type of subject matter must be made, papers submitted to the journals I (*a*) and (*b*) should conform as far as possible to the following pattern:

Title.—This should be concise and explanatory of the purpose of the paper. Where a related series of papers is submitted each individual paper should have the same general heading, followed by a series number and title of the part.

Synopsis.—A short synopsis of the work, drawing attention to salient points, and intelligible without reference to the paper itself, should be given separately at the beginning of the paper.

Introduction.—The aim of the investigation should be given and also a brief statement of previous relevant work with references.

Experimental.—The methods and materials used should be clearly stated in sufficient detail to permit the work to be repeated if desired. Only new techniques need be described in detail, but known methods should have adequate references.

Results.—These should be presented concisely, using tables or illustrations for clarity. Adequate indication of the level of experimental error and the statistical significance of results should be given. Only in exceptional cases will tables *and* graphs derived from them be accepted for publication.

Discussion.—In general, the discussion and interpretation of results should follow their presentation, in a separate section.

Conclusions.

Acknowledgments.

References.

IV. Typescript

(*a*) All manuscripts should be typed in double spacing on one side of the paper only and adequate margins should be left. One copy should be retained by the author, and the top copy should be sent to the appropriate Editor.

(*b*) The address where the work described in the paper was carried out should be given at the end of the paper immediately after any Acknowledgments.

(*c*) Tabulated matter should be clearly set out and the number of columns in each table kept as low as possible.

(*d*) Drawings intended for reproduction should be clear and drawn to a scale which, when reduced to the appropriate size, will produce a legible figure. They should be drawn in Indian ink on plain white paper or board, tracing cloth, tracing paper or faintly blue-lined paper; *any lettering, whether on the margin or in the body of the drawing, should not be in ink but inserted clearly and lightly in blue pencil (blue does not appear when photographing for reproduction).* The author's name and the title of the paper should be written on the back of each drawing. Legends and captions should be typed on a separate sheet.

V. Nomenclature and References to Literature

(*a*) Symbols, formulae and equations must be written with great care. The symbols recommended in the Report by the Symbols Committee of the Royal Society, 1951 (obtainable from the Chemical Society), should be employed. If it is essential that formulae which require special setting (e.g. complex ring structures) should be used, their number should be kept to a minimum.

(*b*) References to the literature should be indicated in the text by a small superior figure referring to a list given at the end of the paper (*see* papers published during 1950 for examples). The names of the journals should be abbreviated in accordance with the 'World List of Scientific Periodicals, 1900–1950', 1952, 3rd ed. (London: Butterworths Scientific Publications). If the name of the journal is not in this list, it should be given in full. The abbreviated title of the journal should be underlined to indicate italic type and followed by the year, the volume number in arabic numerals underlined with a wavy line to indicate bold type, then by the number of the first page in arabic numerals. When books are mentioned in the References, the order should be: author(s), initials, title, year, volume number, edition, page, followed (in brackets) by place of publication and name of publisher.

VI. Proofs and Reprints

(*a*) The address to which the author's proofs are to be sent must be clearly indicated. Authors living abroad may give an address in Great Britain of a person who will undertake to correct their proofs, and his or her name and address should be clearly set out.

(*b*) Proofs will be despatched to the author directly the type has been set by the printer, and authors are requested to return them corrected without delay to the Editor of the Journal concerned. Failure to do this will result in delay in publication.

(*c*) Correction of proofs by authors must be restricted to printer's and similar errors. Any modification of the original text is to be avoided.

(*d*) Twenty-five reprints of author's papers are normally supplied without charge, and further copies may be ordered on a form which is sent with the author's proofs.

VII. Copyright

(*a*) Copyright of all papers submitted to the Society remains the property of the author unless paid for by the Society, but the Society has the sole right of publication for a period of six months from the date of publication.

(*b*) Papers appearing in the publications of the Society of Chemical Industry may be published elsewhere six months after the date of publication by the Society, provided acknowledgment of the original source of publication is given.

Printed in Great Britain by Butler & Tanner Ltd., Frome and London

RESIDUES OF TETRACHLORONITROBENZENE ON WARE POTATOES

By A. R. WILSON* and J. A. DAWSON†

Polarographic determinations have been made of 2 : 3 : 5 : 6-tetrachloro-1-nitrobenzene (TCNB) residues on ware potatoes treated in autumn with 10 lb./ton of 3% TCNB dust to reduce sprouting in clamp. Results indicate that after normal commercial grading and transport following 4–5 months' storage, concentrations of TCNB on tubers are likely to be about 2 p.p.m. When the storage period is increased to 6 months, a further reduction to approximately 1 p.p.m. is probable. The original concentration applied (134 p.p.m.) is reduced mainly by handling before and after clamping, but losses by evaporation have been shown to occur during storage. Washing still further reduces residues and no TCNB at all was found in the flesh of peeled tubers.

Introduction

Considerable loss in both dry matter and water content commonly results from the sprouting of maincrop ware potatoes in late spring in both store and clamp. These losses can be prevented by holding potatoes in cold store, but as facilities for this are rarely available it is now generally recognized that some other means of inhibiting or reducing sprouting is desirable. Various chemical treatments have been applied to the tubers for this purpose, among the more promising of which is a dust containing 2 : 3 : 5 : 6-tetrachloro-1-nitrobenzene (TCNB). The value of this treatment was first demonstrated in Britain by Brown,[1] and shortly afterwards a proprietary dust containing 3% of TCNB by weight was marketed by Bayer Products Ltd. (now marketed by Bayer Agriculture Ltd.) and named 'Fusarex'. Successful results with Fusarex in the control of late-season sprouting in potatoes have since been reported by other workers in Britain,[2, 3] Australia,[4, 5] Sweden,[6, 7] Denmark[8] and Holland.[9, 10] Fusarex has been used to a limited extent commercially in Britain on ware potatoes for the past five years.

Three aspects of the chemical treatment of foodstuffs are of particular importance: (1) the toxicity of the particular chemical to man and animals, (2) the amount of residue present when the foodstuff is used and (3) the presence or absence of off-flavours attributable to treatment. Buttle & Dyer[11] showed that a daily dose of TCNB of 215 mg./kg. of body-weight for mice and 57 mg./kg. for rats produced no ill-effects. Abrams, Scorgie & Willis[12] found that the toxicity of TCNB to pigs is of the same order as it is to rats. Pigs given an average daily dose of 7·1 g. of TCNB per head showed at most a very slight retardation of growth in the early stages. Post-mortem, chemical and histological studies indicated no abnormality and confirmed the findings arrived at on the basis of the growth-rate data. The results quoted above indicate that TCNB is of a low order of toxicity. Potatoes do, however, form a staple item of diet; consequently it is of considerable interest, when interpreting animal-feeding trials in relation to man, to know the amount of TCNB that may reach the consumer. A series of experiments were undertaken in the 1949–50 and 1950–51 storage seasons to obtain data on: (1) the amount of TCNB present on the skin after varying periods of commercial clamp storage, (*a*) when removed from clamp and (*b*) after riddling and transport in open-mesh hessian sacks as in commercial practice, (2) the effect of accidental over-dosage on the residue and (3) the effect of surface moisture at the time of treatment on the immediate retention and eventual residue of TCNB on tubers treated with an excess of the dust. The results obtained form the subject of the present paper.

Experimental method

Rate and method of application of TCNB dust.—Fusarex brand of TCNB dust [nominal 3% by weight of 2 : 3 : 5 : 6 : tetrachloro-1-nitrobenzene in an inert carrier (kaolin)] was used throughout the experiments and, unless otherwise noted, was applied at the rate of 10 lb./ton (134 p.p.m. of TCNB), according to the manufacturer's recommendation. For all large-scale clamp trials dust was applied to the load either gradually, by hand, as the carts were filled in the field, or at two stages—when the carts were half full and full respectively. Either method, followed by the tipping of the load on to the clamp site, appears to give satisfactory results, but the distribution achieved by gradual application is more even. Treatment of

* Agricultural Research Council Potato Storage Investigation, c/o University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics.

† Fungicide and Insecticide Research Co-ordination Service, Cunard Building, 15 Regent Street, London, S.W.1

Table I

Residue of TCNB on the surface of potato tubers after varying periods of clamp storage following autumn application of 3% TCNB dust (Season 1949/50)

Source	Variety	Rate of application, lb./ton (p.p.m. of TCNB)	Treatment of sample	Residue of TCNB after storage, p.p.m.					
				1st sampling (*c.* 30/12/49)		2nd sampling (*c.* 16/2/50)		3rd sampling (*c.* 20/5/50)	
				Single tubers	Mean	Single tubers	Mean	Single tubers	Mean
1*	Home Guard	5 (67)	Hand picked: paper sack	1·7 4·2 4·8	3·6	0·8 4·2 3·6	2·9	— — —	—
			Riddled: hessian sack	2·1 2·3 1·9	2·1	1·6 2·7 2·3	2·2	— — —	—
2*	King Edward	10 (134)	Hand picked: paper sack	1·3 3·5 2·0	2·3	9·5 53·0 13·2	25·2	— — —	—
			Riddled: hessian sack	1·7 2·0 1·0	1·6	4·1 2·0 2·6	2·9	0·6 0·1 0·1	0·3
3	Dr. McIntosh	10 (134)	Hand picked: paper sack	14·1 8·7 8·8	10·5	3·9 4·2 19·9	9·3	— — —	—
			Riddled: hessian sack	3·4 2·3 7·9	4·5	2·7 2·4 3·0	2·7	— — —	—
4	Majestic	10 (134)	Hand picked: paper sack	0·2 0·6 0·6	0·5	18·0 16·0 6·1	13·3	— — —	—
			Riddled: hessian sack	0·2 0·3 0·4	0·3	1·6 1·8 2·6	2·0	— — —	—
5	Majestic	10 (134)	Hand picked: paper sack	1·1 1·7 1·6	1·5	1·0 0·3 0·9	0·7	— — —	—
			Riddled: hessian sack	0·8 0·8 0·7	0·8	0·6 0·6 0·6	0·6	— — —	—
6	King Edward	10 (134)	Hand picked: paper sack	0·5 1·4 0·8	0·9	13·8 6·4 22·8	14·3	0·9 0·4 0·6	0·6
			Riddled: hessian sack	0·4 0·5 0·6	0·5	2·3 1·9 2·5	2·2	0·7 0·8 0·8	0·8
7	Doon Star	5 (67)	Hand picked: paper sack	0·7 0·3 0·4	0·5	0·1 0·2 0·1	0·1	— — —	—
			Riddled: hessian sack	0·3 0·1 0·2	0·2	0·3 0·2 0·2	0·2	— — —	—
8	Majestic	10 (134)	Hand picked: paper sack	3·4 0·7 2·2	2·1	0·7 0·7 0·5	0·6	— — —	—
			Riddled: hessian sack	0·4 0·4 0·3	0·4	0·6 0·8 1·2	0·9	— — —	—
9†	King Edward	10 (134)	Hand picked: paper sack	8·6 6·7 5·8	7·0	43·7 25·5 45·3	38·0	1·6 0·4 0·8	0·9
			Riddled: hessian sack	1·7 3·0 1·0	1·9	10·6 5·0 6·6	7·4	0·9 0·9 1·4	1·1

* Barn storage

† The TCNB present in the residues in the sacks used for the transport of the first batch of samples was:
Paper sack 24·15 mg. ≡ 10·5 p.p.m. (on total weight of potatoes)
Hessian sack 0·53 mg. ≡ 0·25 p.p.m. (on total weight of potatoes)

tubers in the clamps listed in Table I was carried out by the growers as normal practice and was not supervised. The clamps listed in Table II formed part of a larger experiment and treatment of the tubers was carried out by the 'gradual-addition' method mentioned above, under supervision, with the result that reasonably even distribution is known to have been achieved. All tubers clamped were treated in autumn, mainly during the months of September and October. For the small-scale experiments, dust was applied by hand at the rate of 10 lb./ton (Table IV) or; where it was desired to simulate the overdosing liable to occur through poor distribution, at the rate of 30 lb./ton (402 p.p.m. of TCNB) (Table III), or in excess (Table V). Treatments were carried out on varying dates as indicated.

Storage.—All clamps were constructed roughly according to standard Lincolnshire practice, unless otherwise noted, but on bases varying from 6 to 8 ft. The tonnage clamped varied from 2½ (experimental clamps—Table II) upwards. In the experiments recorded in Tables III and IV the potatoes were stored in standard refuse-bins, lined with straw at the bottom and with a covering of straw on top of the tubers to prevent greening when bins were left open (Table III) or condensation moisture reaching the tubers when bins were closed (Table IV).

Sampling.—On each sampling date the clamps were opened at random in one place and the outer layer of tubers removed to a depth of 6–8 in.; two samples of approximately 5 lb. each were then taken at random from below. One of these was placed in a treble-walled paper bag, care being taken to avoid, as far as possible, disturbance of any surface deposit. The other was treated in a fashion simulating commercial handling, namely, riddled (shaken 50 times on a 1¾-in.-mesh circular hand-riddle) and packed into small open-mesh hessian sacks. Samples were then forwarded by rail for chemical examination. The figures for the tubers transported in paper bags represent the maximum residue that could reach the retailer. The figures for tubers transported in hessian sacks take account of losses during riddling and from the bags during transport. In only one instance (Table I, Source 9) were analyses made of the residues in the bags.

In the small-scale experiment reported in Table III, a 6–8-in. layer of tubers was removed in each case from the top of the bin and two 5-lb. samples taken at random from below, care being taken to avoid disturbance of any surface deposit. The original top layer of tubers was then replaced. Samples were treated as they were for the clamps. In the remaining small-scale experiments (Tables IV and V), tubers were carefully hand-picked to avoid disturbance of surface deposit but no special method of sampling was used. Part of the last sample taken in the experiment reported in Table V was riddled before examination.

Analytical work.—The estimation of TCNB residues on the surface of the potatoes was carried out at the analytical laboratory of the Fungicide and Insecticide Research Co-ordination Service at Woolwich.

Normally three, but sometimes six or seven tubers, were taken at random from each bulk sample for separate determination of the residues present. Each tuber was washed carefully with light petroleum and the TCNB in the washings was estimated polarographically by the method developed by Webster & Dawson.[13]

Discussion of results

In Table I the results refer to samples taken from commercially treated crops. Marked differences were found between the TCNB residues on individual tubers from the same clamp; these are considered to be due to uneven distribution of the dust at the time of treatment. There were also considerable differences between the average residue figures for crops treated at the same rate. These are more difficult to explain but may be due partly to variations in adherence of the dust. It is apparent from the greater residue on tubers that had not been riddled and that had been transported in paper sacks that removal of the TCNB during handling subsequent to storage can account for a considerable reduction in residues. In one instance (see footnote to Source 9, Table I), the TCNB on the potatoes and in the paper bags was determined and the total found to be far less than might have been expected from the initial rate of treatment; this suggested that considerable loss of TCNB from the tubers must take place before removal from storage, either during tipping of the loads into clamp or by evaporation.

Results given in Table II, as noted previously, are from a supervised trial where care was taken to ensure even distribution of the dust within the limits of the method used. As might be expected, the results in this trial were more uniform. Residues were again very small, having regard to the initial rate of treatment. The evidence suggests that an increase in the period of storage led to a decrease in TCNB residues.

In the small-scale experiment reported in Table III, which was carried out on 1-cwt. lots in metal refuse-bins, distribution of the dust could be controlled more accurately than was

Table II

Residue of TCNB on the surface of potato tubers after varying periods of clamp storage following autumn application of 3% TCNB dust (Season 1950/51)

Source	Variety	Rate of application, lb./ton (p.p.m. of TCNB)	Treatment of sample	Residue of TCNB after storage, p.p.m.			
				1st Sampling (16/3/51)		2nd Sampling (6/6/51)	
				Single tubers	Mean	Single tubers	Mean
1	Majestic	10 (134)	Hand picked: paper sack	2·5 3·0 3·1	2·9	0·2 0·0 0·1	0·1
			Riddled: hessian sack	2·6 2·5 1·2	2·1	0·1 0·2 0·2	0·2
	Record	10 (134)	Hand picked: paper sack	0·6 1·1 0·6	0·8	— 0·1 —	0·1
			Riddled: hessian sack	1·2 0·9 —	1·1	0·1 0·1 0·1	0·1
	Doon Star	10 (134)	Hand picked: paper sack	14·0 1·9 1·0	5·6	0·3 0·6 —	0·5
			Riddled: hessian sack	1·2 1·4 0·8	1·1	0·2 0·6 0·1	0·3

possible in clamps. The results show that even here the residues on analysis were far less than would be expected from the dosage rates applied. This discrepancy occurs in the first samples taken on the day of treatment: the total TCNB recovered from tuber surfaces and paper sacks was only a small fraction of the quantity applied. Losses of this order by evaporation during transport are unlikely; without further investigations, no explanation other than low initial retention can be offered. Figures again show marked loss of TCNB during riddling and transport in hessian sacks. Differences in the residues on individual tubers receiving the same treatment are marked and it is impossible to demonstrate any clear effect of length of storage. Residues on tubers subject to gross overdosage, although higher, were still relatively low; removal of TCNB through riddling etc. reduced these to a level little above (in the third sampling, actually below) that found on tubers receiving the normal level of application.

Table III

Residue of TCNB on the surface of potato tubers after varying periods of storage in 1-cwt. experimental bins following application of 3% TCNB dust on 13 December (in unheated store at Sutton Bonington; variety King Edward; Season 1949/50)

Rate of application, lb./ton (p.p.m. of TCNB)	Treatment of sample	Residue of TCNB after storage, p.p.m.						Residue of TCNB in sacks: 1st sampling only, p.p.m. on total weight of tubers
		1st Sampling (13/12/49)		2nd Sampling (17/2/50)		3rd Sampling (6/6/50)		
		Single tubers	Mean	Single tubers	Mean	Single tubers	Mean	
10 (134)	Hand picked: paper sack	11·5 4·1 3·9	6·5	24·5 27·0 24·3	25·3	4·5 6·9 3·1	4·8	6·5
	Riddled: hessian sack	1·2 0·7 1·1	1·0	7·0 4·5 1·5	4·3	4·3 5·6 2·6	4·2	0·1
30 (402)	Hand picked: paper sack	8·6 12·3 9·7 22·6 5·6 16·6 6·2	11·7	97·2 41·0 88·9	75·7	5·9 35·9 55·7	32·5	20·8
	Riddled: hessian sack	1·9 1·7 1·6	1·7	20·2 9·1 4·7	11·3	2·5 0·7 1·2	1·5	1·5

The results presented in Table IV are from a trial similar in most respects to that reported in Table III, except that the tubers were stored in closed bins at the laboratory where the

Table IV

Residue of TCNB on the surface of potato tubers after varying periods of storage in a 1-cwt. experimental bin following application of 10 lb./ton of 3% TCNB dust (134 p.p.m. of TCNB) on 2 February (in laboratory at Woolwich; variety King Edward; Season 1949/50; samples hand-picked with minimum disturbance of surface deposit)

Residue of TCNB (p.p.m.)

1st Sampling (2/2/50)		2nd Sampling (28/2/50)		3rd Sampling (21/3/50)		4th Sampling (11/4/50)		5th Sampling (15/5/50)	
Single tubers	Mean	Single tubers	Mean	Single tubers	Mean	Single tubers	Mean	Single tubers	Mean
24·2	44·3	17·8	24·1	64·2	33·4	90·9	45·6	22·0	49·0
10·3		44·8		27·3		93·2		207·0	
81·0		16·0		29·8		18·6		27·0	
84·0		18·0		14·0		17·0		13·0	
44·0		19·0		17·0		16·5		14·0	
14·5		28·7		48·3		37·6		11·0	

Table V

Residue of TCNB on the surface of potato tubers after varying periods of storage in an open 14-lb. container following application of excess of 3% TCNB dust on 5 April (in laboratory at Woolwich; variety King Edward; Season 1949/50; tubers damped before treatment; hand-picked samples taken with minimum disturbance of surface deposit)

Residue of TCNB (p.p.m.)

1st Sampling (5/4/50) Hand picked		2nd Sampling (15/5/50) Hand picked		3rd Sampling (12/6/50) Hand picked		Riddled		Washed in water	
Single tubers	Mean	Single tubers	Mean	Single tubers	Mean	Single tubers	Mean	Single tubers	Mean
407·0	450·0	304·0	272·0	162·5	158·5	91·0	43·4	21·5	11·2
636·0		165·0		161·9		29·6		5·9	
388·0		151·0		308·3		9·6		6·3	
353·0		160·0		61·0					
547·0		203·0		180·0					
372·0		652·0		57·5					

analyses were carried out; consequently losses during transport are eliminated. Variation in amount of residue on individual tubers is again high, and it is impossible to detect any effect of length of storage period. Figures for the first sampling date, which coincided with the date of treatment, again suggest that retention might be a limiting factor.

The results of the trial presented in Table V suggest that retention may be greater where tubers are damp at the time of treatment; unfortunately no direct comparison of the retention of TCNB dust by damp and dry tubers, at the standard dosage level, is available. Figures in Table V indicate that, in this experiment, there was progressive loss of TCNB during storage and also a marked reduction on riddling and washing with water.

Conclusions

Losses of TCNB which occur under commercial conditions in clamp storage are difficult to assess because of the widely different deposition rates on individual tubers inherent in the methods of treatment used. From the evidence provided by the experiments under review, it would appear that at the recommended rate of application, namely 10 lb./ton, only a small proportion of the dust is retained initially by the tubers when they are clamped, the rest presumably settling down on the floor of the clamp (or container). It is possible that greater retention would be obtained if a different carrier were used; no direct evidence on this point is available. Results suggest that retention may be greater if the tubers are damp when treated but further work is necessary to determine the effect of this factor under field conditions. Loss of TCNB by evaporation occurs both in clamps and in open bins, but its extent and relation to storage temperature, as reflected in losses at different dates of sampling, cannot be determined from the data available, for the reasons set out above.

The most important factor in the reduction of surface residues of TCNB on tubers would appear to be removal of the dust during normal commercial methods of handling before and after storage. If the recommended rate and method of treatment (10 lb./ton of 3% dust applied to the load in the cart) are followed and the tubers are stored in a clamp for four to five months, they may be expected to retain, after riddling and transport to the retailer, approximately 2 p.p.m. of the TCNB. After a storage period of over six months, residues

are likely to be reduced to less than 1 p.p.m. Taking the figure of 2 p.p.m. of surface residue of TCNB as a basis, were a man to eat 12 oz. daily of raw, unpeeled, unwashed potatoes, he would ingest approximately 1/10,000 of the daily dose shown by Abrams, Scorgie & Willis[12] to be non-toxic to pigs. The experimental evidence (Table V) shows that washing further reduces residues.

Small amounts of TCNB (less than 1 p.p.m.) were occasionally detected in the layer immediately below the skin, i.e. a layer that would normally be removed as part of the peelings. A limited number of analyses of peeled, treated potatoes were carried out and in these no TCNB at all was found.

Acknowledgments

Thanks are due to Bayer Agriculture Ltd. for providing lists of growers using Fusarex, to these growers for the facilities given to sample their clamps, and to Mr. P. T. G. Twiss, who took the samples.

Facilities provided by the Chief Chemical Inspector, Ministry of Supply, at the Woolwich Laboratory are gratefully acknowledged, also the assistance provided by Mr. J. G. Webster in the earlier part of this work.

Received 16 February, 1953

References

[1] Brown, W., *Ann. appl. Biol.*, 1947, **34**, 422
[2] Luckwill, L. C., *Rep. agric. hort. Res. Sta. Bristol*, 1948, p. 124
[3] Glasscock, H. H., *Gdnrs' Chron.*, 1949, **125**, 54
[4] Downie, W. A., *J. Dep. Agric. Vict.*, 1949, **47**, 301
[5] Downie, W. A., *J. Dep. Agric. Vict.*, 1952, **50**, 61
[6] Emilsson, B., Lillieroth, C. G. & Nilsson, R., *K. LantbrAkad. Tidskr.*, 1949, **88**, 487
[7] Emilsson, B., Lillieroth, C. G. & Nilsson, R., *K. LantbrAkad. Tidskr.*, 1951, **90**, 421
[8] Olsen, H. K., *Beretn. Faellesforsøg i Landbo- og Husmandsforeningerne*, 1950, p. 55
[9] Mooi, J. C., *Landbouwk. Tijdschr.*, 1949, **61**, 628
[10] de Jong, W. H. & Hofstra, D., *Landbouwk. Tijdschr.*, 1950, **62**, 680
[11] Buttle, G. A. H. & Dyer, F. J., *J. Pharm. Pharmacol.*, 1950, **2**, 371
[12] Abrams, J. T., Scorgie, N. J. & Willis, G. A., *Brit. vet. J.*, 1950, **106**, 413
[13] Webster, J. G. & Dawson, J. A., *Analyst*, 1952, **77**, 203

THE DETERMINATION OF ARSENIC IN GELATIN* BY MEANS OF THE MOLYBDENUM-BLUE REACTION

By J. E. EASTOE and B. EASTOE

An absorptiometric method, based on the molybdenum-blue reaction, for the determination of arsenic in gelatin is described. The method is suitable for the simultaneous treatment of a number of samples and is more accurate than existing methods based on the Gutzeit test.

Gelatin is digested with dilute hydrochloric acid and the arsenic, after reduction, is separated as the hydride by the action of nascent hydrogen liberated by zinc. The arsine is absorbed in iodine solution, which, after the addition of ammonium molybdate and 1-amino-2-naphthol-4-sulphonic acid reagents, is then heated at 100°. The optical density of the resulting blue solution, measured photoelectrically, is proportional to the concentration of arsenic over the range 0–25 μg. The use of the aminonaphtholsulphonic acid as reducing agent has been shown to eliminate a source of error that is encountered if hydrazine is used, when the test solutions are matched visually. In addition the aminonaphtholsulphonic acid confers on the colour improved stability to light.

The procedure has been checked against a second method in which the gelatin is completely oxidized with nitric acid. This shows that arsenic, in the forms normally present in commercial gelatins, is made available for separation by digestion with dilute hydrochloric acid on a steam bath. Claims in the literature[1] that tervalent arsenic can be completely recovered from hydrochloric acid solution containing only stannous chloride and potassium iodide by the action of zinc have not been confirmed. Only 50% of the arsenic was recovered from such solutions, but the addition of certain organic materials increased the percentage recovery. Reproducible recoveries, ranging from 96% for 5 μg. of arsenic to 88% for 25μg., were obtained in the presence of gelatin. Error due to incomplete recovery of arsenic can be avoided by carrying out an actual separation of arsenic for the preparation of a calibration curve.

The overall accuracy of the method is ± 2% when a spectrophotometer is used for a range of 0–5 p.p.m. of arsenic in gelatin. The accuracy varies from ± 3–6%, with selenium-photocell absorptiometers, to ± 5–10%, when the solutions are matched visually.

* The final 'e' is omitted, in conformity with the practice of British chemical journals; in this paper 'gelatin' denotes the manufactured product, consisting of the derived protein, together with inorganic ions and other impurities

Routine analytical control of the arsenic content of commercial gelatins, as sold for edible purposes, has been carried out over a considerable period in the factory laboratories of many gelatin manufacturers. Until recently the arsenic content of gelatin, like that of all foods, has been controlled, somewhat indirectly, by law under successive Food and Drug Acts. These stated that food, as sold, should be ' pure food ', which legally implies that it should not be ' injurious to health '. A solid food is normally considered as not complying with this requirement if it contains more than 1 grain of arsenic, expressed as arsenious oxide, per pound (i.e. 1·083 p.p.m. of elementary arsenic). This limit was suggested in the report, published in 1903, of the Royal Commission on Arsenic in Foods. In 1948 a limit of 1 p.p.m. of elementary arsenic, applying specifically to edible gelatin, was directly enforced by the Ministry of Food.[2] More recently, in 1951, this limit has been raised to 2 p.p.m.[3]

The Gutzeit test

The procedures hitherto used in practice for the determination of arsenic in gelatin have been based almost invariably on the Gutzeit test.[4, 5] This test involves conversion of the arsenic into the gaseous arsenic hydride, which is carried in a stream of hydrogen over moistened mercuric chloride or bromide paper. The intensity (or sometimes length) of the yellowish stain produced on the paper is compared with standard stains, from known amounts of arsenic. The Gutzeit method suffers from several disadvantages which limit its accuracy and reliability: (1) The visual comparison of stains is subjective in nature, difficult for yellow colours and complicated on paper by the influence of texture; (2) Small variations in technique have a relatively large effect on the stains produced from a given weight of arsenic; (3) As the method is comparative, the intensity of stains of constant size cannot be expressed in definite units, and the length of stains cannot be used as a measure, in the absence of any guarantee of constant intensity. Consequently a study of the effect of the many possible variations in technique cannot be made, nor can the overall recovery of arsenic be measured; (4) Gelatin is not normally added during the preparation of standard stains. Consequently the stains from unknown and standards are not formed under identical conditions. This difference has been shown, in this study, to produce errors; (5) Different laboratories frequently obtain results in poor agreement on the same sample. Because of these drawbacks, the Gutzeit test was not considered to be a suitable basis for an accurate method for either research purposes or routine control.

The molybdenum-blue method

A colorimetric method, for which the final measurements were made with a suitable absorptiometer, appeared to be the most likely to give the required sensitivity and accuracy. In this way, disadvantages (1) and (3) could be overcome and a basis for the systematic investigation of the remaining difficulties be obtained.

The molybdenum-blue method appeared the most promising, on account of its high sensitivity and the fact that it has already been widely investigated. Both orthophosphate and arsenate ions react with molybdate ions in sulphuric acid solutions of suitable concentration to form the colourless complex ions phosphomolybdate and arsenomolybdate respectively. A variety of reducing agents have been used to convert these complexes into soluble blue derivatives of indefinite composition, usually referred to as ' molybdenum blue '. This type of reaction has recently been developed to a state approaching perfection for the accurate determination of small quantities of phosphate,[6] the intensity of the blue colour being proportional to concentration over a wide range. The reaction has also been applied to the micro-determination of arsenic.[1, 7, 8]

Since phosphates normally occur in gelatin in greater amounts than arsenic, it was essential to separate the arsenic before applying the colorimetric reaction, in order to avoid interference. Fortunately, arsenic can be readily separated by conversion into the gas phase, either as the gaseous hydride or as the easily volatile chloride or bromide. Separation as the hydride was found to be practicable and simple (' preferred method '), but a second method (' reference method '), in which the separation as chloride or bromide, after digestion of the gelatin with nitric acid, was used, will be described also, since it served to provide figures for comparison.

Preparation of the sample

The preparation of samples of organic materials for analysis before the separation of arsenic presents some difficulty. Since dry combustion is inapplicable as the first stage in the determination owing to the volatility of arsenic compounds, methods of wet digestion must be used. These methods fall into two categories: those in which the organic matter

is completely oxidized to carbon dioxide and water by powerful oxidizing agents (e.g. nitric acid, perchloric acid or hydrogen peroxide); and those in which the organic matter is broken down only sufficiently for it to dissolve completely.[4] The former type of wet digestion has been generally held to be the more reliable, since the destruction of organic matter prevents subsequent interference, and also it is assumed that all the arsenic is converted into the ionic form. It suffers, however, from the disadvantage that the digestion is usually a lengthy and troublesome process, where comparatively large amounts of organic matter are to be destroyed. Dilute-acid digestion, in which the organic matter is dissolved but not oxidized, is simpler to carry out and requires smaller quantities of reagents, thus helping to reduce the reagent blank.

For a water-soluble material such as gelatin, the dilute-acid digestion offers the advantages of ease of operation and low blank-values. Its reliability depends on proof that all the arsenic is rendered soluble and available for quantitative separation. In the present study, a procedure (the 'reference method') involving complete oxidation of organic matter was developed first. This was then used as a basis of comparison for the completeness of recovery of arsenic, as normally found in commercial gelatin, in the 'preferred method' where the gelatin was digested with dilute acid.

The 'reference method'

The 'reference method', which is described in detail in the Experimental section, was developed from the British Standard, Method 1,[4] by replacing the final Gutzeit stage with the molybdenum-blue procedure of Rodden.[7] The gelatin, dissolved in boiling sulphuric acid, was completely oxidized by gradual addition of nitric acid. After residual nitric acid had been removed with ammonium oxalate, hydrochloric acid and a mixture of sodium chloride, potassium bromide and hydrazine sulphate were added, to reduce the quinquevalent arsenic to the volatile trihalides. The arsenic was separated by distillation of the trichloride into dilute nitric acid. The nitric acid solution was evaporated to dryness and the residue dissolved in an acid solution of ammonium molybdate containing hydrazine sulphate. When the solution was heated to 100° for 10 minutes, a blue colour developed whose intensity was proportional to the arsenic concentration.

This method was successful with samples of commercial gelatin and complete recovery of added arsenic was obtained. The procedure was not considered suitable for general use, however, on account of two disadvantages: (i) the large and variable blank, and (ii) the length and unsatisfactory nature of the digestion and distillation stages.

The large blank arose mainly from arsenic contained in the large volumes of acids (35 ml. of nitric acid and 10 ml. of sulphuric acid) required for the digestion. Thus acids containing less than 0·05 p.p.m. of arsenic (the AnalaR limit) could introduce up to 2·25 μg. of arsenic, corresponding to 0·45 p.p.m. of gelatin. In addition, some arsenic was dissolved from the resistance-glass digestion flasks. Pretreatment of the flasks with boiling hydrochloric acid containing sodium chloride and hydrazine sulphate reduced but did not eliminate contamination of the digest. The blank value, even from the treated flasks, was variable. This source of contamination was eliminated by using quartz flasks, but these were too expensive where a number of determinations were carried out simultaneously. Table I shows the optical density obtained for the complete blank determinations in various types of flask. The high and variable blank rendered especially difficult the accurate determination of small quantities ($<$ 0·5 p.p.m.) of arsenic in gelatin.

Table I

Optical density of the blank with various types of flask ('reference method')

Type of flask	Optical density (4-cm. cell, No. 608 filter)
Untreated resistance-glass	0·153
Treated resistance-glass	0·04–0·07
Quartz	0·037

The digestion stage took 1–1½ hours to complete and required constant attention. Moreover, it was difficult to attend to more than four samples at once. To achieve rapid oxidation, it proved necessary to maintain a low concentration of nitric acid in the boiling solution. Addition of too much nitric acid lowered the boiling point and slowed down the rate of reaction. On the other hand, boiling off all the nitric acid resulted in charring, causing reduction of the arsenic to the tervalent state and possible loss by volatilization.

The distillations could be carried out only singly, as constant care was required, with the form of apparatus used, to prevent sucking back of the distillate during the later stages. An attempt was made to omit the digestion stage, by distilling the arsenic directly from an acid

solution of gelatin ; this failed, owing to distillation of organic matter, and consequent formation of suspended carbon in the final test solution.

The ' preferred method '

The ' reference method ' having been established as a standard for comparison, attention was turned to the development of a more convenient method having a low blank, so as to be suitable for low as well as high arsenic concentrations. The procedure investigated was that of Kingsley & Schaffert,[1] who recently reported that arsenic in biological materials could be completely recovered, after digestion with dilute hydrochloric acid. Their procedure consisted in treating the acid digest with stannous chloride and potassium iodide to reduce the arsenic to the tervalent state. Zinc was then added to liberate hydrogen and arsine. The mixed gases, after removal of hydrogen sulphide with lead acetate, were passed through iodine solution to absorb the arsine. The arsenic, oxidized to the quinquevalent state by the iodine, was estimated photoelectrically by a modification of the molybdenum-blue method, using hydrazine as reducing agent.

Colorimetry

The final, colorimetric stage of the procedure was first investigated and found to be satisfactory for use with photoelectric instruments, but unsuitable for visual measurements. The spectrophotometer curve (Hilger Uvispek spectrophotometer) of the test solution is shown in Fig. 1. The solution showed an absorption maximum in the near infra-red at 835 mμ and a falling but appreciable absorption in the red and orange. The absorption rose again in the violet part of the spectrum. The form of the curve showed that the optical density could be measured, for the determination of arsenic, with the spectrophotometer at 835 mμ or with the Hilger Spekker or Biochem absorptiometers, fitted with Ilford Spectrum Red No. 608 light filters. This conclusion was found to be correct, Beer's law being obeyed over the range 0–25 μg. of arsenic, with all three instruments. The optical density of a given solution measured at 835 mμ was about three times greater than when measured with the selenium photocell absorptiometers. An attempt to increase the sensitivity of these instruments, by using infra-red-sensitive selenium photocells, failed, as the lower electrical resistance of these photocells increased the damping of the galvanometer.

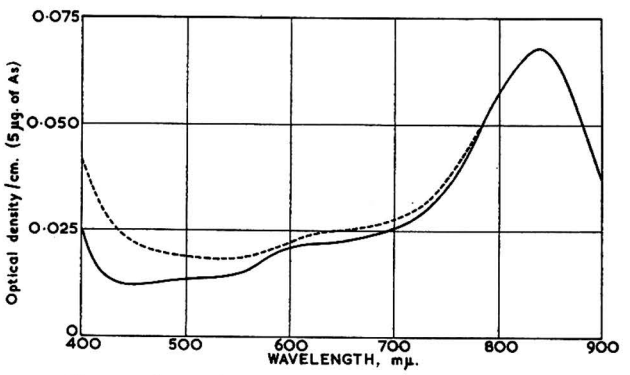

FIG. 1.—*Spectrophotometer curves for arsenic test-solutions*
1-Amino-2-naphthol-4-sulphonic acid (full line)
Hydrazine sulphate (broken line)

At a later stage, errors were noted when attempting to match, visually, directly prepared test solutions with solutions prepared by separation of arsenic as arsine. The latter solutions always appeared more green and less blue to the eye than the corresponding standards ; this led to the arsenic content of unknown solutions being assessed as too low. This error was not shown when the optical densities of the solutions were measured photoelectrically. The origin of this discrepancy was finally traced to the passage of hydrogen through the iodine solution, which carried away the iodine vapour and reduced the iodine concentration when compared with the standards. This resulted in an increased concentration of residual hydrazine sulphate in the final test solution, thereby producing increased absorption in the blue and violet parts

of the spectrum, but no change at the red end. The error in matching by eye was therefore explained, whereas no error would arise with the absorptiometers, which utilized only red or infra-red radiation.

The difficulty was overcome by replacing the hydrazine sulphate reducing agent with the 1-amino-2-naphthol-4-sulphonic acid reagent of Fiske & Subbarow,[9] as modified by Yuen & Pollard.[6] This reagent contains a large excess of sodium sulphite, which instantly reduces the iodine, in the cold, leaving the concentration of the aminonaphtholsulphonic acid unchanged. Although the colour develops in 45 minutes with phosphate ions at room temperature,[6] maximum colour development with arsenate ions was found to take about 12 hours at room temperature, the rate of formation of colour increasing with the intensity of the incident light. Colour development was, however, completed in three minutes at 100°, independently of the intensity of illumination.

The blue solutions with 1-amino-2-naphthol-4-sulphonic acid also showed an absorption maximum at 835 mμ, the optical density near this wavelength being almost identical with solutions reduced with hydrazine sulphate (Fig. 1). Beer's law was obeyed at all wavelengths previously investigated (Fig. 2). The colorimetric blank, with the particular sample of reagent used, was slightly higher than with hydrazine sulphate. The blue solutions reduced with the sulphonic acid possessed the additional advantage of being far more stable to light than those treated with hydrazine.

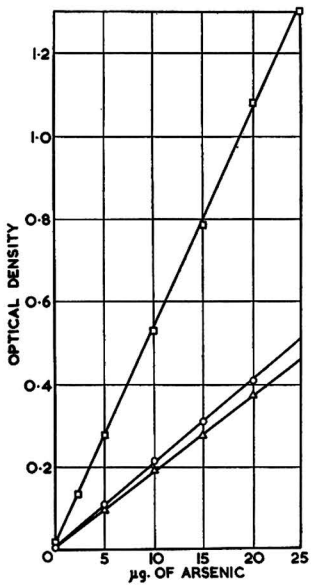

FIG. 2.—*Arsenic calibration curves ('preferred method'): directly prepared solutions; 25 ml. of solution; 4-cm. cell*

□ Uvispek spectrophotometer at 835 mμ
○ Spekker absorptiometer, 609 filter
△ Spekker absorptiometer, 608 filter

Recovery of arsenic in the separation stage

The recovery of arsenic, added as known quantities of arsenious oxide to the dilute hydrochloric acid, was investigated, after separation according to the method of Kingsley & Schaffert.[1] The apparatus used (Fig. 3) was modified to permit the use of rubber bungs and avoid the use of sintered discs, which are awkward to rinse. The findings of Kingsley & Schaffert, who claimed quantitative recovery under these conditions, were not confirmed. In the present study only 50% of the arsenic was recovered in the range 0–25 μg.; this figure was not increased, although a number of changes in conditions was tried, including the use of an apparatus with standard glass joints. Five different types of zinc were tested (including the 'mossy zinc' specified) without marked effect on the recovery figures. This suggested that the zinc was sufficiently 'activated' by deposition of tin from the stannous chloride. The samples of zinc, alloyed with copper, which evolved hydrogen very rapidly did not give higher recoveries than the pure zinc.

These experiments were carried out with solutions containing hydrochloric acid, stannous chloride and potassium iodide, to which arsenious oxide was added, but no organic matter was present. In a further experiment, various organic materials were added as well as known quantities of arsenic. The recovery of arsenic was determined, making allowance for any arsenic present in the added material, by running blanks (Table II).

Table II

Recovery of arsenic in the presence of organic materials

Material added	None	Sucrose	Horse serum	Gelatin
Recovery of 10 μg. of arsenic, %	about 50	about 60	70–75	92

Thus the recovery markedly increased in the presence of organic matter, especially gelatin. This effect was not explained, although it was noticed that the evolution of hydrogen was much slower with gelatin present and the bubbles of gas remained longer below the surface. The difference in percentage recovery in the presence and absence of gelatin would be expected to lead to an error in the Gutzeit determination, where the standard stains are usually prepared without addition of gelatin.

The increased recovery, in the presence of gelatin, was confirmed over a range from 2·5 to 25 μg. of arsenic, the recovery decreasing from 96 to 88% over this range. Although a number of variations were made in the technique, these figures were not exceeded. The cause of the loss of arsenic was not traced, but an investigation of the effect of variations in the conditions of the procedure suggested that the low recoveries resulted, possibly, from the cumulative effect of a number of small losses. The percentage recovery of a given amount of added arsenic was constant, within the limits of experimental error, for experiments carried out during a period of several months (Table III).

Table III

Percentage recovery of various weights of arsenic added to gelatin ('preferred method')

μg. of As added	Percentage recovery					Mean
	(i)	(ii)	(iii)	(iv)	(v)	
5	96	98	94	96	96	96
10	93	90	93	93	93	92
15	90	90	89	90	91	90
20	89	88	88	89	91	88
25	89	88	87	89	89	88
					Overall mean	91

This constancy of recovery enabled an absorptiometer calibration-curve to be plotted relating the optical density of the blue solutions to the weight of arsenic added before separation as arsine. Such a curve has a slightly lower slope than that for directly prepared standard solutions (Fig. 4) and automatically allows for the decreased recovery with any given amount of arsenic, when reading off the arsenic content of an unknown sample.

When the solutions are matched visually, it is normally too time-consuming to prepare the standards by actual separation of the arsenic. Preparation of standards by direct addition of standard arsenic solution, and use of a correction factor, ranging from 1·04 to 1·13, according to the arsenic concentration, is recommended.

Recovery of other forms of arsenic

Quinquevalent arsenic, added as sodium arsenate, was recovered in slightly lower amounts (85–91%) than tervalent arsenic. This showed that the reduction with stannous chloride and hydriodic acid was reasonably effective, although not completely quantitative.

A number of organic arsenical compounds were subjected to the dilute-acid digestion and treatment with zinc. Only about 5% of the arsenic was recovered from both *n*-propylarsonic acid and phenylarsonic acid. Kingsley & Schaffert[1] reported quantitative recovery from *N*-*p*-arsonophenylglycine amide, but, in the present study, a recovery of only 60% was obtained with *p*-aminophenylarsonic acid. It is evident that the extent to which organic arsenic compounds are broken down by dilute acid varies greatly with small differences of structure. Evidence was obtained to suggest that arsenic attached to aromatic nuclei would not be estimated even by methods involving nitric acid digestion. There is, however, little reason to expect the occurrence of such compounds in gelatin.

Comparison of the 'preferred method' with the 'reference method'

The arsenic contents of about 30 samples of gelatin were determined by the 'preferred method'. Results from duplicate determinations gave good agreement and the method failed in one instance only, where the very large volume of hydrogen sulphide evolved, some of which escaped absorption in the lead acetate trap, caused turbidity in the test solution. It is clear that the sample giving off such large quantities of hydrogen sulphide was abnormal and should have been rejected as an edible gelatin, on other grounds.

A comparison of the results for the arsenic contents of a number of gelatin samples, determined by the 'preferred method' and the 'reference method', showed good agreement (Table IV).

This agreement supported the statement of Kingsley & Schaffert[1] that arsenic from biological materials is completely extracted by digestion with dilute acid. Investigations with ion-exchange resins have shown that a substantial proportion of the arsenic in gelatin is absorbed on strong acid–strong base mixed-bed resins, suggesting that such arsenic is in ionic form. It would therefore be extracted by acids during digestion.

A comparison of results obtained using the 'preferred method' in our laboratory and two industrial laboratories showed that good agreement could be obtained between different workers on the same samples.

Table IV

Comparison of results obtained with 'preferred' and 'reference' methods on commercial gelatin

Sample	Arsenic content, p.p.m.	
	'Preferred method'	'Reference method'
A	< 0·04	< 0·10
B	0·36	0·37
C	0·51	0·51
D	3·08	3·04

Experimental

(*a*) *'Preferred method'*

Reagents

Stock standard arsenic solution (1000 *p.p.m. of arsenic*).—Arsenious oxide, A.R. (0·1320 g.), was dissolved in 2 ml. of N-sodium hydroxide solution, the solution was diluted to about 50 ml. with water, neutralized by the addition of 2 ml. of N-hydrochloric acid and diluted to exactly 100 ml. This solution kept for several months.

Dilute arsenic solutions (5 *p.p.m. of arsenic and* 2 *p.p.m. of arsenic*).—These were prepared, when required, by dilution of 5 ml. and 2 ml. respectively of the stock standard arsenic solution to 1 l. with water.

Arsenic-free gelatin.—This was prepared by the de-ionization of gelatin of initially low arsenic-content with mixed-bed ion-exchange resins (I.R. 120 and I.R.A. 400). Suitable gelatin is also available from the British Gelatine Works, Ltd., 46 Wilton Place, Knightsbridge, London, S.W.1.

Hydrochloric acid, AnalaR (*sp. gr.* 1·18).

Potassium iodide solution (15%).—This was prepared, as required, by dissolving 7·5 g. of potassium iodide, A.R., in water and diluting to 50 ml.

Stannous chloride solution (20%).—This was freshly prepared by dissolving 5 g. of stannous chloride, A.R., in hydrochloric acid, AnalaR, and diluting to 25 ml. with the acid.

Zinc, granulated (*arsenic-free*); *Lead acetate solution* (*saturated*); *Cotton wool* (*absorbent, non-medicated*).

Stock iodine solution (0·025N).—Iodine (3·175 g.), A.R., and 10 g. of potassium iodide, A.R., were dissolved in about 30 ml. of distilled water and diluted to 1 l.

Dilute iodine solutions, A and B (0·00125N *and* 0·0025N *respectively*).—These were prepared by diluting 50 ml. and 100 ml. respectively of stock iodine solution to 1 l. with water. The dilute solutions kept for at least three months.

Ammonium molybdate solution (1% *in* 5N-*sulphuric acid*).—Sulphuric acid, AnalaR (140 ml.), was added with stirring, to 600 ml. of distilled water and allowed to cool, then 10·0 g. of ammonium molybdate, $(NH_4)_6Mo_7O_{24},4H_2O$, A.R., was dissolved in the acid and the solution diluted to 1 l.

1-*Amino-2-naphthol-4-sulphonic acid solution.*—Sodium metabisulphite (12·5 g.), 5 g. of crystalline sodium sulphite, A.R., and 0·5 g. of 1-amino-2-naphthol-4-sulphonic acid were dissolved in water. The solution was filtered and diluted to 250 ml. The solution kept for about a week.

Apparatus

The apparatus for the arsenic separation is shown, to scale, in Fig. 3. It was constructed of resistance glass and the 100-ml. conical flask had a standard B.19 joint to ensure that the rubber bung fitted closely. The capillary tube had an internal diameter of 0·5–1·0 mm. and the 25-ml. graduated test-tubes were fitted with standard B.19 stoppers. The exact size of the apparatus is not of critical importance.

Procedure

Approximately 5 g. of powdered gelatin was accurately weighed into the 100-ml. conical flask, no particles being left on the neck, and 20 ml. of water was added, followed by 10 ml. of hydrochloric acid. The contents of the flask were mixed and heated on a steam bath for one hour. The digest was cooled to room temperature and a further 10 ml. of hydrochloric acid added, followed by 2 ml. of potassium iodide solution and 1 ml. of stannous chloride solution. The solution was mixed and allowed to stand for 15 minutes.

During this interval, 20 ml. of dilute iodine solution A (0·00125N) was measured into the absorption tube and the hydrogen sulphide trap assembled. A piece of cotton wool was pressed firmly into the bottom of the trap and moistened with a few drops of lead acetate

solution. Two more pieces were gently pressed on top of the first, and similarly moistened, the cup portion of the trap being approximately one-quarter filled with cotton wool. A water seal was made between the upper bung and the trap to prevent leakage of gas.

After the solution had been standing for 15 minutes, 3 g. (± 0·5 g.) of zinc was added and the trap, with the delivery tube, fitted immediately. A water seal was made between the rubber bung and the flask. The delivery tube was placed resting lightly on the bottom of the absorption tube and the gas was allowed to bubble through the iodine solution for two hours. The delivery tube was then raised and rinsed into the absorption tube with a few drops of water.

Ammonium molybdate reagent (2·5 ml.) was added to the solution in the absorption tube and the contents were mixed. To this 0·5 ml. of 1-amino-2-naphthol-4-sulphonic acid solution was added, the solution diluted to the mark with distilled water and mixed. The tubes, without stoppers, were placed in a partitioned wire-basket, which was then immersed in boiling water for exactly ten minutes. The level of the boiling water was above that of the solutions in the tubes. The basket was then placed in cold water and the solutions were allowed to cool to room temperature (15–22°).

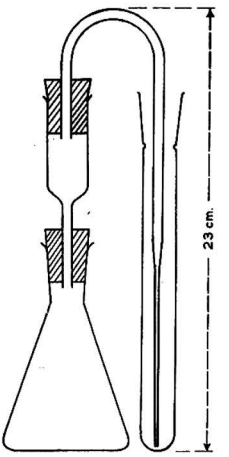

FIG. 3.—*Apparatus for separation of arsenic*

The optical densities of the solutions were measured in glass cells of optical path 4 cm., by means of the Hilger Uvispek spectrophotometer at 835 mμ (0·1-mm. slits); alternatively, either the Hilger Spekker absorptiometer or Biochem absorptiometer fitted with Ilford Spectrum Red No. 608 light-filters may be used. The arsenic content of the solution, expressed in μg., was read off from a calibration curve, similar to the lower curve in Fig. 4. This calibration curve had been prepared by adding 0, 0·5, 1·0, 2·0, 3·0, 4·0 and 5·0 ml. of 5-p.p.m. arsenic solution (corresponding to 0–25 μg. of arsenic) to 5-g. portions of arsenic-free gelatin, separating the arsenic as described above and determining the optical densities of the solutions. The curve needed only occasional checking.

The weight of arsenic in the solution (in μg.), as determined from the calibration curve, divided by the weight of the gelatin sample (in g.) gave the arsenic content of the gelatin in parts per million.

Where no photoelectric instrument is available, the solutions may be matched visually against standard blue solutions. These are freshly prepared each day by adding 0, 1·0, 2·0, 3·0, 4·0, 5·0, 7·5 and 10·0 ml. of 2·0-p.p.m. arsenic solution (corresponding to 0–20 μg. of arsenic) to a series of absorption tubes, adding 10 ml. of dilute iodine solution B (0·0025N), diluting to approximately 20 ml. and developing the colour in the usual way. The unknown solution in a Nessler tube is either matched with one of the standards or identified as being midway in tint between adjacent standards. In calculating the arsenic content of the gelatin, when the solutions are matched against directly prepared standards, a correction should be applied, in the form of a factor, to allow for incomplete recovery in the separation stage:

$$\text{Concn. of As in gelatin (p.p.m.)} = \frac{\text{Wt. of As in standard } (\mu g.)}{\text{Wt. of gelatin (g.)}} \times F$$

where F varies from 1·04 for 5 μg. of As to 1·13 for 20 μg. of As.

Effect of variations in technique

Preliminary digestion.—No increase in the recovery of arsenic from commercial gelatin was observed on increasing the time of digestion beyond one hour. When the digestion period was less than one hour some samples tended to froth, after the addition of zinc, and spoil the determination.

Identical recoveries, within the limits of experimental error, were obtained when known amounts of arsenic were added to arsenic-free gelatin solutions either before or after the digestion, showing that no loss of arsenic occurred during heating on the water bath. Vigorous boiling over a Bunsen flame resulted in loss of arsenic.

Contamination from resistance glass.—Contamination of solutions has already been discussed in connexion with the nitric acid oxidation in the ' reference method '. Determinations

carried out by the 'preferred method' with de-ionized gelatin in (i) an untreated flask and (ii) a flask which had been boiled with a solution for removing arsenic gave values of 0·026 and 0·022 respectively for the optical density at 835 mμ. The small difference showed that contamination was negligible.

Effect of the type of zinc used.—Much work has been carried out in the past on the effect of the 'activity' of zinc on the recovery of arsenic. In this method the use of a tin salt effectively activated a fairly pure zinc, and alloying the zinc with copper resulted in an unnecessarily high rate of evolution of gas. In the present method, the zinc almost completely dissolved in two hours. A recovery of 92% of arsenic was obtained with May and Baker 'zinc, arsenic free', and of 89% with both B.D.H. 'zinc, AnalaR' and 'mossy zinc, c.p.'[1]

Effect of hydrogen sulphide trap.—No significant difference in recovery was observed when several large pieces of cotton wool (as specified) were substituted for the very small pieces used in earlier experiments. It was concluded that the trap did not absorb arsenic. The larger trap was much more efficient for absorbing hydrogen sulphide where large volumes were evolved.

Effect of jet size.—Jet size did not markedly affect the recovery of arsenic; 91% recovery of 10 μg. of arsenic was obtained with a fine jet (0·1 mm. inside diameter) and 92% recovery with a jet of the diameter specified (0·7 mm.). Unconstricted tubing (3 mm. inside diameter) gave a recovery of 89% of arsenic.

Effect of depth and concentration of iodine solution.—Various volumes and concentrations of iodine solution were used during these investigations, ranging from 5 ml. of 0·001N to 20 ml. of 0·0025N, without any marked effect on recovery. Doubling the iodine concentration from the level specified did not markedly affect the recovery (Table V). The finding of Milton & Duffield[8] that addition of sodium bicarbonate to the iodine improved the recovery was not confirmed for quantities of arsenic below 25 μg.

Table V

Effect on recovery of arsenic of increasing the specified iodine concentration

μg. of As added	Recovery with 20 ml. of 0·00125N-I_2 (specified concn.), %	Recovery with 20 ml. of 0·0025N-I_2, %
5	96	91
10	92	92
15	90	91
20	88	90
25	88	90

Effect of cooling the iodine solution to 0°.—Kingsley & Schaffert[1] found that recovery was improved by cooling the iodine solution in ice. This was not confirmed in the present study, the recovery actually being lower at 0° than at 20° for amounts of arsenic ranging from 5 to 25μg. (Table VI).

Table VI

Effect of temperature of the iodine solution on recovery

μg. of As added	Recovery with iodine at 20°, %	Recovery with iodine at 0°, %
5	96	85
10	92	84
15	90	79
20	88	79
25	88	79

Loss of arsenic at the beginning of evolution.—The possibility of loss of arsenic, immediately after adding the zinc and before insertion of the stopper, was investigated. Such loss could occur only if most of the arsine were evolved soon after adding the zinc. A series of determinations were made in which the gas was allowed to escape for various times before allowing it to bubble through the iodine solution for the specified two hours (Table VII).

Table VII

Recovery after allowing escape of hydrogen, following addition of zinc

Time for which gas was allowed to escape, min.	0	0·5	1	2	4	8
Recovery of 10 μg. of As, %	94	94	93	86	74	27

No loss of arsenic occurred during the first half minute. Thus there was no risk of error arising in the determination, as the stopper could be inserted in a few seconds.

Effect of gas-evolution time on recovery.—The hydrogen was allowed to bubble through the iodine solution for varying periods. Two hours' evolution (as specified) showed a large increase in recovery over the one-hour period suggested by Kingsley & Schaffert ;[1] overnight evolution increased the recovery only slightly over the two-hour period (Table VIII).

Table VIII

Recovery of arsenic after varying times of hydrogen evolution

Absorption time, h.	Recovery of 10 μg., %
0·5	65
1	75
2	92
18	94

Completeness of absorption by iodine.—An experiment was carried out in which the gas was bubbled through a second tube of iodine solution after leaving the first. No arsenic was recovered in the second tube, the optical density of the solution being identical with the colorimetric blank.

Stability of the test solution to light.—No change was shown in the optical density, at the absorption maximum, of solutions containing 1-amino-2-naphthol-4-sulphonic acid after 24 hours' exposure to daylight indoors near a window. A slight yellowing of the solutions was, however, noticed on visual comparison with freshly prepared standards. Solutions kept in the dark showed no visible change after 24 hours but became somewhat yellow after four days. Solutions prepared with hydrazine sulphate (1 ml. of 0·15% solution) in the early stages of this investigation faded rapidly in daylight.

Heating time.—The period for which the test solutions were heated for colour development was not of critical importance. The colour intensity increased rapidly for 2–3 minutes, continued to increase slightly for up to 5 minutes, and then remained practically constant for the rest of the 10-minute heating period.

Temperature of solution during optical-density measurement.—The test solution showed a small variation in optical density with temperature. This was reversible and was ignored, provided that the laboratory temperature did not show extreme variations (Table IX).

Table IX

Optical density of test solution at various temperatures

Temp., °c	Optical density of test soln. containing 10 μg. of As
20	0·536
30	0·526
37	0·515

Chemical interference

Phosphate.—Since orthophosphate ions give the molybdenum-blue reaction, it was important to ensure that phosphates did not give rise to phosphine on addition of zinc, so leading to high results. Sodium phosphate added to arsenic-free gelatin in the maximum proportion considered likely to be present in commercial gelatin (0·1%) did not affect the result.

Sulphur dioxide.—Sulphur dioxide is reduced by zinc and acid to hydrogen sulphide which is evolved with the hydrogen. Tests in which sulphur dioxide was added (as sodium sulphite) at the level of the edible limit for gelatin (1000 p.p.m. of SO_2) showed that the lead acetate trap could easily remove the hydrogen sulphide completely. Incomplete removal of hydrogen sulphide leads to reduction of iodine and production of a turbidity in the test solution.

Checking of technique

An advantage of the molybdenum-blue method over the Gutzeit test is that it provides a means of systematically checking the analytical technique. This may be done in the following way :

(i) *Blank.*—The blank for the colorimetric part of the procedure should be an almost colourless solution (optical density less than 0·02 at 835 mμ, and less than 0·01 with Spectrum Red filter). This shows that the 1-amino-2-naphthol-4-sulphonic acid, molybdate and iodine reagents are sufficiently free from arsenic and phosphates.

A blank for the complete determination on arsenic-free gelatin should almost match the colorimetric blank (optical density less than 0·025 at 835 mμ), showing that arsenic contents of the acid, zinc and gelatin are sufficiently low.

(ii) *Recovery.*—Where solutions are matched visually, the recovery of known weights of arsenic, added to arsenic-free gelatin, should be occasionally checked by comparison with standard blue solutions, prepared in the ordinary way. The solutions containing separated arsenic should show 90% of the colour intensity of the direct standards.

For the absorptiometric method described, the method of preparing the calibration curve automatically compensates for losses in recovery. It is advisable, however, to check the calibration curve against another curve relating optical density with weight of arsenic, for directly prepared solutions. A recovery of about 90% should be shown (Fig. 4).

Accuracy

An accuracy of ± 2% was obtainable with 10 μg. of As, by using the spectrophotometer at 835 mμ. With the Spekker absorptiometer and Spectrum Red No. 608 filter an accuracy of ± 3–4% was obtainable; with the Biochem instrument and the same filter the accuracy was ± 5–6%. Visual matching gave results within ± 5–10%.

Sensitivity

A weight of 0·2 μg. of arsenic, corresponding to 0·04 p.p.m. of arsenic on the 5-g. gelatin sample, gave an optical density increment of 0·010 at 835 mμ, which could be measured to ± 0·001. The sensitivity with red filters was about one-third of this value.

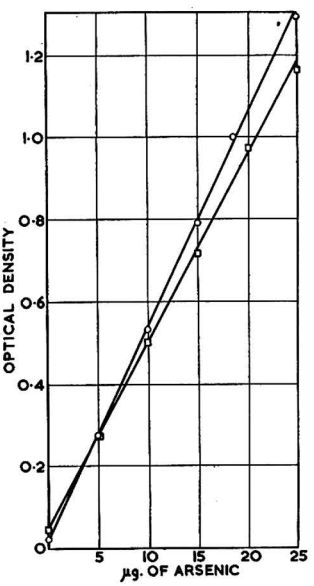

FIG. 4.—*Calibration curves for separated arsenic and direct colorimetric standards: 25 ml. of solution; 4-cm. cell; Uvispek spectrophotometer at 835 mμ*

○ Directly prepared standards
□ Arsenic separated as arsine

Procedure where the arsenic content exceeded 5 p.p.m.

The determination was repeated by using 1 or 2 g. of the gelatin of high arsenic-content together with 4 or 3 g. respectively of arsenic-free gelatin.

(b) 'Reference method'

Reagents

Reagents mentioned under 'procedure', and not given below, were identical with those used in the 'preferred method'.

Nitric acid, AnalaR (sp. gr. 1·42); Sulphuric acid, AnalaR; Ammonium oxalate solution (A.R., saturated).

Chloride–hydrazine–bromide reagent.—Sodium chloride, A.R. (100 g.), 10 g. of hydrazine sulphate, A.R., and 0·4 g. of potassium bromide, A.R., were powdered and mixed together.

Dilute nitric acid.—Nitric acid, AnalaR, was diluted in the proportion 1 : 5 with water.

Hydrazine sulphate solution.—A 0·15%-solution of the A.R. salt was used.

Hydrazine–molybdate reagent.—Ammonium molybdate solution (10 ml.) was diluted to 90 ml., 1 ml. of hydrazine sulphate solution was added and the solution diluted to 100 ml. This solution was prepared just before use.

Solution for removing arsenic from glass.—Hydrochloric acid, sp. gr. 1·18, saturated with sodium chloride and containing 0·1% of hydrazine sulphate was used.

Procedure

Gelatin (5 g.) was placed in a 250-ml. quartz or treated-glass Kjeldahl flask and 10 ml. of nitric acid added. The mixture was warmed until the evolution of brown fumes slackened. Sulphuric acid (10 ml.) was added and the mixture was gently boiled until charring just began. Nitric acid was added, in small portions, from time to time to check the darkening of the liquid, charring being avoided. When the liquid remained colourless after the evolution of brown fumes ceased, heating was continued until sulphuric acid fumes were evolved. This generally required about 1½ hours' heating and the addition of about 35 ml. of nitric acid. Ammonium oxalate solution (15 ml.) was added to

the cooled solution and the mixture was heated until sulphuric acid fumes were again evolved. A further 15 ml. of ammonium oxalate solution was added and the solution was evaporated to fuming once more.

The cooled solution was quantitatively transferred to a distillation apparatus[4] by the use of 7 ml. of water. After the solution had been cooled, 5 g. of chloride–hydrazine–bromide reagent was added through a wide-necked funnel. Hydrochloric acid (10 ml.) was added and the condenser was fitted at once. The liquid was distilled into 10 ml. of dilute nitric acid contained in a boiling tube externally cooled with water. The lower end of the air condenser dipped below the liquid surface. Distillation was continued until sucking-back made continuance impracticable; about 7 ml. of distillate was collected.

The distillate was transferred to a 50-ml. beaker which had previously been subjected to prolonged boiling with the solution for removing arsenic. Nitric acid (8 ml.) was added and the solution was evaporated to dryness on a hot-plate. The residue was heated at 130° for 30 minutes to remove nitric acid.

The residue was dissolved in molybdate–hydrazine reagent, the solution transferred to a 25-ml. graduated tube and made up to volume with the reagent. The solution was mixed and the tube heated for 10 minutes in boiling water. After the blue solution had been cooled to room temperature its optical density was measured in the way described for the 'preferred method'. The arsenic content was read off from a calibration curve that had been constructed by the use of data from measured volumes of standard arsenic solution, evaporated to dryness with nitric acid and heated with molybdate–hydrazine reagent. A blank was run for the entire procedure.

Acknowledgment

The authors wish to thank the Director and Council of the British Gelatine and Glue Research Association for permission to publish this paper.

The British Gelatine and Glue Research Association
2a Dalmeny Avenue
London, N.7

Received 5 March, 1953

References

[1] Kingsley, G. R. & Schaffert, R. R., *Analyt. Chem.*, 1951, **23**, 914
[2] Statutory Instruments, 1948, No. 2460; Edible Gelatine Order, 1948 (London: H.M.S.O.)
[3] Statutory Instruments, 1951, No. 1196; The Food Standards (Edible Gelatine) Order, 1951 (London: H.M.S.O.)
[4] B.S. 757 : 1944
[5] Smith, C. R., *U.S. Dept. of Agric., Bur. Chem., Circ.* 102 (1912)
[6] Yuen, S. H. & Pollard, A. G., *J. Sci. Fd Agric.*, 1951, **2**, 36
[7] Rodden, C. J., *J. Res. nat. Bur. Stand.*, 1940, **24**, 7
[8] Milton, R. & Duffield, W. D., *Analyst*, 1942, **67**, 279
[9] Fiske, C. H. & Subbarow, Y., *J. biol. Chem.*, 1925, **66**, 375

THE SEPARATION OF GLYCERIDE CONCENTRATES FROM NATURAL FATS BY CRYSTALLIZATION FROM SOLVENTS

By J. S. CAMA, M. M. CHAKRABARTY, T. P. HILDITCH and M. L. MEARA

The limits of experimental accuracy of systematic crystallization of fats and fatty oils from solvents as a method of determining glyceride constitution are critically considered. Examination of artificial fat mixtures of approximately known composition prepared from concentrates of various types of mixed glycerides has given results generally accordant with the proportions of glyceride categories known to be present. More conclusive evidence of the validity of the crystallization procedure is, however, to be gathered from the facts (*a*) that the trisaturated glyceride content of a fat is the same, whether it is determined chemically (by removal of all unsaturated glycerides by permanganate–acetone oxidation) or physically (by crystallization); and (*b*) that the amount of a simple triglyceride (e.g. trisaturated, triolein, trilinolein etc.) in a seed fat, determined by the crystallization procedure, is related to the proportion of the acid concerned (e.g. saturated, oleic, linoleic etc.) in the total fatty acids of the fat in a perfectly regular manner, irrespective of the nature of the acid concerned (saturated or unsaturated).

**

Introductory

Preliminary resolution of natural fats into simpler mixtures of mixed triglycerides, by systematic crystallization from solvents at temperatures from — 70° upwards, has proved to be a most useful procedure in determining the approximate proportions of the different categories of mixed glycerides, and even of those of individual mixed glycerides, present in the original fats or fatty oils. The process is not an easy one, mainly owing to the effect of the increased solubility of a relatively insoluble glyceride in a solvent (e.g. acetone) which already contains in solution more-soluble glycerides. This mutual-solubility effect is naturally the more marked when the mixture of glycerides in a natural fat is very complex. It is therefore necessary to conduct the crystallizations with these factors in mind: to select those conditions of solvent, concentration and temperature which give the most adequate separations, and to repeat the crystallization of intermediate fractions until little or no further alteration between precipitate and solute is observable. After many operations and recrystallizations the original fat will be thus divided into a large number of fractions (from six up to sixteen or more, according to the nature of the fat), each of which is sufficiently simple for its glyceride structure to be determined from the proportions of its (relatively few) component acids.

Procedures of this kind have been carried out with many natural fats, both in our laboratories and in those of a number of American and Indian investigators, with satisfactory results. However, doubt has sometimes been expressed as to how far such separations are effective, and Kartha[1] in particular has asserted that the method is untrustworthy and that ' the limits of accuracy of which the fractional crystallization method is capable do not seem to have been studied in detail '. Kartha, having himself failed (ref. [1] pp. 8–10) to effect adequate separation by crystallization of an extremely simple synthetic mixture (oleodistearin and mixed oleolinoleins), apparently maintains that the crystallization method must be held suspect until it has been shown that glyceride mixtures of known composition can be accurately analysed with its aid. It is hoped that the work briefly summarized in this paper may serve in some measure to meet such criticisms.

A primary difficulty in devising mixtures of glycerides that simulate different kinds of natural fats is the complex nature of the vast majority of individual natural component glycerides: it is little use, for example, to employ mixtures of tripalmitin, triolein and similar simple glycerides that are known not to be present to any extent in natural fats. We have therefore waited until we were able to accumulate—by crystallization from selected fats rich in one or other mixed glyceride—sufficient concentrates very rich in particular components (e.g. palmitostearins, oleopalmitostearins, palmitodioleins, oleolinoleins etc.) to serve as more or less adequate sources of different types of glycerides in artificial mixtures. Each concentrate was itself analysed, of course, so that the proportions of minor components present with the individual mixed glyceride that formed the bulk of the material were also known.

The experimental part of this communication is largely devoted to the application of the crystallization procedure to several mixtures that more or less resemble in fatty acid composition various types of natural fats, both solid (relatively saturated) or liquid (relatively unsaturated). These results are, however, preceded by the comparatively easy and accurate separation of the simple mixture of oleodistearin and oleolinoleins that was inadequately dealt with by Kartha. The discussion of our findings will be followed by a comparison of data for trisaturated glycerides determined in a fat either by chemical isolation or by the crystallization technique (both methods giving identical results), and by other figures which show that, for a given proportion of a fatty acid (e.g. linoleic, oleic, elaeostearic etc.) the amounts of simple triglycerides (e.g. trilinolein, triolein, trielaeostearin etc.) recorded by the crystallization technique follow the same course as those observed with trisaturated glycerides.

In order to avoid possible further misunderstanding, it is desired to define the probable limits of accuracy in determinations of component glycerides, or glyceride categories (e.g. tri-, di-, and mono-saturated, triunsaturated etc.). When, as in the crystallization technique, the determination ultimately depends on the observed proportions of component acids in each glyceride fraction, it is evident that an experimental error in the amount of a component acid may be trebled in the corresponding glyceride. Although, therefore, the probable error in a component acid determination should not exceed 1 unit per cent. (and is probably in modern work much less than this), the corresponding error in any one glyceride category may not be less than $\pm$ 2% of their percentages as recorded. It is further believed that, except in the few cases of fats that consist of an unusually simple mixture of only two or three fatty acids, it is not possible by any current technique to obtain precision of greater order than this in the determination of the proportions of individual glycerides, or categories of glycerides, in natural fats. Knowledge within these limits has proved amply sufficient to delineate the

main types of glyceride structure in natural fats, and to indicate the approximate proportions of their chief constituents. It may be well, however, to emphasize that fractional precision in analyses of this kind is unlikely to be achieved.

Methods

Fairly large quantities of a number of concentrates of different glyceride types were prepared from suitable sources by intensive application of the crystallization technique. These concentrates (the component acids and glyceride categories in which are shown in Table I) were as follows:

Trisaturated (palmitostearins) from a beef depot fat.
Disaturated-mono-unsaturated (i) 'Oleopalmitostearin' from a beef depot fat.
(ii) 'Oleodipalmitin' from piquia fat.
(iii) Oleodistearin from *Allanblackia floribunda* seed fat.
Monosaturated-diunsaturated (i) 'Palmitodiolein' from palm oil.
(ii) 'Stearodiolein' from Baku fat.
Triunsaturated (oleolinoleins) from sunflower-seed oil.

Table I

Component acids and glyceride categories of the concentrates

	Trisaturated	Disaturated			Monosaturated		Triunsaturated
	'PS_2'	'OPS'	'OP_2'	'OS_2'	'PO_2'	'SO_2'	'OL_2'
Component acids, % (mol.)							
Myristic	2·0	3·7	—	—	—	—	—
Palmitic	34·0	24·3	68·3	—	39·8	3·4	7·1
Stearic	61·3	36·9	—	66·1	—	33·8	—
Arachidic	—	1·5	—	—	—	0·7	—
Hexadecenoic	0·2	1·7	—	—	—	—	—
Oleic	2·5	30·3	31·7	33·9	45·1	60·9	20·5
Linoleic	—	1·6	—	—	15·1	1·2	72·4
Component glyceride categories, % (mol.)							
Trisaturated	91·9	—	—	—	—	—	—
Disaturated	8·1	99·2	95·1	98·3	19·4	13·7	—
Monosaturated	—	0·8	4·9	1·7	80·6	86·3	21·3
Triunsaturated	—	—	—	—	—	—	78·7

These concentrates were used to make up 'artificial fats' as indicated in Table II.

Table II

Artificial fats prepared from concentrates in Table I

	Fat A Wt.		Fat B Wt.		Fat C Wt.	
	g.	%	g.	%	g.	%
Concentrates used						
'PS_2'	36·2	18·0	10·2	5·1	—	—
'OPS'	60·1	29·9	40·0	20·0	—	—
'OP_2'	18·0	9·0	14·2	7·1	—	—
'PO_2'	60·3	30·1	130·0	64·8	42·9	26·8
'SO_2'	26·1	13·0	—	—	—	—
'OL_2'	—	—	6·0	3·0	117·1	73·2
	200·7		200·6		160·0	
	% (mol.)		% (mol.)		% (mol.)	
Glyceride categories (calc.)*						
Trisaturated	16·9		4·7		—	
Disaturated-mono-unsaturated	47·4		33·7		5·2	
Monosaturated-diunsaturated	35·7		59·2		41·8	
Triunsaturated	—		2·4		53·0	

* Calculated from the component glycerides in the respective concentrates (as given in Table I)

Each artificial fat was systematically crystallized from acetone (or, in the cases of the least soluble fractions obtained in the later stages, from ether), 10 ml. of solvent being usually employed per gram of glycerides; the first crystallizations were conducted at the lowest temperature used (— 60° or — 70°) and the temperatures of succeeding crystallizations of the deposited solids were gradually increased until, in the final stages with the solid fats A and B, crystallizations at 0° and at + 15° were undertaken. The general scheme of procedure

has been fully described in several recent communications.[2] Its application to the 'artificial' fats now described may be sufficiently indicated by the following summary:

	Fat A	Fat B	Fat C
Number of separate crystallizations performed	15	16	12
Final number of glyceride fractions analysed	10	11	8

The component acids in each glyceride fraction were determined by our usual procedure and the saturated-unsaturated glyceride categories present in each fraction were calculated on the assumption that the intensive crystallization had been sufficient to ensure that not more than two of the categories of trisaturated, disaturated, monosaturated or triunsaturated glycerides were present in any one fraction.

Results

I. *Kartha's failure*[1] *to resolve a mixture of oleodistearin and oleolinoleins by crystallization.*—Before discussing the results obtained with the comparatively complex fat mixtures A, B and C we desire to record our experience with the simple mixture of glycerides studied by Kartha.[1] Kartha made an artificial mixture consisting of 16·6% of oleodistearin (crystallized from *Garcinia indica* seed fat) with 83·4% of oleolinoleins (prepared by esterification with glycerol of the unsaturated acids from sesame oil). He submitted the mixture (8·65 g.) to a *single crystallization* from acetone (20 ml. per g. fat) at 0° for three days, when crystals (1·18 g., 13·6%) were deposited, leaving in solution 7·52 g. (86·4%). He thus estimated that his analysis showed 14% of disaturated, 6% of monosaturated and 80% of triunsaturated glycerides instead of the known proportions of 17% of disaturated-mono-unsaturated and 83% of triunsaturated glycerides. He concludes that 'this experiment shows that quantitative separation of monosaturated from triunsaturated glycerides cannot be obtained by crystallization under the simplest possible circumstances'; to us it shows only that this investigator has failed completely to understand the nature of the crystallization technique and the manner in which it should be applied.

We have without difficulty resolved a similar mixture satisfactorily by means of *three* crystallizations. The concentrate 'OS_2' [Table I, 4·9 g., iodine value (I.V.) 29·1] was mixed with concentrate 'OL_2' (24·9 g., I.V. 143·4), thus giving a mixture similar to that used by Kartha (calc.: mono-unsaturated 16, diunsaturated 18, triunsaturated glycerides 66%). The mixture was crystallized from acetone (10 ml. per g.) at − 40°, the deposited solids were again crystallized similarly at − 40°, and the solids then deposited given a third crystallization from acetone (10 ml. per g.) at − 20°, with the following results:

	Wt., g.	I.V.
Glycerides left in solution at − 40° (1st crystn.)	12·9	144·1
" " " " − 40° (2nd crystn.)	9·8	142·0
" " " " − 20°	2·2	138·1
" insoluble at − 20°	4·9	29·4

There were thus recovered 4·9 g. of solid glycerides of I.V. 29·4 and 24·9 g. of liquid glycerides of average I.V. 142·2.

II. *Component glycerides in the artificial fat mixtures A, B and C.*—The component acid and glyceride data for these mixtures are given in Tables III, IV and V.

Table III

Glyceride fractions from fat A (I.V. 38·5)

	A	B	C	D	E	F	G	H	I	J	Total
Weight, g.	26·7	8·6	42·8	18·7	21·7	34·9	23·3	8·9	4·9	3·3	193·8
Iodine value	1·8	6·0	28·2	33·2	41·6	55·6	64·7	56·6	64·7	74·4	38·5
Glycerides, % (wt.)	13·8	4·4	22·1	9·7	11·2	18·0	12·0	4·6	2·5	1·7	100·0
" % (mol.)	13·7	4·5	22·0	9·8	11·1	17·9	12·1	4·7	2·5	1·7	100·0
Component acids, % (mol.)											
Saturated	97·9	93·2	68·3	63·1	54·4	41·5	35·0	52·2	45·5	31·6	60·5
Oleic	2·1	6·8	31·7	36·0	44·3	53·3	58·1	33·6	37·2	49·8	36·0
Linoleic	—	—	—	0·9	1·3	5·2	6·9	14·2	17·3	18·6	3·5
Component glyceride categories, increments % (mol.)											
Trisaturated	12·8	3·6	1·0	—	—	—	—	—	—	—	17·4
Disaturated	0·9	0·9	21·0	8·8	6·9	4·3	0·5	2·1	1·1	—	46·5
Monosaturated	—	—	—	1·0	4·2	13·6	11·6	2·6	1·4	1·7	36·1

Table IV

Glyceride fractions from fat B (I.V. 53·8)

	A	B	C	D	E	F	G	H	I	J	K	Total
Weight, g.	7·4	4·3	5·6	32·9	37·7	28·1	18·1	37·1	9·7	5·0	7·9	193·8
Iodine value	3·0	10·3	29·3	32·7	51·9	63·5	57·4	65·3	70·6	85·2	98·6	53·8
Glycerides, % (wt.) ..	3·8	2·2	2·9	17·0	19·5	14·5	9·3	19·1	5·0	2·6	4·1	100·0
,, % (mol.) ..	3·8	2·2	2·9	17·0	19·3	14·5	9·3	19·2	5·1	2·6	4·1	100·0
Component acids, % (mol.)												
Saturated	96·2	89·1	68·9	64·7	43·7	43·5	49·7	41·7	44·1	32·7	22·7	50·1
Oleic	3·8	10·9	29·9	32·8	50·7	43·6	37·9	46·1	38·0	39·9	42·8	39·9
Linoleic	—	—	1·2	2·5	5·6	12·9	12·4	12·2	17·9	27·4	34·5	10·0
Component glyceride categories, increments % (mol.)												
Trisaturated	3·5	1·6	0·2	—	—	—	—	—	—	—	—	5·3
Disaturated	0·3	0·6	2·7	16·0	5·9	4·4	4·5	4·8	1·8	0·1	—	41·1
Monosaturated	—	—	—	1·0	13·4	10·1	4·8	14·4	3·3	2·5	2·7	52·2
Triunsaturated	—	—	—	—	—	—	—	—	—	—	1·4	1·4

Table V

Glyceride fractions from fat C (I.V. 121·0)

	A	B	C	D	E	F	G	H	Total
Weight, g.	6·0	5·7	6·8	10·5	7·7	5·5	40·8	65·4	148·4
Iodine value	78·7	81·4	83·2	86·0	106·3	123·2	129·9	133·9	121·0
Glycerides, % (wt.) ..	4·0	3·8	4·6	7·1	5·2	3·7	27·5	44·1	100·0
,, % (mol.) ..	4·1	3·9	4·6	7·2	5·2	3·7	27·4	43·9	100·0
Component acids, % (mol.)									
Saturated	35·5	33·7	34·5	35·5	26·0	14·1	11·9	11·9	17·3
Oleic	41·0	40·2	38·0	37·2	31·0	29·9	28·4	23·5	28·1
Linoleic	23·5	26·1	27·5	33·3	43·0	56·0	59·7	64·6	54·6
Component glyceride categories, increments % (mol.)									
Disaturated	0·3	—	0·1	0·5	—	—	—	—	0·9
Monosaturated	3·8	3·9	4·5	6·7	4·0	1·5	9·8	15·7	49·9
Triunsaturated	—	—	—	—	1·2	2·2	17·6	28·2	49·2

In another experiment a mixture of equal parts of an Indian poppy-seed and an Indian sesame oil (the glycerides in each of which had been determined by the crystallization procedure[3, 4]) was crystallized systematically from acetone, with the results shown in Table VI.

Table VI

Glyceride fractions from a 50/50 mixture of poppy-seed and sesame oils

	A	B	C	D	E	F	G	H	I	J	K	Total
Weight, g.	10·3	5·7	10·6	19·5	47·2	21·9	18·9	11·4	4·2	3·0	5·8	158·5
Iodine value	68·8	88·5	99·0	116·2	126·6	134·3	141·1	141·0	133·1	131·7	131·4	126·4
Glycerides, % (wt.) ..	6·5	3·6	6·7	12·3	29·7	13·8	11·9	7·2	2·7	1·9	3·7	100·0
,, % (mol.) ..	6·7	3·7	6·8	12·5	29·9	13·9	12·0	7·2	2·5	1·7	3·1	100·0
Component acids, % (mol.)												
Saturated	47·1	35·2	28·9	18·3	11·3	8·5	8·7	10·2	8·1	9·2	4·5	15·5
Oleic	27·3	30·6	29·3	32·1	32·1	24·9	22·0	21·5	26·3	28·0	27·9	28·3
Linoleic	25·6	34·2	41·8	49·6	56·6	66·6	69·3	68·3	65·5	62·8	67·6	56·2
Component glyceride categories, increments % (mol.)												
Disaturated	2·8	0·2	—	—	—	—	—	—	—	—	—	3·0
Monosaturated	3·9	3·5	5·9	6·8	10·1	3·5	3·2	2·2	0·6	0·5	0·4	40·6
Triunsaturated	—	—	0·9	5·7	19·8	10·4	8·8	5·0	1·9	1·2	2·7	56·4

Discussion

The proportions of saturated-unsaturated glycerides found in each of the artificial mixtures A, B and C, and in the mixture of equal parts of poppy-seed and sesame oil, are compared in Table VII with the proportions calculated from the known amounts of constituent concentrates (or oils) used to make up the mixtures.

Having regard to the complex mixture of concentrates employed in fats A and B, we consider that these results offer satisfactory evidence of the general reliability of the low-temperature crystallization procedure which has been employed in these laboratories and elsewhere. The accordance between observed and calculated proportions is excellent in all

Table VII

Comparison of observed and calculated proportions of glyceride categories

	Fat mixture A		Fat mixture B		Fat mixture C		Poppy/sesame oil mixture	
	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.
Trisaturated	17	17	5	5	—	—	—	—
Disaturated	47	47	41	34	1	5	3	3
Monosaturated	36	36	52	59	50	42	41	43
Triunsaturated	—	—	1	2	49	53	56	54

four glyceride categories of the mixture A and the poppy-seed–sesame mixture, and also in the trisaturated and triunsaturated glycerides of mixture B. It is reasonably good for the disaturated and triunsaturated glycerides in mixture C, but is several units per cent. different in the disaturated-mono-unsaturated and the monosaturated-diunsaturated glycerides in mixture B, and the monosaturated-diunsaturated glycerides in mixture C. The corresponding figures for categories of individual unsaturated (oleo- or linoleo-) glycerides, which were available in some of the mixtures, showed less accordance than the majority of those for the saturated-unsaturated categories.

Although three of the sixteen values recorded in Table VII could be interpreted to suggest that the crystallization procedure is liable to unduly great possibilities of experimental error, we are of opinion that the results of these trials, in other cases accordant, do not justify this adverse conclusion. The preparation and storage in quantity of the unsaturated glyceride concentrates needed to prepare mixtures of complexity comparable with natural fats is a matter of difficulty. The many crystallizations involve the risk of oxidative changes, and the natural protective antioxidants of the original fat employed are largely removed or destroyed in the course of the operations, thus causing the more unsaturated concentrates to be more vulnerable than the majority of fats in their natural state. It seems significant in this connexion that the poppy-seed–sesame oil mixture (in which no prior crystallization of the component natural fats was necessary) and the fat mixture A (57% of which consisted of concentrates of trisaturated glycerides and mono-oleo-disaturated glycerides, and another 13% of a diunsaturated glyceride concentrate containing only traces of linoleoglycerides) showed very close agreement between the respective observed and calculated proportions of all the glyceride types present. In spite of the generally good accordance shown in Table VII, therefore, we are not convinced that this procedure of preparing artificial fat mixtures of approximately known composition is altogether satisfactory as a method of testing the accuracy of the crystallization technique as applied to natural fats.

A better method, it would seem, lies in comparing the proportions of trisaturated glycerides found in a number of fats by the crystallization procedure with those found in the same fats by the entirely different method of isolation of fully saturated glycerides by the chemical process of oxidation to acidic derivatives of all glycerides containing unsaturated groups by potassium

Table VIII

Trisaturated glycerides found in natural fats by (a) chemical (oxidation), (b) crystallization procedure

	By chemical method (oxidation)			By physical method (crystallization)		
	Ref. No.	Saturated acids % (mol.)	Trisaturated glycerides % (mol.)	Ref. No.	Saturated acids % (mol.)	Trisaturated glycerides % (mol.)
Vegetable fats						
Coconut	5	92·9	84	6	94·0	82
Palm kernel	5	85·3	66	6	86·8	62
Stillingia tallow	7	68·4	24	8	73·1	21
Borneo tallow	9	62·9	5	10	62·9	5
Cacao butter	11	59·8	3	12	60·6	2
Kokum butter	13	59·0	2	14	59·2	1
Palm oil, Cameroons	15	49·1	8	16	52·5	8
„ „ Belgian Congo	15	49·6	6	17	49·6	6
Animal body fats						
Sheep	18	61·0	27	19	60·6	28
Cow, English	20	57·7	18	21	58·7	17
Pig, perinephric	22	51·0	11	23	50·5	9
„ back	22	42·9	7	23	44·1	5
Animal milk fats						
Buffalo, Indian	24	74·9	42	25	72·3	40
Cow, Indian	26	67·9	34	25	68·9	35

permanganate in acetone solution. Fortunately there are a number of instances in which the determination of trisaturated glycerides has been carried out by both methods, as will be seen from Table VIII. Since different specimens of the same fat may vary somewhat in fatty acid composition, the proportions of saturated acids in the total fatty acids are given as well as those of the trisaturated glycerides; the numbers in the reference columns refer to the literature references at the end of this paper.

The figures in Table VIII show unequivocally that, for a given saturated acid content in the total fatty acids, the same proportions of trisaturated glycerides are recorded either by the direct chemical (oxidation) method or by the crystallization technique. The validity of the latter procedure having thus been established for trisaturated glycerides, it becomes possible to assess its reliability in regard to other simple triglycerides, such as triolein, trilinolein, trielaeostearin, and others which are present in some natural fats. If, as in Table IX, we compare the proportions of a given simple triglyceride in relation to the proportion of its component fatty acid in the total acids of a seed fat, it is seen that a remarkably regular sequence results, whether or not the glyceride whose amount has been determined by the crystallization technique is trisaturated or triunsaturated. This is strong evidence that the method is capable of giving reliable results for unsaturated, as well as fully saturated, types of glycerides.

Table IX

Content of (simple) triglycerides in relation to content of fatty acid concerned (determined by crystallization procedure)

Vegetable fat	Acid in total acids	% (mol.)	Triglycerides	% (mol.)
Dika fat[27]	Saturated	97·9	Trisaturated	94
Coconut fat[6]	,,	94·0	,,	82
Castor oil[28]	Ricinoleic	91·6	Triricinolein	75
Palm-kernel fat[6]	Saturated	86·8	Trisaturated	62
Tung oil[29]	Elaeostearic	83·9	Trielaeostearin	56
,, ,, [29]	,,	79·0	,,	45
Safflower-seed oil[30]	Linoleic	76·5	Trilinolein	31
Olive oil[31]	Oleic	76·4	Triolein	29
Poppy-seed oil[3]	Linoleic	73·2	Trilinolein	27
Stillingia tallow[8]	Saturated	73·1	Trisaturated	21
Sunflower-seed oil[30]	Linoleic	72·4	Trilinolein	24
Tobacco-seed oil[32]	,,	70·9	,,	19
Sunflower-seed oil[30]	,,	67·3	,,	8
Olive oil[31]	Oleic	66·8	Triolein	5
Conophor oil[33]	Linolenic	65·7	Trilinolenin	10
Sunflower-seed oil[30]	Linoleic	63·2	Trilinolein	7
Borneo tallow[10]	Saturated	62·9	Trisaturated	5
Maize oil[34]	Linoleic	60·8	Trilinolein	1
Cacao butter[13]	Saturated	60·6	Trisaturated	2
Kokum butter[14]	,,	59·2	,,	1
Groundnut oil[35]	Oleic	59·1	Triolein	6
Dhupa fat[36]	Saturated	57·3	Trisaturated	1
Linseed oil[33]	Linolenic	56·2	Trilinolenin	5
Sunflower-seed oil[30]	Linoleic	51·1	Trilinolein	1
Palm-kernel fat[6]	Lauric	50·6	Trilaurin	1

Conclusions

1. The degree of segregation of different categories of saturated and unsaturated glycerides by means of systematic crystallization over an appropriate range of temperatures has been tested by submitting complex mixtures of mixed glycerides of approximately known composition to the procedure. The results were reasonably, and in most instances closely, accordant with the known proportions of the different saturated-unsaturated glyceride categories.

2. In eight vegetable and six animal (body or milk) fats in which the content of fully saturated glycerides has been determined by different workers (*a*) by isolation after removal of all unsaturated glycerides by permanganate–acetone oxidation and (*b*) by the method of systematic crystallization, the results by either procedure are substantially identical.

3. The contents of simple triglycerides (when present) of unsaturated acids such as oleic, linoleic, linolenic, elaeostearic or ricinoleic recorded by the crystallization procedure, arranged in relation to the proportion of such acid in the total acids of the fat concerned, form a regular sequence which is the same as that for trisaturated glycerides in relation to the proportion of saturated acids in the total acids of the fat.

4. A simple mixture of trisaturated and triunsaturated glycerides can readily be completely and quantitatively separated by correct application of the crystallization technique.

5. It is of course to be understood that the application of systematic crystallization to the segregation of a fat or fatty oil must be conducted with due regard to the precautions that have been repeatedly indicated by others as well as ourselves in the published literature.

The University
Liverpool

Received 17 March, 1953

References

[1] Kartha, A. R. S., 'Studies on the Natural Fats', Part I (Thesis, 1951, University of Madras)
[2] cf. Crawford, R. V. & Hilditch, T. P., *J. Sci. Fd Agric.*, 1950, **1**, 230, 372; Gupta, S. S., Hilditch, T. P. & Meara, M. L., *J. chem. Soc.*, 1950, p. 3145; Gupta, S. S. & Hilditch, T. P., *Biochem. J.*, 1951, **48**, 137
[3] Bridge, R. E., Chakrabarty, M. M. & Hilditch, T. P., *J. Oil Col. Chem. Ass.*, 1951, **34**, 354
[4] Chakrabarty, M. M. & Hilditch, T. P., *J. Sci. Fd Agric.*, 1951, **2**, 255
[5] Collin, G. & Hilditch, T. P., *J. Soc. chem. Ind., Lond.*, 1928, **47**, 261T
[6] Dale, A. P. & Meara, M. L., privately communicated
[7] Hilditch, T. P. & Priestman, J., *J. Soc. chem. Ind., Lond.*, 1930, **49**, 397T
[8] Gupta, S. S. & Meara, M. L., *J. chem. Soc.*, 1950, p. 1337
[9] Hilditch, T. P. & Priestman, J., *J. Soc. chem. Ind., Lond.*, 1930, **49**, 197T
[10] Bushell, W. J. & Hilditch, T. P., *J. Soc. chem. Ind., Lond.*, 1938, **57**, 48
[11] Hilditch, T. P. & Lea, C. H., *J. chem. Soc.*, 1927, p. 3106
[12] Meara, M. L., *J. chem. Soc.*, 1949, p. 2154
[13] Vidyarthi, N. L. & Rao, C. J. D., *J. Indian chem. Soc.*, 1939, **16**, 437
[14] Hilditch, T. P. & Murti, K. S., *J. Soc. chem. Ind., Lond.*, 1941, **60**, 16
[15] Hilditch, T. P. & Jones, E. E. (Miss), *J. Soc. chem. Ind., Lond.*, 1930, **49**, 363T; Banks, A., Dean, H. K. & Hilditch, T. P., *J. Soc. chem. Ind., Lond.*, 1935, **54**, 77T
[16] Hilditch, T. P. & Maddison, L., *J. Soc. chem. Ind., Lond.*, 1940, **59**, 67
[17] Meara, M. L., *J. chem. Soc.*, 1948, p. 722
[18] Collin, G., Hilditch, T. P. & Lea, C. H., *J. Soc. chem. Ind., Lond.*, 1929, **48**, 46T
[19] Hilditch, T. P. & Shrivastava, R. K., *J. Amer. Oil Chem. Soc.*, 1949, **26**, 1
[20] Hilditch, T. P. & Longenecker, H. E., *Biochem. J.*, 1937, **31**, 1805
[21] Hilditch, T. P. & Paul, S., *Biochem. J.*, 1938, **32**, 1775
[22] Banks, A. & Hilditch, T. P., *Biochem. J.*, 1932, **26**, 298
[23] Hilditch, T. P. & Pedelty, W. H., *Biochem. J.*, 1940, **34**, 971
[24] Achaya, K. T. & Banerjee, B. N., *Biochem. J.*, 1946, **40**, 664
[25] Achaya, K. T. & Hilditch, T. P., *Proc. roy. Soc.*, 1949, [B] **137**, 187
[26] Bhattacharya, R. & Hilditch, T. P., *Analyst*, 1931, **56**, 161
[27] Meara, M. L. & Patel, C. B., *J. Sci. Fd Agric.*, 1950, **1**, 48
[28] Gupta, S. S., Hilditch, T. P. & Riley, J. P., *J. Sci. Fd Agric.*, 1951, **2**, 245
[29] Hilditch, T. P. & Mendelowitz, A., *J. Sci. Fd Agric.*, 1951, **2**, 548
[30] Barker, C. & Hilditch, T. P., *J. Oil Col. Chem. Ass.*, 1950, **33**, 6
[31] Hilditch, T. P. & Maddison, L., *J. Soc. chem. Ind., Lond.*, 1941, **60**, 258
[32] Crawford, R. V. & Hilditch, T. P., *J. Sci. Fd Agric.*, 1951, **1**, 230
[33] Hilditch, T. P. & Seavell, A. J., *J. Oil Col. Chem. Ass.*, 1950, **33**, 24
[34] Doerschuk, A. P. & Daubert, B. F., *J. Amer. Oil Chem. Soc.*, 1948, **25**, 425
[35] Crawford, R. V. & Hilditch, T. P., *J. Sci. Fd Agric.*, 1950, **1**, 372
[36] Baliga, M. N. & Meara, M. L., *J. Soc. chem. Ind., Lond.*, 1949, **68**, 52

ACACIA MOLLISSIMA WILLD. III.*—The Purification of Native Black-wattle Wood Lignin

By P. R. ENSLIN

An adsorption chromatographic method for the separation of isolated native black-wattle wood lignin from impurities such as tannins and phlobaphenes is described. The pure native lignin is characterized by its elementary analysis, methoxyl content, molecular weight, ultra-violet and infra-red absorption spectra and behaviour on paper chromatograms. The results of nitrobenzene oxidation indicate that the lignin is of the guaiacyl-syringyl type.

A small percentage of the total lignin in wood was isolated by Brauns[1-4] and obtained in an unchanged state by extraction with ethanol at room temperature, and without the aid of an acid catalyst. The main limitation in the use of this so-called 'isolated native lignin'

* Part II: *J. Sci. Fd Agric.*, 1952, **3**, 37

in studying the chemical structure of lignin is that it represents only 1–10% of the total lignin in the wood. Recently, however, Nord *et al.*[5–7] demonstrated that a considerable proportion of the lignin in wood could be extracted by Brauns' method after it had been liberated by enzymes in the action of certain ' brown rot ' fungi. The liberated lignins were found to be identical with the ' isolated native lignins '.

A remaining criticism against Brauns' native lignin is that, in certain woods, it is contaminated with substances closely resembling lignin, e.g. tannins and phlobaphenes.[8] Such impure native lignins have been isolated from redwood,[9] Douglas fir,[10] cork[11] and wattle wood.[12]

Recently, Hess[13] investigated the homogeneity of isolated native black-spruce lignin and concluded that it was a mixture of substances which differed both in molecular structure and chemical constitution.

In this paper, an adsorption chromatographic method for the separation of isolated wattle native lignin from impurities such as tannins and phlobaphenes is described.

Fresh samples of the heartwood and sapwood from *Acacia mollissima* Willd. were separately extracted with 96% alcohol, and the lignins isolated and purified by Brauns' method. The heartwood yielded 0·24% of crude lignin (about 7–9% methoxyl) and the sapwood 0·15% of crude lignin (10–14% methoxyl).

These lignins were shown to be heterogeneous on paper chromatograms, using strips of No. 1 Whatman filter paper with (i) 50% aqueous methanol and (ii) anhydrous acetone as solvents. With (i) the lignin and phlobaphenes (?) moved as a well defined spot (R_F 0·82) which showed a dark purple fluorescence under ultra-violet light, and produced an intense red spot after spraying with a 0·5% solution of phloroglucinol in 50% aqueous hydrochloric acid. At R_F values between 0·00 and 0·50, several brightly fluorescent blue and yellow spots were visible under ultra-violet light. With (ii) the lignin and the brightly fluorescent materials moved with the solvent front, whereas the phlobaphenes streaked slightly from the starting line (brown colour in visible light, dark purple fluorescence in ultra-violet light).

After many experiments with different adsorbents and solvent systems, the following procedure was finally adopted for the purification of the lignin on a preparative scale : A sample of 5 g. of crude lignin dissolved in acetone containing 2–5% of water was adsorbed on 200 g. of Hyflo Super Cel which was spread out and dried at 50°. The adsorbent was packed in a column and eluted with anhydrous acetone until a negative phloroglucinol–hydrochloric acid lignin test was obtained. The bulk of the dark-coloured phlobaphene remained in the column and could be recovered with 50% aqueous acetone. The lignin solution was concentrated and again adsorbed on fresh Hyflo Super Cel. The purification was repeated until no further improvement in the colour of the lignin was observed and no further increase in its methoxyl content occurred. The lignin still contained some brightly fluorescent material which was subsequently removed by chromatography on cellulose powder (Hyflo Super Cel was unsuitable) with 70% aqueous methanol. A ratio of 1 part of lignin to 1000 parts of cellulose was satisfactory. The development of the chromatogram could be followed under ultra-violet light ; the lignin moved downwards as a purple fluorescent band which gradually separated from a brightly fluorescent yellow band. The lignin eluate was evaporated to dryness under reduced pressure and tested for its homogeneity on paper chromatograms. When necessary, the cellulose powder chromatogram was repeated.

The unchromatographed lignins were reddish brown to dark brown and dissolved to form dark-coloured solutions in organic solvents, such as alcohols, acetone and methyl ethyl ketone, when small amounts of water were present. The purified lignins, on the other hand, were pure white in colour when precipitated and light yellow when dried as foams ; they dissolved easily in anhydrous alcohols, acetone and methyl ethyl ketone to give yellow solutions. The crude heartwood lignin was the more difficult to obtain absolutely pure (see below) and was slightly more coloured than the sapwood lignin. The phlobaphenes, when once isolated and dried, were almost insoluble in water and organic solvents.

Both purified heartwood and sapwood lignins behaved identically on paper chromatograms and were homogeneous, moving as well defined spots, showing a purple fluorescence under ultra-violet light and an intense red colour with phloroglucinol–hydrochloric acid ; average R_F values were : 75% methanol (0·87), 50% methanol (0·82), 50% ethanol (1·00), 25% ethanol (0·83), anhydrous acetone (1·00), 20% aqueous acetone (0·80), 20% aqueous methyl ethyl ketone (0·80), 10% aqueous methyl ethyl ketone (0·70), distilled water (0·00).

The heartwood lignin gave C, 60·6 ; H, 6·1 ; OMe, 20·5 ; molecular weight, 900 ; and the sapwood lignin C, 61·1 ; H, 6·2 ; OMe, 21·0 ; molecular weight 830, 760, 780.

Molecular weights were determined in a modification of the micro-Menzies–Wright ebullioscopic apparatus of Ketchum,[14] with absolute alcohol as solvent.

The identity of the heartwood and sapwood lignins was further supported by their identical ultra-violet absorption spectra (in 96% alcohol) with a band at 280 mμ ($E^{1\%}_{1\,cm.}$, 130), a broad flat band at 320–340 mμ ($E^{1\%}_{1\,cm.}$, 70) and minima at 260 mμ ($E^{1\%}_{1\,cm.}$, 92) and 300 mμ ($E^{1\%}_{1\,cm.}$, 65).

The two infra-red absorption spectra were determined with a Perkin–Elmer model-12C spectrometer. Films were prepared on rock-salt plates by evaporation of acetone and of alcohol solutions. The spectra shown (Fig. 1) are the result of choosing wave-band regions as appropriate having regard to the presence of traces of solvents even after evacuation to 10^{-4} mm. for 5 hours. As can be seen, the spectra are identical and they are very similar to those of native lignins from other woods.[6, 15, 16]

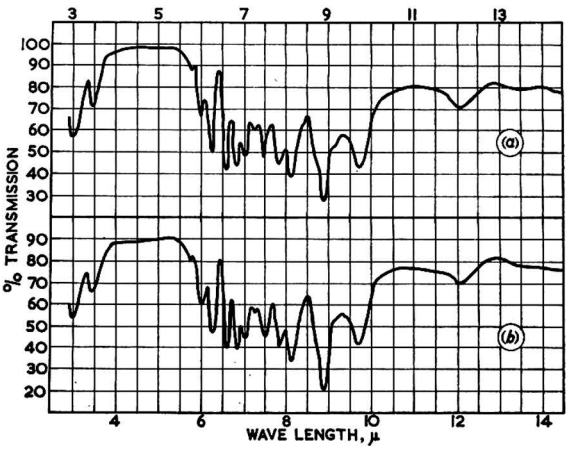

FIG. 1.—*Infra-red absorption spectra: (a) sapwood lignin, (b) heartwood lignin*

The following structural features can be tentatively assigned to certain of the bands by analogy with previous work: 2·97 μ (bonded hydroxyl groups), 3·48 μ, 6·85 μ and 7·04 μ (saturated aliphatic groups), 6·03 μ (aldehyde or keto group), 6·28 μ and 6·60 μ (phenyl ring with possible *para*-substitution), 7·83 μ and 8·12 μ (aromatic C–O–C, aromatic C–O and phenyl OH and CHO groups), 12·02 μ (phenyl ring substitution).

The alkaline nitrobenzene oxidation of the lignins was carried out by the method given by Stone & Blundell;[17] the reaction products were estimated spectroscopically. The unchromatographed sapwood lignin (9·7% OMe) yielded 9·3% of vanillin and 8·3% of syringaldehyde (ratio vanillin : syringaldehyde 1·0 : 0·9), whereas the chromatographed sapwood lignin (21·1% OMe) yielded 22·6% of vanillin and 20·6% of syringaldehyde (ratio vanillin : syringaldehyde 1·0 : 0·9). The isolated native lignin from wattle wood can therefore be designated as a 'guaiacyl–syringyl hardwood lignin'.[6]

A study of the lignin enzymically liberated from wattle wood by the action of brown rot fungi is now in progress.

Acknowledgments

The author is indebted to Dr. H. Shaw of the Wattle Research Institute, Pietermaritzburg, for samples of wattle timber, to Dr. D. A. Sutton for valuable suggestions and to the Council for Scientific and Industrial Research for permission to publish this work. Analyses were carried out by the Micro-analytical Laboratory (Mr. F. W. Schöning and Miss H. Theron) and the infra-red absorption spectra by Mr. C. A. J. Hoeve.

National Chemical Research Laboratory
South African Council for Scientific and Industrial Research
Pretoria
South Africa

Received 15 December, 1952

References

[1] Brauns, F. E., *J. Amer. chem. Soc.*, 1939, **61**, 2120
[2] Brauns, F. E., *J. org. Chem.*, 1945, **10**, 211
[3] Buchanan, M. A., Brauns, F. E. & Leaf, R. L., junr., *J. Amer. chem. Soc.*, 1949, **71**, 1297
[4] Brauns, F. E. & Seiler, H., *T.A.P.P.I.*, 1952, **35**, 67
[5] Schubert, W. J. & Nord, F. F., *J. Amer. chem. Soc.*, 1950, **72**, 3835
[6] Kudzin, S. F., DeBaun, R. M. & Nord, F. F., *J. Amer. chem. Soc.*, 1951, **73**, 4615
[7] Kudzin, S. F. & Nord, F. F., *J. Amer. chem. Soc.*, 1951, **73**, 4619
[8] Brauns, F. E., 'Nature of the Chemical Components of Wood', *T.A.P.P.I. Monogr. Ser.*, 1948, **6**, 108
[9] Buchanan, M. A., Lewis, H. F. & Kurth, E. F., *Industr. Engng Chem.* (*Industr.*), 1944, **36**, 907
[10] Graham, H. M. & Kurth, E. F., *Industr. Engng Chem.* (*Industr.*), 1949, **41**, 409
[11] DeBaun, R. M. & Nord, F. F., *J. Amer. chem. Soc.*, 1951, **73**, 1358
[12] Stephen, A. M., *J. Sci. Fd Agric.*, 1952, **3**, 37
[13] Hess, C. L., *T.A.P.P.I.*, 1952, **35**, 312
[14] Ketchum, D., *Industr. Engng Chem.* (*Anal.*), 1947, **19**, 504
[15] Jones, E. J., *T.A.P.P.I.*, 1949, **32**, 167
[16] De Stevens, G. & Nord, F. F., *J. Amer. chem. Soc.*, 1951, **73**, 4622
[17] Stone, J. E. & Blundell, M. J., *Analyt. Chem.*, 1951, **23**, 771

VITAMIN T: GROWTH AND PROTEIN UTILIZATION IN THE RAT

By A. E. BENDER and ELIZABETH J. TUNNAH

An attempt was made to evaluate the so-called vitamin-T complex Goetsch. A laboratory preparation had no effect on the growth rate of young rats, and a commercial preparation had no effect on the protein utilization of the adult rat.

Introduction

Numerous reports by Goetsch[1–3] claim that the so-called vitamin-T complex has a stimulating effect on growth and protein utilization. Originally reported[1] as a substance not indispensable for the maintenance of life but capable of causing accelerated development in insects, it was nevertheless labelled as a vitamin. It was later claimed[3] to accelerate the growth of mice, frogs and salamanders, to promote growth in backward children,[4, 5] to promote wound healing and to assist in rearing premature infants.[6]

Pototschnig[6] suggested that the mechanism of these effects was improved utilization of dietary proteins and increased synthesis of body proteins.

Many of the claims appear to be based on inadequate data. For example, Goetsch[7] deduced that vitamin T caused increased growth in mice from an experiment with only three mice per group, without offering evidence as to the significance of the experimental results. Other claims appear to lack precise experimental evidence. For these reasons experiments were designed: (1) to find the effect of the vitamin T on the rate of growth and food utilization of young rats fed on an adequate diet, and (2) to determine the effect of the material on the biological value of proteins.

First experiment

In this experiment, vitamin T was prepared as recommended by Goetsch[2] by concentrating the culture medium from a 10-day culture of *Torula utilis* grown on Czapek–Dox malt medium. Four litters totalling 30 rats of a black-and-white hooded strain were divided into four groups and fed on stock diet supplemented as follows:

Group I. 8 rats. Thiamine hydrochloride 5 mg. Riboflavin 10 mg.
Pyridoxine 5 mg. Calcium pantothenate 30 mg.
Nicotinic acid 10 mg. Inositol 50 mg.
Folic acid 2 mg. *p*-Aminobenzoic acid 15 mg.
Choline chloride 100 mg. Biotin 200 μg. (per kg. of diet).

Group II. 8 rats. Yeast culture-medium concentrate containing 1 mg. of dry matter per rat per day.

Group III. 8 rats. As in group II, using 100 mg. of dry matter per rat per day.

Group IV. 6 rats. No supplement.

The stock diet consisted of maize 54·5%, dried yeast 3%, grass meal 5%, linseed cake-meal 2%, meat-and-bone meal 5%, skim milk 10%, wheat germ 15%, arachis oil 5% and sodium chloride 0·5%, with the addition of cod-liver oil.

Results

Table I shows that the addition of the so-called vitamin-T complex to a diet already adequate in known factors had no effect on the growth rate of young rats, nor on the efficiency of utilization of food. The similarity of growth rates in Groups I and IV indicates that the stock diet contained a supply of vitamins adequate for rapid growth.

Table I

Growth rate [± Standard Error (S.E.)] and food consumption (four bucks and four does per group)

Diet	Initial wt., g.		Wt. after 25 days		Wt. gain, g.		Food consumption, g.		Wt. gain per g. of food consumed	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
I. Stock diet with vitamin supplement ..	46·5±1·6	46·7±1·2	144·3±6·8	124·0±5·7	97·8	77·3	327·8	315·0	0·30	0·25
II. Stock diet and yeast-culture medium. 1 mg. of dry matter/rat/day	48·5±1·9	46·5±1·3	146·3±2·1	123·3±3·2	97·8	76·8	333·0	308·0	0·29	0·25
III. Stock diet and yeast-culture medium. 100 mg. of dry matter/rat/day ..	47·2±1·8	47·5±1·0	127·8±12·9	125·8±4·2	80·6	78·3	295·8	303·0	0·27	0·26
IV. Stock diet ..	49·3±0·7	46·3±1·2	132·7±12·7	123·3±3·7	83·4	77·0	288·7	310·7	0·29	0·25

Second experiment

In this experiment, designed to investigate the claims of increased protein synthesis, a commercial preparation of vitamin T (Pharmazell 'T vitamin Goetsch') was used. The nutritive value of the proteins of wheat gluten and dogfish meal, with and without the addition of vitamin T, were compared by the repletion method of Frost & Sandy.[4] In this method rats are depleted of protein for seven days, when considerable weight loss occurs, and then fed on the test protein for five days at two levels, namely 0·12 g. and 0·24 g. of nitrogen per day. The gain in weight is a measure of protein resynthesis and serves to evaluate the protein. Three diets were fed at each of the two nitrogen levels: (1) unsupplemented, (2) supplemented with 0·01 ml. of the vitamin-T preparation per rat per day, and (3) supplemented with 0·25 ml. of vitamin-T preparation per rat per day.

Results

Table II shows that vitamin T had no effect on the rate of protein repletion of adult rats with either animal or vegetable protein. Protein repletion of the same rats with commercial casein is shown for the purposes of comparison.

Table II

Mean wt. regain (± S.E.) after five days' protein feeding, following seven days' protein depletion (four rats per group)

Diet	0·12 g. of N/rat/day			0·24 g. of N/rat/day		
	Unsupplemented	+ 0·01 ml. of vitamin T	+ 0·25 ml. of vitamin T	Unsupplemented	+ 0·01 ml. of vitamin T	+ 0·25 ml. of vitamin T
Dogfish meal ..	13·3 ± 0·7	15·8 ± 0·5	11·8 ± 1·9	26·3 ± 2·1	26·8 ± 1·3	26·0 ± 5·0
Wheat gluten ..	4·0 ± 1·2	2·0 ± 1·1	4·0 ± 1·3	5·5 ± 1·3	6·5 ± 2·2	6·0 ± 0·9
Casein (control) ..	13·8 ± 0·8			22·2 ± 1·5		

Conclusions

The claims of Goetsch[7] and Pototschnig,[6] that the vitamin-T complex accelerates growth, improves utilization of food, and improves the assimilation of proteins, are not substantiated.

If the effect of vitamin T was either to improve a poor diet or to increase the rate of protein synthesis in undernourished animals, then no result would be expected in the first experiment where the diet was already fully adequate, but some effect should have been observed in the second experiment where both a poor diet (0·12 g. of nitrogen per day) was fed and the rats were undernourished (deprived of protein for seven days).

These findings are in keeping with those of Schmidt[9] and Herzheim[10] who failed to confirm any effect on undernourished children, but are contrary to those communicated privately by the Pharmazell Co. where rats that failed to grow and eventually lost weight and died on a purified diet grew moderately when vitamin T was added. The quantity of vitamin T used in the Pharmazell experiment was 0·6 ml. per day (corresponding to 840 ml. per 70-kg. adult), compared with 0·25 ml. in the second experiment.

The Crookes Laboratories Ltd.
Park Royal
London, N.W.10

Received 30 January, 1953

References

[1] Goetsch, W., *Öst. zool. Z.*, 1946, **1**, No. 1/2
[2] Goetsch, W., *Öst. zool. Z.*, 1947, **1**, No. 3/4
[3] Goetsch, W., *Experientia*, 1947, **3**, 326
[4] Goetsch, W. & Nussbaumer, G., *Ärztl. Prax.*, 1950, **2**, 10
[5] Nussbaumer, G., *Med. Klinik*, 1949, **44**, 636
[6] Pototschnig, H., *Med. Klinik*, 1951, **46**, 987
[7] Goetsch, W., *Öst. zool. Z.*, 1948, **1**, 533
[8] Frost, D. V. & Sandy, H. S., *J. Nutr.*, 1949, **39**, 427
[9] Schmidt, G. W., *Arch. Kinderheilk.*, 1951, **142**, 113
[10] Herzheim, J., *Dtsch. med. Wschr.*, 1951, **76**, 1019

SOME TYPICAL ANALYSES OF RED SEAWEEDS

By ALAN G. ROSS

The seaweeds with which this paper is concerned all belong to the group Rhodophyceae, the best known of which are *Gelidium* spp. and other genera, from which agar is obtained, and *Gigartina* spp. and *Chondrus* spp., collectively known as Irish moss. This work was carried out under the auspices of the Institute of Seaweed Research with a view to obtaining a comparative survey of the general analytical characteristics of the group as a whole, although particular attention has been devoted to the distribution of the various sugars present (as shown in Table II).

Experimental

All analyses were carried out on dried and milled samples.

Moisture and volatile matter.—The sample was dried to constant weight at 105°. All other figures are given on the dry-weight basis.

Total sulphate.—The sulphate was determined by precipitation with barium chloride after hydrolysis with 30% hydrochloric acid for five hours.

Ash.—The sample was ignited at a dull red heat in a platinum crucible to constant weight. In some cases the ash figures may be slightly low owing to the presence of volatile inorganic salts.

Nitrogen.—Nitrogen was determined by the Kjeldahl method in a micro-apparatus.

Fats and pigments soluble in carbon tetrachloride.—This figure was obtained by Soxhlet extraction with boiling solvent for 18 hours, followed by removal of the solvent and weighing.

Alcohol-soluble matter.—The residue from the previous estimation was extracted with boiling ethanol for 18 hours and the soluble matter dried and weighed.

Uronic anhydride.—The uronic anhydride was estimated by distillation with 19% hydrochloric acid and absorption of the carbon dioxide produced in sodium hydroxide solution, according to the method of McCready, Svenson & Maclay.[1] A slight modification was used owing to the presence of carbonates in some of the samples. Each sample was allowed to stand in 19% hydrochloric acid overnight and the flask evacuated at room temperature for ten minutes to remove carbon dioxide from inorganic sources. Allowance (approximate) was also made on the final percentage for carbon dioxide produced from the sugars present.

Cellulose.—The method used was that for the determination of ' residual fibre ', consisting essentially in successive boilings with acid and alkali and weighing of the insoluble matter. Ash determinations were carried out on all cellulose residues to determine the percentage of silicious matter present, for which allowance was made, where necessary, along with the moisture in the final percentages. This silicious matter is probably derived from adherent sand and mud particles in the sample.

Total reducing sugars.—The samples were hydrolysed with N-sulphuric acid, neutralized with barium carbonate, filtered and washed and the filtrate and washings made up to appropriate dilutions; the reducing sugars present were then determined by means of alkaline

hypoiodite solution. The results were calculated to anhydro-hexose or -pentose, according to the predominant sugar.

Distribution of sugars.—Small samples were hydrolysed in sealed tubes for three hours with N-sulphuric acid, and after neutralization with barium carbonate the sugar contents were investigated by means of the paper chromatogram.

Results

Table I summarizes the results obtained, with the methods of analysis given above, for 26 different species of seaweeds. The samples are arranged in increasing order of sulphate content, as this figure appears to be one of the main differences between the various species. Table II shows the distribution of the sugars present and an approximate guide to their abundance. No quantitative chromatograms were carried out on the hydrolysates of the samples, but visual estimates of the intensities of the sugar spots on the chromatogram were made; the results were arranged in four categories, as in Table II, namely 'strong', 'medium', 'weak' and 'trace', representing approximately over 80%, 10–50%, 5–10% and less than 5% of the total sugars respectively.

Table I

Species	Source	Ash	Total sulphate	Nitrogen	Fats and pigments soluble in CCl_4	Alcohol-soluble	Uronic anhydride	Cellulose	Reducing sugars
		%	%	%	%	%	%	%	%
Corallina officinalis	North Berwick, 6/11/52	50·0	0·5	1·5	0·4	3·6	4·5	5·1	10·8
Rhodochorton floridulum	Plymouth, 1/11/52	53·3	0·5	2·6	1·6	3·1	1·6	7·6	16·7
Rhodymenia palmata	North Berwick	21·2	0·7	3·5	2·4	7·9	3·3	2·4	36·2 anhydro-pentose; 3·5 anhydro-hexose
Ahnfeltia spp. ..	Plymouth, 4/11/52	25·6	1·6	3·9	0·7	3·1	3·4	8·8	41·4
Gelidium pristoides	Strandfontein, S. Africa, 22/6/52	19·8	2·2	3·4	1·0	6·0	2·6	6·5	38·7
,, *cartilageam*	St. James, S. Africa, 26/1/52	22·6	2·4	3·3	0·8	4·8	2·4	9·0	33·9
,, *coulteri* ..	California, U.S.A., 8/52	23·1	2·7	2·8	1·6	5·2	4·1	8·5	42·8
Suhria vittata ..	Kommetje, S. Africa, 10/4/52	18·9	2·9	3·8	1·6	6·2	2·9	7·9	40·9
Gracilaria confervoides	Langebaan, S. Africa, 10/6/52	31·0	2·8	3·8	1·1	6·4	1·6	3·8	32·0
,, *foliifera*	Plymouth, 3/11/52	38·0	4·5	2·6	1·1	6·8	2·4	4·6	28·1
Pterocladia pyramidale	California, U.S.A., 8/52	28·6	4·3	2·9	2·7	4·7	4·6	8·2	31·6
Ptilota plumosa ..	Dunbar	34·4	4·4	4·1	1·2	7·0	1·2	4·7	30·0
Porphyra umbilicalis	North Berwick, 24/4/52	21·8	5·2	4·4	3·2	7·4	3·9	3·2	43·2
Ceramium rubrum	,, ,, 10/6/52	27·5	5·3	3·1	3·2	10·6	5·8	4·7	36·1
Dilsea edulis ..	Plymouth, 3/11/52	19·8	6·7	3·1	0·8	4·9	2·1	3·1	45·4
Endocladia muricata	California, U.S.A., 8/52	18·1	7·8	3·4	2·6	2·2	2·4	1·0	31·3
Polysiphonia fastigiata	North Berwick, 4/3/52	24·9	9·0	4·8	2·2	10·7	4·2	1·2	35·6
Membranoptera spp.	Dunbar	33·7	9·0	3·8	1·6	12·0	1·1	4·4	28·3
Phycodrys spp. ..	,,	41·4	10·6	3·6	1·5	12·7	0·8	3·4	23·2
Furcellaria fastigiata	Galway Bay, 15/12/51	22·7	10·8	2·8	1·3	5·0	2·6	5·7	37·2
Gigartina stellata ..	North Berwick, 30/1/52	21·2	14·2	3·6	1·6	4·8	3·8	2·3	38·9
,, *radula* ..	Kommetje, S. Africa, 6/6/52	28·3	18·2	2·7	0·5	5·6	1·5	1·8	35·2
,, *stiriata* ..	Kommetje, S. Africa, 10/4/52	28·5	17·3	2·9	1·0	7·6	1·3	1·1	36·3
,, *cristata* ..	California, U.S.A., 8/52	24·5	19·1	2·4	2·5	4·1	2·7	4·1	37·4
Chondrus crispus	Aberayron, 22/2/52	20·8	16·7	3·1	1·2	7·2	3·2	2·0	39·3
Rhodoglossum affine	California, U.S.A., 8/52	23·5	18·3	2·1	0·9	2·7	3·1	3·0	37·1

Conclusion

The results given in Table I reveal a number of facts and give a general idea of the composition of red seaweeds. The most noticeable point is the wide variation in the sulphate

content of the various species, the minimum figure being associated with a low galactose content or with none. The sulphate increases to a figure representing approximately one sulphate group per hexose unit. The ash content is fairly steady (20–30%) except in one or two exceptional cases such as *Corallina*, which contains a large amount of carbonate within the structure of the plant, and *Rhodochorton*, which grows in muddy areas of the shore. As a result of these high figures for ash the carbohydrate content as hexose is very low. The sugar and nitrogen contents are, like the ash, comparatively consistent, being mainly in the regions 30–40% and 2–4% respectively.

The particular sugars present (Table II) are also of interest since every species with any appreciable sulphate content contains galactose as the primary sugar. The genera *Rhodymenia* (xylose), *Rhodochorton* (xylose) and *Corallina* (glucose) are the exceptions. Also worth noting is the comparatively high glucose content of *Dilsea edulis*, which was used as the source of Floridean starch by Barry, Halsall, Hirst & Jones.[2]

The presence of fucose and xylose in almost every sample, and of mannose in some samples (though seldom in any quantity), indicates that these sugars are in some way connected with the growth of the plant.

Table II

Species	Galactose	Glucose	Xylose	Mannose	Fucose
Corallina officinalis	Medium	Strong	Trace	—	Trace
Rhodochorton floridulum	Weak	Weak	Strong	—	—
Rhodymenia palmata	,,	,,	,,	—	—
Ahnfeltia spp.	Strong	,,	Weak	Trace	Trace
Gelidium pristoides	,,	Trace	,,	,,	Weak
,, *cartilageam*	,,	,,	,,	,,	,,
,, *coulteri*	,,	Weak	,,	,,	Trace
Suhria vittata	,,	Trace	,,	—	Weak
Gracilaria conforvoides	,,	—	,,	—	Trace
,, *foliifera*	,,	Trace	Trace	—	Weak
Pterocladia pyramidale	,,	Weak	Weak	Trace	Trace
Ptilota plumosa	,,	,,	Trace	—	,,
Porphyra umbilicalis	,,	—	Weak	Weak	Weak
Ceramium rubrum	,,	Weak	,,	Trace	Trace
Dilsea edulis	,,	Strong	,,	,,	Weak
Endocladia muricata	,,	Trace	Trace	,,	,,
Polysiphonia fastigiata	,,	—	Weak	,,	Trace
Membranoptera spp.	,,	Weak	,,	,,	Weak
Phycodrys spp.	,,	,,	,,	,,	Trace
Furcellaria fastigiata	,,	,,	,,	—	Weak
Gigartina stellata	,,	Trace	,,	—	—
,, *radula*	,,	,,	,,	—	—
,, *stiriata*	,,	,,	,,	—	—
,, *cristata*	,,	Weak	,,	Trace	Trace
Chondrus crispus	,,	Trace	,,	—	—
Rhodoglossum affine	,,	Weak	,,	Trace	Trace

The main differences between these seaweeds and the brown seaweeds (Phaeophyceae) lie in the absence of (*a*) glucose in the form of laminarin and (*b*) alginic acid; these substances are replaced by galactan sulphuric esters containing more or less sulphate.

Acknowledgments

The author wishes to thank the following for the provision of samples for analysis: The Institute of Seaweed Research; Dr. Mary Parke, Marine Biological Station, Plymouth; Dr. S. P. Ligthelm, University of Cape Town, South Africa; Dr. P. O'Colla, University College, Galway; Professor Lily Newton, University College, Aberystwyth and Dr. E. Yale Dawson, University of Southern California, U.S.A.

The work described in this paper forms part of the programme of research and development on seaweed undertaken by the Institute of Seaweed Research, to whom the author is indebted for permission to publish.

Chemistry Department
University of Edinburgh

Received 9 January, 1953

References

[1] McCready, R. M., Svenson, H. A. & Maclay, W. D., *Industr. Engng Chem.* (*Anal.*), 1946, **18**, 290

[2] Barry, V. C., Halsall, T. G., Hirst, E. L. & Jones, J. K. N., *J. chem. Soc.*, 1949, p. 1468

SULPHUR IN SOILS. I.—Determination of Readily Soluble Sulphates in Soil

By R. C. LITTLE

A review is given of techniques proposed for the determination of sulphates in soils. Classical methods for sulphate determination are not, in general, suitable for determining readily soluble sulphates in soils. Sulphate in a 0·001N-hydrochloric acid extract of soil (40 g. of soil in 100 ml. of acid) can be directly titrated with 0·02N-barium chloride by using sodium rhodizonate as an internal indicator. Bromocresol purple used to screen the rhodizonate indicator increases the sensitivity and the ease of detection of the end-point. The method is simple, rapid and free from serious interference. Results were within the limits of $\pm$ 0·5 mg. of SO_4 per 100 g. of soil for soils having a sulphate content of about 10 mg. of SO_4 per 100 g. of soil; from soils of higher sulphate content, errors amounting to less than 5% can be expected. The method is applicable to soils of a wide range of organic matter and sulphate contents.

Introduction

Sulphur exists in several modes of combination in soils. In ordinary agricultural and forest soils the total percentage of sulphur is low—about 0·2% of the dry weight of the soil. This is distributed among a mixture of organic and inorganic colloids and other matter, usually including a high proportion of silicious minerals. A study of soil sulphur therefore demands the application of several analytical techniques, each appropriate to the determination of combined sulphur in fractions of about 0·1% occurring in mixtures which not only are highly heterogeneous but which vary greatly in composition. Even for the determination of total sulphur in soil, the preparatory techniques normally applied to other materials (solution in acid, bomb oxidation and alkali fusion) are either not applicable or introduce serious, if not crippling, complications; and the difficulties of determining the sulphur fractions in soils are increased by the scarcity of colorimetric or other reactions suitable for estimating combined sulphur in low concentration.

The present work was undertaken as part of a programme which aimed at devising analytical methods capable of estimating the chief fractions of sulphur in soil and of studying the distribution of those fractions in the field. It included trials of some of the published methods devised for the determination of low concentrations of soluble sulphate.

Review

Probably few gravimetric reactions have been more thoroughly studied than that based on the low solubility of barium sulphate. However, this reaction requires that the solution shall be free from all colloidal matter if the results are to be accurate; nor is the determination very reliable when the dissolved sulphate is in low concentration. Evans & Rost[1] and Wiklander & Hallgren[2] have used a barium sulphate gravimetric method for determining the sulphate content of normal soils, but only after concentrating water extracts. For saline soils (in which the soluble-sulphate content may be high—approaching that of sea-water) concentration is not necessary, and the colloidal content of water extracts of saline soils is relatively low; but such soils are not of the first agricultural importance.

Gedroiz[3] pointed out the errors induced by organic colloids in gravimetric determination of soil sulphate when organic matter was not completely destroyed by heating the soil with acid. He gave no method for the determination of water-soluble sulphate but restricted himself to gravimetric determination of sulphate after treatment of soil with boiling hydrochloric acid. This technique avoids trouble from inorganic colloids, which are often troublesome in water extracts.

Several diverse methods have been suggested for the turbidimetric determination of sulphates in dilute solution. Most (e.g.[4, 5]) are based on the comparison of the turbidity of the unknown solution with that of a known solution. At least one paper describes a method in which the time required for a solution to become turbid after the precipitating reagent has been added is measured. Other workers have measured the depth of the turbid liquid required to obscure a light (e.g.[6]). The turbidimetric method has been applied to many analytical problems and has proved satisfactory in some of them. There are, apparently, many disadvantages in applying it to soils; the chief one is probably the difficulty of maintaining uniform precipitating conditions. Again, as with the gravimetric method, extracts free from all colloidal matter are essential.

The turbidimetric technique has been employed as a 'quick' method in diagnostic work for the determination of sulphates in soil extracts, but in most of its forms it cannot be considered satisfactory for the determination of sulphate in water extracts of soils.

Chesnin & Yein[5] used barium chloride of controlled crystal-size in an attempt to maintain uniform precipitating conditions when determining the sulphate content of soil extracts (Morgan's sodium acetate–acetic acid extracting reagent). They claim that their method gives consistent results over the range 0–40 p.p.m. of sulphur. It is possible that this variation in the turbidimetric technique is of value in the determination of sulphates in soil extracts that are free from all colloidal matter, but it was ruled out in the present work on account of the difficulty in obtaining clear and colourless water extracts of soils.

By adding a solution of benzidine hydrochloride to a solution containing sulphate ions, slightly soluble benzidine sulphate can be precipitated. This reaction has frequently been used for the determination of sulphates, and the possibility of applying it to the present work was specially investigated. The many references to the method in the literature indicate that it has been the subject of extensive investigation, mostly in the analysis of materials other than soils; concentration of impurities, concentration of reagents, pH of solution and the method of separation and washing of the precipitate have all been examined. Workers appear to disagree about some points of procedure, and especially about the best method of washing the precipitate. Various methods of estimating the quantity of precipitate have been used, e.g. titration of the sulphate with alkali,[7–9] titration of the benzidine with permanganate,[10, 11] and diazotizing and coupling the benzidine, followed by a colorimetric estimation.[12–15]

Christie & Martin[10] and Hibbard[11] have applied the benzidine method to the determination of sulphates in soil extracts. Both these sets of workers preferred a potassium permanganate titration for the estimation of the benzidine sulphate precipitate.

Other published methods for the determination of sulphate in soils (not tested in the present work) include that used by Cantino[16] and by Hirst & Greaves,[17] who precipitated the sulphate with an acid solution of barium chromate, the excess of barium chromate being removed by making the solution alkaline, and the residual chromate estimated by titration with thiosulphate after the addition of potassium iodide.

Puri & Ashgar[18] used a method based on the reaction of an alkali-metal sulphate with barium carbonate, the determination being completed by titrating the resulting alkali-metal carbonate to the neutral point of thymolphthalein. They found their method useful for the determination of sulphate in saline soils.

More recently[19, 20] methods have been used which involve the reduction of sulphate to hydrogen sulphide, which in turn can be estimated either colorimetrically or volumetrically. This method appears to be sensitive but rather time-consuming.

Indicator techniques, based on use of an internal indicator such as rhodizonic acid or tetrahydroxyquinone, have been the object of special study in this investigation, and are therefore reviewed separately (see Experimental, section C).

Experimental

The work described in this paper relates only to the determination of sulphate present as such and removed by essentially aqueous extraction of air-dried soil. The extractant added to the soil was either distilled water or a very dilute solution of hydrochloric acid.

The investigation was devoted to finding a method of determination of soluble sulphate whose accuracy should not be materially influenced by substances present in such extracts.

(*A*) *Barium precipitation*

Precipitation of the sulphate as barium sulphate was first studied, and it was found that reasonably reproducible results could be obtained from soils containing more than 100 mg. of SO_4 per 100 g. of soil, with an extraction ratio of 40 g. of soil to 100 ml. of water. The filtered extract had to be heated on the water bath and refiltered—not to concentrate the extract, but to remove colloidal matter. With soils of lower sulphate content (about 10 mg. of SO_4 per 100 g. of soil) it was necessary to use larger quantities of soil and extractant; the filtered extract had to be concentrated on the water bath to give about 200 ml. of solution containing at least 0·25 mg. of SO_4 per ml. If the colloidal matter was not removed the precipitate was invariably coloured, and higher results (up to 20% higher) were obtained.

The barium sulphate gravimetric method was tedious to perform, and it was thought that its accuracy under these conditions was only relative. The method appears to be most suited to saline soils and ordinary field soils, i.e. those not yielding much organic colloid to an extractant. Peaty and other soils, very rich in organic matter, might yield anomalous results if examined by the simple technique described. This point was not specially tested; but it may be mentioned that the obvious methods of completely destroying colloidal organic matter

by oxidation are excluded, since they would include sulphur not originally present as soluble sulphate.

(B) *Benzidine precipitation*

Several forms of the benzidine-precipitation method were tried. The final estimation of the amount of benzidine sulphate precipitate presented no difficulty; most techniques were satisfactory—colorimetric estimation as described by Cuthbertson & Tompsett[12] being especially useful for small quantities of precipitate.

No variation of technique in benzidine precipitation was found to be very satisfactory. Even when pure sulphate solutions were used, the resulting precipitate tended to float in the vessel during separation and washing. Washing proved difficult. If acetone was used as the wash-liquid low results were obtained, owing to the slight solubility of benzidine sulphate in acetone. The use of water saturated with benzidine sulphate gave high and inconsistent results, owing presumably to varying amounts of the wash-liquid being held by the precipitate. Christie & Martin[10] used water, but they stressed that if less than 15 ml. were used high results were obtained, and if more than 20 ml. were used the slight solubility of benzidine sulphate led to low results. When this method of washing was tried, better but still rather inconsistent results were obtained; the improvement may have been due to accidental compensation of errors.

The best wash-liquid tried was a mixture of four parts by volume of acetone with one part of glacial acetic acid, which, it is believed, has not been previously recommended. Use of this led to consistent results with solutions of pure sulphate of low concentration.

All these preliminary trials with benzidine were made on pure solutions of potassium sulphate. The effect of adding other ions likely to be found in soil extracts, e.g. calcium, magnesium, potassium, sodium, aluminium, iron and phosphate, was next investigated.

Phosphate (as potassium dihydrogen phosphate) produced high results for sulphate when determined by precipitation as benzidine sulphate in acid solution (about pH 3·0), centrifuging, washing the precipitate with the acetic acid–acetone mixture and developing a red colour with thymol.[12] Other ions mentioned tended to lower the results when added to give concentrations of the same order as those expected in soil extracts; 10 p.p.m. of calcium depressed the results by about 10%. When an investigation was made of the combinations of the various ions, at much lower individual concentrations than those which had previously been found to interfere, precipitation was found to be slow and low results were obtained. It was concluded that the precipitation methods reviewed above are unreliable for the determination of sulphates in complex mixtures such as soil extracts.

(C) *Indicator techniques*

Use of internal indicators such as rhodizonic acid and tetrahydroxyquinone in the direct titration of sulphate with barium chloride showed promise, and a study of these methods was therefore made.

(a) *Review*

In 1929 Strebinger & Zombory[21] used rhodizonic acid as an internal indicator in the titration of sulphate. The method employed a back-titration. An excess of standard barium chloride was added to the sample, followed by a small amount of disodium rhodizonate. The excess of barium was then titrated with a standard sulphate solution. The presence of excess of barium caused precipitation of the bright red barium rhodizonate, and, on back-titration with sodium sulphate, all the barium was precipitated as barium sulphate and the yellow disodium rhodizonate was formed. For solutions about 0·2N with respect to sulphate, Schroeder[22] found this method satisfactory, though not as accurate as claimed by Strebinger & Zombory.[21] Schroeder also found that tetrahydroxyquinone was more satisfactory than disodium rhodizonate in dilute solutions and could be used in the direct titration of sulphates. He claimed that hydroxide, chloride, silicate, calcium, magnesium and aluminium ions did not interfere unless they were present in high concentrations. Iron was found to interfere and was removed. In order to speed up the precipitation of barium sulphate it was found desirable that a fairly high concentration of alcohol should be present. In the method that Schroeder finally adopted, 25 ml. of ethyl alcohol was added to a 25-ml. aliquot.

Sheen & Kahler[23] employed direct titration for the estimation of sulphates with tetrahydroxyquinone. They found that up to 60 p.p.m. of phosphate could be tolerated, and that ferrous and ferric ions had to be maintained at a concentration below 5 p.p.m. If iron was not removed the tetrahydroxyquinone was first coloured green and then destroyed.

Sheen, Kahler & Cline[24] proposed the use of the titration method for the determination of sulphur in rubber after the oxidation of the sulphur to sulphate by one of the recognized methods. On comparison with the gravimetric method they found that the average percentage difference was 0·01% on the percentage of sulphur, referred to the weight of rubber taken.

One of the chief difficulties of the rhodizonate method appears to be obtaining a reproducible end-point. Hallet & Kuipers[25] claimed that reproducible end-points could be obtained if the titration vessel was illuminated with a 60-w 'daylight' bulb. If the operator had his eye on a level with the solution he could see the colour-change better. The light was then coming up through the solution, and by tilting the flask the colour-change became more obvious. They maintained that a colour standard to which the end-point can be matched is essential for accurate work.

Ogg, Willits & Cooper[26] determined the end-points by using an apparatus in which the titration vessel and a colour filter are mounted side by side on an opal glass plate. The illumination, preferably fluorescent, was from below, with no overhead lighting. They claimed that results were reproducible to within 0·02 ml. of the mean titration value, the average deviation being 0·003 mg. on 1 mg.

de Sallas & Valle[27] used sodium rhodizonate as an internal indicator in the determination of sulphate in natural waters and claimed high accuracy in 57 samples containing from 7 to 238 mg. of sulphate per l. Siegfriedt, Wiberley & Moore[28] used tetrahydroxyquinone in the titration of sulphate with barium chloride, after combustion in a small oxygen bomb of organic compounds containing sulphur. They found that the use of a magnetic stirrer gave reproducible end-points more readily than did either shaking or stirring by hand. An orange filter was used to select identical end-points.

After trial and adaptation of several features derived from these methods, a new technique was evolved that has been found quite suitable for determining soluble sulphate in soils. Very dilute hydrochloric acid is used as an extractant; precision of end-point is obtained by the use of 'screened' rhodizonic acid.

(*b*) *Method*

(i) *Reagents.*—*1* Approximately 0·1N-hydrochloric acid. Dilute 9 ml. of concentrated hydrochloric acid (AnalaR) to 1 l. with distilled water.

2 Approximately 0·001N-hydrochloric acid. Dilute 10 ml. of approximately 0·1N-hydrochloric acid to 1 l. with distilled water.

3 Ethyl alcohol (96%).

4 Bromocresol purple indicator (B.D.H. Indicator solution).

5 Dilute ammonium hydroxide. Concentrated ammonia (sp. gr. 0·88) diluted with an equal volume of distilled water.

6 Rhodizonic acid. Saturated solution of disodium rhodizonate in distilled water which must be freshly prepared at least every three hours.

7 0·02N-barium chloride. Dissolve 2·4432 g. of $BaCl_2,2H_2O$ in 1 l. of distilled water.

8 0·02N-potassium sulphate. Dissolve 1·7426 g. of K_2SO_4 in 1 l. of distilled water.

(ii) *Procedure.*—40 g. of air-dried soil (passing a 2-mm. sieve) is weighed out into a shaking bottle of 150-ml. capacity, 100 ml. of 0·001N-hydrochloric acid is added, and the bottle is shaken on a 'to-and-fro' shaker for two hours. The suspension is then filtered (twice if necessary) through a Whatman No. 42 filter paper. The first filtrate may be slightly cloudy, but this does not interfere with the titration, although very cloudy extracts should be refiltered. A volume of 20 ml. of the filtrate is pipetted into a 100-ml. beaker and made up to 25 ml. with distilled water. Ethyl alcohol (25 ml.) and 3 drops of bromocresol purple are added, followed by dilute ammonium hydroxide until the indicator just turns to blue. One drop of sodium rhodizonate indicator is then added, and the titration carried out. The barium chloride is run in (about 0·1 ml. at one time), the solution being well stirred between each addition to ensure that the patches of barium rhodizonate formed as the barium chloride enters the solution are well dispersed. As the end-point is approached, the barium chloride is added drop by drop. The end-point is taken when the red colour spreads throughout the whole solution.

In the majority of ordinary agricultural soils the titre is low, often below 0·5 ml. For such soils, it is advisable to add 1·00 ml. of the standard potassium sulphate solution to the

20 ml. of filtrate before making up to 25 ml. with distilled water. Slightly better end-points are obtained if the addition of the rhodizonate is delayed until the amount of barium chloride run in is about equivalent to that required by the 1 ml. of standard sulphate solution added.

The sulphate content of the soils (as fine earth passing a 2-mm. sieve) is given by the expression :

$$\text{mg. of } SO_4/100 \text{ g. of soil} = (T - B) \times 0{\cdot}96 \times 100/40 \times 100/20$$

where T is the total titration of soil extract plus added potassium sulphate in ml. of standard barium chloride and B is the titration of standard potassium sulphate in ml. of standard barium chloride.

When it is unnecessary to add the 1 ml. of standard potassium sulphate, B can be taken as the blank.

A blank should always be estimated for each fresh batch of indicator, and in addition a titration of standard sulphate solution should be carried out as a check on the technique. Corrections for stoniness of mineral soils and for volume/weight ratios of soils rich in organic matter are made as usual if desired.

(iii) *Discussion.*—After a little experience, the end-points are detected with certainty and relative ease. (A laboratory assistant, with little previous experience of titrations in general, was trained to carry out the titration. In a short time her results were consistent and reliable.) All determinations are in duplicate ; that is, two titrations of aliquots of the same extract are made, and the mean is taken as the result of one analysis. It has been found that the duplicate titration mostly (95%) agreed to within one drop of 0·02N-barium chloride. Replicate analyses of the same soils have given results within the limits $\pm$ 0·5 mg. per 100 g. of soil for soils with a sulphate content of about 10 mg. per 100 g. of soil ; and in soils of higher sulphate content the error is less than 5%.

Since, for most agricultural soils, the titre is often less than 0·5 ml. of 0·02N-barium chloride, a premature end-point is obtained. In order to have a titre of reasonable magnitude, 1 ml. of standard potassium sulphate is always added when the sulphate content is expected to be small. For some horticultural and most greenhouse soils (which have soluble-sulphate contents as high as 200 mg. of SO_4/100 g. of soil) the addition of the standard sulphate solution is unnecessary and smaller aliquots of the extract may be sufficient to give a satisfactory titration. Using a semi-micro burette of 5- or 10-ml. capacity, with divisions of 0·01 ml., a titration of 1–2 ml. is considered desirable when factors such as the length of time required for the titration, ease of detecting the end-point and the required accuracy are taken into account.

Ogg *et al.*[26] stated that the titre should not be less than 3 ml. in any titration, standard or unknown, otherwise the apparent normality of the barium chloride varies. This has not been the author's experience. If the indicator blank (usually about 0·10 ml.) is taken into account, then, as can be seen from the results (Table I), the apparent normality remains constant. Without considering the indicator blank, a graph such as that shown in the paper by Ogg *et al.*[26] would be obtained. Since this work was done, an opinion concordant with its conclusions on this point has been expressed by Siegfriedt *et al.*[28]

Table I

The constancy of apparent normality of barium chloride

Sulphate present, mg.	0	1·0	2·0	3·0	4·0	5·0
Titre, ml. of $BaCl_2$ soln.	0·10	1·11	2·12	3·10	4·11	5·10

With an extraction ratio of 40 g. of soil to 100 ml. of reagent and with a 20-ml. aliquot, the range covered by the suggested limits of the titration is 1–25 mg. of SO_4/100 g. of soil (assuming that 0·10 ml. is the lowest reading possible with accuracy). By varying the size of the aliquot (and the extraction ratio in sulphate-rich soils) all concentrations of sulphate likely to be encountered in soils can be brought within range of the titration.

Either rhodizonic acid or tetrahydroxyquinone has been used as the indicator by various workers. After preliminary work, it was decided that rhodizonic acid is the more suitable of the two. It can be dissolved in water, so that the quantity of indicator added can be measured in drops. A solution of disodium rhodizonate will fade, but it was found that the reagent was sufficiently stable for three or four hours.

The actual colour change of the indicator is from the yellow of the disodium rhodizonate to the bright red of the barium rhodizonate. Unfortunately, as the concentration of the sulphate decreases, then the barium added has more opportunity of being precipitated as rhodizonate

instead of sulphate. Therefore, when the end-point is approached, a gradual change in colour takes place. However, if the titration is done slowly near the end-point, there is a quite sudden change from yellow to a reddish-violet.

Of the many indicators tested for screening the rhodizonate indicator, bromocresol purple was found to be most effective. Before the titration is started, the solution is made just alkaline to bromocresol purple. Addition of the rhodizonate indicator produces a green colour, and the colour change is from green to violet. This change is much easier to detect than that of the unscreened indicator.

Much has been written about the most suitable lighting conditions for this titration. In the present work, it was found that a 'white' light coming up through the solution from below was sufficient to enable the end-point to be detected satisfactorily. Alternatively, if the solution was held up to a window, out of direct sunlight, the detection of the end-point was made easier.

Water extracts of soils tend to give very cloudy filtrates unless special filtration methods are adopted. This cloudiness, if at all dense, tends to reduce the sensitivity of the end-point. Much clearer filtrates were obtained when 0·001N-hydrochloric acid was used as the extracting reagent. Table II shows the results of a comparison of the two extractants. On the basis of these results, 0·001N-hydrochloric acid was adopted as extracting reagent.

The effect of varying the length of the time of shaking was studied. Two soils were taken and a number of equal weights of each soil were weighed out into shaking bottles, and 100 ml. of 0·001N-hydrochloric acid was added to each. The bottles were then shaken for varying periods, the suspensions filtered immediately on removal from the shaker and sulphate was determined in the extracts. It was found that most of the sulphate is brought into solution after only five minutes' shaking, two hours being sufficient to give complete extraction.

Interference from other ions has received considerable attention. Some workers have maintained that iron cannot be tolerated in concentrations greater than 5 p.p.m. For example, Sheen & Kahler[23] put the limits of tolerance of iron, aluminium and phosphate at approximately 5, 6, and 60 p.p.m. respectively. These figures appear to hold good only when tetrahydroxyquinone is used as internal indicator. de Sallas & Valle,[27] who used rhodizonate, described the effect of iron and aluminium as negligible. The effect of iron and aluminium was studied, in view of these differences of opinion, and because both those elements may be present in soil extracts, though perhaps in small quantities only.

Table II

0·001N-Hydrochloric acid and water as extracting reagents (specimen results)

Soil sample No.*	Sulphate in mg. of SO_4/100 g. of soil extracted by 0·001N-HCl	Water
L 264	7·7	8·5
L 265	7·5	8·0
L 266	5·5	5·5
L 268	6·9	6·7
L 270	5·4	5·9
L 271	5·2	5·5
L 316	4·3	4·1
L 323	6·3	6·1
L 325	7·8	7·7
90048	253·1	248·0
90049	142·0	146·3
90050	321·9	315·6
90051	101·3	103·2
Mean of 9 L samples given	6·3 ± 0·3	6·4 ± 0·3
Mean of 32 L samples compared†	5·5 ± 0·3	5·3 ± 0·3
Mean of 4 greenhouse soils given†	204·6 ± 2·5	203·3 ± 2·5

* Samples prefixed by L were ordinary field soils; the others were greenhouse soils

† Coefficient of correlation for 36 samples compared = 0·9998

When a sulphate solution is titrated with barium chloride in presence of excess of aluminium, and rhodizonate screened with bromocresol purple is used as indicator, the titre is reduced. It was found that up to 50 p.p.m. of soluble aluminium (as Al^{3+}) in the extract produced no interference, and that the percentage error was still less than 5% in the presence of 100 p.p.m. of aluminium. Ferric iron, however, tended to increase the titre. It was found that up to 700 p.p.m. of soluble iron (as Fe^{3+}) had no effect on the titration, and that 1200 p.p.m. resulted in a positive error of 5%.

The concentrations of iron and aluminium were determined on a number of 0·001N-hydrochloric acid extracts of diverse soils. The concentration of iron was found to be very low, mostly below 10 p.p.m., and that of aluminium was never found to exceed 30 p.p.m. of the extract.

Table III shows the effect of various concentrations of phosphate on the titration, with pure solutions of potassium sulphate as the source of sulphate. The highest concentration of phosphate (as P_2O_5) which is without effect on the titration is 3 p.p.m. Determination of phosphate in 0·001N-hydrochloric acid extracts of soils revealed that only a small fraction of the phosphate in soil was dissolved by this weak-acid extractant. Only in soils having exceptionally high soluble phosphate contents did 0·001N-hydrochloric acid give extracts with a phosphate content greater than 5 p.p.m. As a matter of experience, soils containing large amounts of soluble phosphate also have a high sulphate content; this makes it possible to take much smaller aliquots for the titration, thus maintaining confidence in the accuracy of the titration, and reducing the content of phosphate in the titration vessel below the level of interference.

Table III

Effect of phosphate on results from pure solution

Sulphate (mg. of SO_4 added)	Phosphate content (p.p.m. of P_2O_5 added)	Sulphate (mg. of SO_4 found)
0·91	0	0·91
0·91	0·5	0·91
0·91	2·5	0·91
0·91	3·0	0·91
0·91	3·5	0·92
0·91	4·0	0·92
0·91	4·5	0·94
0·91	5·0	0·96
0·91	25·0	1·21
0·91	50·0	1·71

Testing against a conventional procedure has been considered. It was thought that to compare the proposed technique against, say, the classical barium sulphate gravimetric procedure would not necessarily provide a real test, on account of the limitations of the gravimetric procedure as applied to soils. If the gravimetric procedure were generally applicable to soils, the present work would not be necessary. If a discrepancy arose between the results of the proposed method and gravimetric procedure, it would be difficult to say where the reason for the discrepancy is to be sought: unless, say, *ad hoc* research were undertaken to investigate the behaviour of barium sulphate precipitation under conditions of microquantities in the presence of a variety of interfering substances, with and without presence of organic and inorganic colloids or their products of oxidation and/or solution in strong reagents.

Some tests were, however, made on pure solutions of potassium sulphate, calcium sulphate, aluminium sulphate and ferric alum (ferric ammonium sulphate), in which the SO_4 radical was estimated in the presence and absence of soil extract by the proposed method and the gravimetric method. In order to reduce the effect of soil colloids as far as possible, and to obtain a soil extract containing a reasonable amount of sulphate, a greenhouse soil (labelled A: original-loss-on-ignition 15%) was chosen for this work. An extract of uniform composition was made by shaking 100 ml. of 0·001N-hydrochloric acid with each of several 40-g. lots of the soil, and bulking the filtered extracts. This uniform bulk was used for the test described in Table IV. The results in that Table show that the figures obtained by the two techniques agreed satisfactorily for this soil extract.

A comparison of the techniques was made with two other soils, one a normal agricultural soil (labelled B: original-loss-on-ignition 10%), the other a hill peat (labelled C: original-loss-on-ignition 75%). The agricultural soil gave an extract with 0·001N-hydrochloric acid which, even after being twice filtered through a Whatman No. 42 paper, obviously contained a certain amount of colloidal matter. This was again evident from the coloured precipitate in the gravimetric estimation. The peat, similarly extracted, gave a clear, but slightly coloured extract and the precipitate in the gravimetric estimation was coloured, showing contamination.

Varying degrees of contamination, by colloidal matter, of the 0·001N-hydrochloric acid extracts of soils, were observed. The extracts of soils A, B, and C were treated to remove colloid; this was done by adding 1 ml. of concentrated hydrochloric acid (10N) to 200 ml. of extract in a beaker and evaporating down to about 10 ml. on a sand bath. This concentrated extract was then filtered, the beaker thoroughly scrubbed, and the filter paper well

Table IV

Comparison of titrimetric and gravimetric techniques for various sulphate compounds and an extract of greenhouse soil, A, not treated to remove colloids

Solution	Titrimetric				Gravimetric			
	Number of ml. used		mg. of SO_4		Number of ml. used		mg. of SO_4	
	Solution	Extract	Found	Calc.	Solution	Extract	Found	Calc.
Soil extract	0	2	1·09		0	75	41·03	
Potassium sulphate ..	1*	0	0·94		10	0	47·25	
,, ,, ..	1	1	1·49	1·50	10	25	60·77	60·93
Calcium sulphate ..	1	0	0·81		50	0	40·29	
,, ,, ..	1	1	1·36	1·36	50	25	54·30	53·97
Ferric alum	1*	0	0·96		20	0	95·99	
,, ,, ...	1	1	1·51	1·50	20	25	110·70	109·67
Aluminium sulphate ..	1	0	1·27		25	0	31·69	
,, ,, ..	1	1	1·82	1·82	25	25	45·87	45·37

* Solution used for titration was that used for the gravimetric determination, but diluted 1 : 5

washed with distilled water. The volume of the filtrate after washing was about 150 ml.

After this procedure, the extract of soil A showed little colloid; soil B was heavily contaminated, mainly with inorganic material, and soil C was also heavily contaminated, but mostly with organic matter.

The sulphate was then determined gravimetrically as for the untreated extract of soil A. The results are given in Table V. It will be seen that the greenhouse soil (A) presented the

Table V

Comparison of titrimetric and gravimetric techniques on extracts of three soils

Label and type of soil used	Sulphate, mg. per 100 g. of soil		
	Untreated extracts		After treating extracts for removal of colloids (gravimetric)
	Titrimetric	Gravimetric	
A, Greenhouse	273·6	274·2	273·9
B, Normal agricultural	8·98	9·45	8·89
C, Hill peat	19·99	21·25	20·04

least problem (as was expected). The organic matter in the extracts burnt off during ignition of the barium sulphate precipitate, so that the increases shown for B and C in the third column must have been due to inorganic matter. The fourth column shows that accurate results for sulphate extractable by 0·001N-hydrochloric acid can be obtained by the gravimetric technique provided care is taken to remove the inorganic colloid before addition of barium chloride. Equally accurate results could, however, be obtained with less trouble by applying the proposed titrimetric technique directly to the filtered extract. This was true even of soil B, which is the type that is most common and also lowest in soluble sulphate.

For the work presented in Table V, to obtain a weighable precipitate of barium sulphate from peat and agricultural soil it was necessary to use 200 ml. of extract and also to add to this a known amount of potassium sulphate. If the addition of pure sulphate were to be avoided it would be necessary to take for a single determination 500 ml. of extract and to concentrate that to approximately 200 ml. As much as 300–360 g. of soil would need to be extracted to obtain that volume (in view of the fact that the volume of filtrate recovered is 60–70 ml. when 40 g. of soil is extracted with 100 ml. of extractant). Such quantities would make the method cumbersome.

The underlying uncertainty is possibly analogous to that prevailing in the work of Brown & Hayes[29] on determination of alginates on rayon, although it arises from a different cause. Brown & Hayes wrote: 'Whilst it is impossible to verify that extraction is quantitative by using a known amount of alginate . . .' In the present work it is intrinsically impracticable to verify the accuracy of the present method for all soils by comparison with an accepted procedure.

The present titrimetric method is offered not simply as a rapid method for determination of sulphates. It is not intended primarily to displace the gravimetric or other well-known methods of sulphate determination where they are applicable; though for some kinds of work its ease and rapidity may commend it for use instead of them.

The titrimetric method described can be used without special difficulty for soils of a wide range of contents of organic matter and soluble sulphate. It has been found as accurate for peaty soils as for ordinary 'mineral' or field soils. The accuracy of the method does not appear to be affected by the treatment or origin of the organic matter, whether that is *in situ* as in hill peats, or has been artificially introduced in the form of added peat or stable manure (e.g. in greenhouse soils) or otherwise. No similarly comprehensive method is known to exist.

It is pertinent that, except for the work of Evans & Rost,[1] virtually all recently published estimations of sulphate in soils have been done either upon recent marine or littoral clays of Dutch and Baltic situations, or upon arid saline soils and other abnormal soils such as the Finnish 'sulphate soils' (*sulfaattimaa*) of Kivinen[30] which contain iron sulphides prone to oxidation after disturbance (cf. [31]). These soils all contain a much higher proportion of sulphate than is normal in ordinary agricultural soil, so that an error in determination of sulphate by the gravimetric method would not be of much consequence. Little study of methods of determination of sulphate has accompanied most of the published analyses; the complete system of analysis provided by Smittenberg *et al.*[20] was presented without evidence of fitness or specimen results: it seems to have been intended for recently reclaimed marine clays rich in sulphate and metallic sulphides and poor in organic matter.

The present titrimetric method has been used in a preliminary survey of the occurrences of soluble sulphate in British hill- and lowland-soils of widely different origins and characters. A few illustrative results are given in the Appendix.

The readily soluble sulphate contents of lowland agricultural soils have been found to be strikingly independent of the manurial treatments known to have been given. For example, on the plots at Rothamsted and Woburn Experimental Stations, dressings of sulphate-containing fertilizers over a period of many years had little or no effect in raising the readily soluble sulphate contents of the soils. It will also be noted that the soluble-sulphate content of untreated hill-peat soils was of the same order as that found in soils of marshes subject to periodic tidal flooding. It is hoped to discuss distribution of sulphate more fully in a later communication.

Appendix

Readily soluble sulphate content of a selection of soils (0–8 *in. depth*)

Location of sample	Soil texture	Sulphate in mg. of SO_4/100 g. of dry fine soil
Agricultural: hill land		
Lephinmore Hill Farm, Argyllshire	Medium loam	9·6
	,, ,,	10·8
	,, ,,	7·7
	Peat	14·4
	,,	37·4
	,,	31·7
Agricultural: lowland		
Broadbalk field,* Rothamsted, Hertfordshire (paths)	Clay loam with flints	3·4
	,, ,, ,, ,,	3·6
	,, ,, ,, ,,	2·6
Permanent barley plots,* Woburn, Bedfordshire	Sand	5·0
	,,	4·8
	,,	7·4
	,,	7·0
Peel Hill Field, Auchincruive, Ayrshire	Medium loam	2·2
	,, ,,	5·5
	,, ,,	5·1
Agricultural: tidal marshland		
Solway shore, Dumfriesshire	Silt	6·1
	,,	12·0
	,,	30·2
	,,	35·7
Horticultural		
Market garden, Ayrshire	Medium loam	13·1
	,, ,,	6·3
Private garden, Dunbartonshire	Medium loam	5·5

Location of sample	Soil texture	Sulphate in mg. of SO_4/100 g. of dry fine soil
Greenhouse		
Market garden, Lanarkshire	Loam	253·1
	,,	321·9
Market garden, Ayrshire	,,	86·3
	,,	56·3

* Variously treated, including control (no manure) and heavy applications of sulphates

Acknowledgments

Some of the trials of benzidine sulphate precipitation were made at the Section for Soil Science, Hungarian State Chemical Institute, Budapest, while the author held a Hungarian Government Scholarship in 1948, under the auspices of the British Council.

The author wishes to thank Professor Hugh Nicol, Dr. C. L. Whittles and Mr. E. K. Schofield-Palmer for their interest and suggestions in this work; Sir William Ogg, Director of Rothamsted Experimental Station, and Dr. H. H. Mann, Hon. Local Director of Woburn Experimental Station, for allowing samples of soil to be obtained from the 'classical' fields under their control.

Soil Laboratory
The West of Scotland Agricultural College
Auchincruive, Ayr

Received 19 January, 1953

References

[1] Evans, C. A. & Rost, C. O., *Soil Sci.*, 1945, **59,** 125

[2] Wiklander, L. & Hallgren, G., *Kgl. LantbrHögsk. Ann.*, 1949, **16,** 811

[3] Gedroiz, K. K., 'Chemische Bodenanalyse', 1926, pp. 69 & 170 (Berlin: Borntraeger)

[4] Boutwell, P. W. & Toepfer, E. W., *Industr. Engng Chem. (Anal.)*, 1932, **4,** 117

[5] Chesnin, L. & Yein, C. H., *Soil Sci. Soc. Amer. Proc.*, 1951, **15,** 149

[6] Jackson, D. D., *J. Amer. chem. Soc.*, 1901, **23,** 799

[7] Cope, C. L., *Biochem. J.*, 1931, **25,** 1183

[8] Drummond, J. C., *Biochem. J.*, 1915, **9,** 492

[9] Power, M. H. & Wakefield, E. G., *J. biol. Chem.*, 1938, **123,** 665

[10] Christie, A. W. & Martin, J. C., *Soil Sci.*, 1917, **4,** 477

[11] Hibbard, P. L., *Soil Sci.*, 1919, **8,** 61

[12] Cuthbertson, D. P. & Tompsett, S. L., *Biochem. J.*, 1931, **25,** 1237

[13] Marenzi, A. D. & Banfi, R. F., *Biochem. J.*, 1939, **33,** 1879

[14] Raiziss, G. W. & Dubin, H., *J. biol. Chem.*, 1914, **18,** 297

[15] Letenoff, T. V. & Reinhold, J. G., *J. biol. Chem.*, 1936, **114,** 147

[16] Cantino, E. C., *Soil Sci.*, 1946, **61,** 361

[17] Hirst, C. T. & Greaves, J. E., *Soil Sci.*, 1922, **13,** 231

[18] Puri, A. N. & Ashgar, A. G., *Soil Sci.*, 1938, **45,** 41

[19] Rancke-Madsen, E., *Acta chem. scand.*, 1949, **3,** 773

[20] Smittenberg, J., Harmsen, G. W., Quispel, A. & Otzen, D., *Plant & Soil*, 1951, **3,** 353

[21] Strebinger, R. & Zombory, L., *Z. anal. Chem.*, 1929, **79,** 1

[22] Schroeder, W. C., *Industr. Engng Chem. (Anal.)*, 1933, **5,** 403

[23] Sheen, R. T. & Kahler, H. L., *Industr. Engng Chem. (Anal.)*, 1936, **8,** 127

[24] Sheen, R. T., Kahler, H. L. & Cline, D. C., *Industr. Engng Chem. (Anal.)*, 1937, **9,** 69

[25] Hallet, L. T. & Kuipers, J. W., *Industr. Engng Chem. (Anal.)*, 1940, **12,** 360

[26] Ogg, C. L., Willits, C. O. & Cooper, F. J., *Analyt. Chem.*, 1948, **20,** 83

[27] de Sallas, S. M. & Valle, A. M., *Rev. Obr. sanit. Nac., B. Aires*, 1948, **12,** 177

[28] Siegfriedt, R. K., Wiberley, J. S. & Moore, R. W., *Analyt. Chem.*, 1951, **23,** 1008

[29] Brown, E. G. & Hayes, T. J., *Analyst*, 1952, **77,** 452

[30] Kivinen, E., *Acta chem. fenn.*, 1950, [A] **3–4,** 85

[31] Black, J., quoted by Walker, J., *Trans. Highl. agric. Soc. Scot.*, 1803, **2,** 34–35

FLAVOUR ASSESSMENT OF SUGAR SOLUTIONS

By GWENETH M. CHAPPELL

An untrained tasting panel reported that sucrose was the most acceptable sugar, maltose the least acceptable, fructose the sweetest and lactose the least sweet.

With added flavouring oils a higher concentration proved acceptable and there was more uniformity of opinion. Flavouring agents differed in their appeal to members of the panel.

The addition of very small amounts of common salt to flavoured sugar solutions apparently increases the strength of the added flavour, the sweetness of the solution and, to some persons, its acceptability. Stronger flavours masked the taste of salt, weaker ones did not. More than half the panel found that higher concentrations of sugar enhanced the flavour of orange and lemon oil.

Introduction

A short study has been made of the comparative sweetening powers and acceptability of different sugars. A tasting panel of 20 members, comprising the Teaching Staff of the Household Arts Department of Queen Elizabeth College and students in training in this College, reported on the sugars. Smaller panels were used for three tests. The Bureau of Human Nutrition and Home Economics, Washington, considers that a small, well-selected, well-trained panel is more accurate than a large, untrained one. This is undoubtedly true when subjective opinions are to be compared with objective measurements. It is generally conceded, however, that non-professional tasters may be used in consumer polls. Since the object of this study was an evaluation of the acceptability of sugar solutions of different concentrations by the average consumer, a larger panel was considered not only justifiable but desirable.

As a result of observations made during experiments designed to test the reliability of tasting panels, great doubt is cast on the authenticity of organoleptic tests.

Reliability of tasting panels

When identical solutions were submitted to the panel on two different occasions, opinions varied from one day to the other. Sucrose solutions were used for this purpose, and the panel was requested to select the most acceptable concentration. Solutions with 0·05 ml. of lemon oil per 100 ml. and solutions without added flavouring were submitted to the panel (Table I).

Table I

Concn. of sucrose soln., %	No. of persons selecting any one soln. as the most acceptable			
	Solns. without flavouring		Solns. with lemon oil	
	First day	Second day	First day	Second day
15	0	0	1	1
20	8	4	4	4
25	6	6	4	4
30	1	6	5	3
35	3	4	2	3
40	0	0	1	0
45	0	0	0	0
50	0	0	0	1

It will be seen that some members of the panel were unable to select an acceptable solution in three series and that 15% and 40–50% concentrations were not popular.

When flavouring was added to sugar solutions, a wider range of sweetness proved acceptable, and the agreement between opinions on different days was closer than when unflavoured solutions were tasted. One person did not necessarily select the same solution on two different days. A second experiment designed to test the reliability of tasting panels involved the use of four identical 35% (w/v) solutions of sucrose, containing 0·05 ml. of oil of ginger per 100 ml. Each member of the panel claimed to have distinguished between the sweetness and flavour of at least two of the solutions and 13 attempted to differentiate between three or four.

Relative sweetening powers of sugars

Maltose, lactose, glucose and sucrose were used as 35% solutions, without added flavouring. A panel of 10 persons all chose sucrose as the sweetest and all but one found glucose

only ' faintly sweet '. Two reported maltose the least sweet, but the majority linked maltose and lactose together as not sweet. Seven members of the panel remarked critically on the flavour of maltose.

Solutions (25%) of maltose, lactose, glucose and sucrose were flavoured with 0·1 ml. of oil of clove per 100 ml. and a panel of 10 persons all voted sucrose the sweetest. Nine disliked the flavour of maltose, five considered the flavour of clove was most pronounced in glucose solutions and three said that lactose was the least sweet.

A panel of 20 persons tasted 25% solutions of maltose, lactose, glucose, sucrose and fructose on two different occasions and reported as follows (Table II).

Table II

Sugar soln., 25%	Test I			Test II		
	Sweetest	Least sweet	Most acceptable	Sweetest	Least sweet	Most acceptable
Maltose	0	1	1	0	1	2
Lactose	0	9	1	0	19	2
Glucose	0	4	1	0	0	1
Sucrose	8	0	12	4	0	8
Fructose	12	0	0	16	0	4

In the first test there were six complaints of the flavour of maltose, and 11 persons disliked the flavour in the second test. The uniformity of opinion on the part of the panel about lactose in Test II is noteworthy. Six persons did not report on lack of sweetness in Test I and it would be interesting to know whether they would have voted for lactose if compelled to decide. It is quite clear that the flavour of maltose is not acceptable to many persons, but it is surprising that at least seven members of the smaller panel reported adversely on the flavour of this sugar, although, when the larger panel was employed, there were proportionately fewer complaints.

In the following experiment, 20% fructose, 25% sucrose and 30% lactose solutions were used, without added flavouring, and submitted to a panel of 20 tasters. Earlier organoleptic reports having shown that the panel found these sugars to decrease in sweetness in that order, the concentration of these solutions was graded in the same order. Sucrose was selected as the sweetest of this series by 14 persons and fructose by only 3. This reversed the findings of the previous experiment and suggests that the difference of 5% in the respective concentrations compensated for differences in sweetening powers. Each member of the panel found lactose less sweet than the other two solutions.

A further comparison was made between unflavoured 25% sucrose and 35% lactose solutions. In this experiment the small panel agreed unanimously that sucrose was the sweeter, three members complaining that it was too sweet. The results of the last two experiments show that the panel found lactose further removed in sweetening power from glucose than is fructose.

The effect of added flavouring oils and other edible substances on the apparent sweetness and acceptability of sugar solutions

A series of tests were made with sugar solutions of varying concentrations, with or without added oils, salt, rennet or lemon juice, to determine the effect of these substances on the apparent sweetness and acceptability of these solutions and to determine the most popular degree of sweetening. Fructose was used in concentrations from 5 to 40% (increments of 5%), with 0·075 ml. of oil of orange (*oleum aurantii*) per 100 ml. A panel of 20 tasters reported as follows: 8 members selected the 15% solution as the most pleasant, 3 selected the 20%, 4 selected the 25%, 3 selected the 30%, 1 voted for the 5% and 1 for the 10%; the 35 and 45% solutions received no vote. All but four members of the panel recognized increasing sweetness in solutions ranging in concentration from 5 to 40%, although individual reports were received of 35% solutions being unsweet and 40% excessively sweet. Half the panel found concentrations of 25% and upwards were too sweet and 5 persons complained that 35 and 40% were too sweet. Three persons objected to the flavour of this series and three more described it as tasting of cough mixture. No one recognized the flavour of orange, and yet no one failed to identify correctly all the flavouring oils, including orange, used with sucrose solutions.

The remaining experiments were made on sucrose solutions. Solutions ranging from 10 to 50% in concentration (increments of 5%) were subdivided as follows: A, 0·05 ml. of oil of

lemon ; B, 0·05 ml. of oil of lemon and 0·5 g. of salt ; C, 0·2 ml. of oil of lemon ; D, 0·2 ml. of oil of lemon and 0·5 g. of salt per 100 ml. A panel of 20 members reported on these solutions (Table III).

Table III

Concn. of sucrose soln., %	No. of persons selecting any one soln. as the most acceptable			
	A	B	C	D
	0·05 ml. lemon oil	0·05 ml. lemon oil and 0·5 g. salt	0·2 ml. lemon oil	0·2 ml. lemon oil and 0·5 g. salt
15	1	0	1	0
20	4	6	4	1
25	4	3	4	2
30	5	6	3	2
35	2	1	3	6
40	1	1	0	2
45	0	0	0	0
50	0	0	1	0

Some reports did not specify which solution was the most acceptable. There was little difference between the opinions of the panel regarding solutions with 0·05 ml. or 0·2 ml. of oil of lemon. Concentrations of 20–30% sucrose were the most popular when only lemon oil was added or when 0·05 ml. lemon oil and salt were added. With 0·2 ml. of lemon oil and salt a sweeter solution was acceptable to more persons. In series A & B, 14 persons said that the flavour of lemon increased as sweetness increased, whereas two persons reported the reverse. In series C & D, only one person found that flavour and sweetness paralleled each other and no one reported to the contrary.

A few reports were received from the panel relating to each series of solutions (Table IV).

Table IV

	A	B	C	D
Sweetest	4	1	2	5
Strongest lemon flavour	1	2	0	8
Most pleasant	3	0	1	6
Salty taste	0	6	0	0

It is clear from these results that the stronger flavour of lemon in series C & D masked the taste of salt and that the salt enhanced the sweetness, flavour and acceptability of these solutions. The presence of salt in solutions containing only 0·05 ml. of lemon oil rendered them less acceptable to the panel and there were six reports of a salty taste, only two of which remarked adversely on this feature. Only one member of the panel considered the unsalted solutions to be most strongly flavoured and half the panel found one or other of the two salted series to have the strongest flavour of lemon.

Because so few persons found solutions containing 40% or more of sucrose to be palatable, the next experiment was made on solutions of 15 to 35% concentration (increments of 5%). Each concentration was subdivided as follows : A, no addition ; B, 0·05 ml. of oil of orange ; C, 0·25 ml. of oil of orange ; D, 0·2 g. of salt and 0·25 ml. of oil of orange per 100 ml. The panel of 20 reported on the most acceptable concentration in each series (Table V).

Table V

Concn. of sucrose soln., %	No. of persons selecting any one soln. as the most acceptable			
	A	B	C	D
	no addition	0·05 ml. orange oil	0·25 ml. orange oil	0·2 g. salt and 0·25 ml. orange oil
15	0	3	1	0
20	7	3	4	3
25	6	6	3	5
30	1	0	4	2
35	3	4	2	1

In the absence of flavouring agents, the panel preferred 20 and 25% concentrations of sucrose. With added flavouring, the 20% solution was less popular ; fewer members of the panel were able to select an acceptable solution and the scatter was wider.

As was found in earlier experiments, there was a tendency for higher concentrations of sucrose to prove acceptable when flavouring was used. Half the panel found the flavour of orange more pronounced as the concentration of sugar increased, while seven found the reverse. In the preceding experiment, with lemon oil, fewer persons found a decreased flavour as sugar solutions increased in concentration. Comparison of one series with another produced the results in Table VI.

Table VI

	A	B	C	D
Sweetest	2	2	1	2
Strongest flavour of orange	3	1	3	5
Weakest flavour of orange	4	0	0	2
Most acceptable	3	0	1	2

Remarks by six members of the panel indicated that series D had a different flavour from the others, although only one person identified it as salt and two conceded that it strengthened the flavour of orange. Series A contained no oil of orange, so that the opinions of the three persons who thought this series had the strongest flavour of orange are valueless. The addition of salt obviously enhanced the flavour of the oil.

The foregoing experiment was repeated but with different quantities of oil of orange added to each 100 ml. of solution, i.e. A, 0·1 ml. of oil of orange; B, 0·15 ml. of oil of orange; C, 0·35 ml. of oil of orange; D, 0·35 ml. of oil of orange and 0·2 g. of salt. The panel of 20 reported on the most acceptable concentration of sugar in each series (Table VII).

Table VII

Concn. of sucrose soln., %	No. of persons selecting any one soln. as the most acceptable			
	A 0·1 ml. orange oil	B 0·15 ml. orange oil	C 0·35 ml. orange oil	D 0·2 g. salt and 0·35 ml. orange oil
15	0	1	1	3
20	3	1	1	0
25	4	9	9	9
30	4	1	3	3
35	3	3	1	0

The popularity of 25% solutions of sucrose was most noticeable in this experiment, although the stronger orange flavouring in series C, and orange with salt in series D, increased the acceptability of 30% solutions. Comparison between the series gave the results in Table VIII.

Table VIII

	A	B	C	D
Most acceptable	4	5	1	1
Most unpleasant	0	0	2	4
Sweetest	1	2	1	4
Strongest flavour of orange	4	1	2	4
Weakest flavour of orange	0	3	0	0

Only one of the four persons who complained of the flavour of series D recognized salt. One thought the series 'unpleasant but quite palatable' and another, 'the sweetest of the series, but unpleasant'. Four members of the panel considered that solutions containing only 0·1 ml. of oil of orange had a stronger flavour than those containing 0·15–0·35 ml. Three persons complained of the taste of series C, identifying it as bitter, sour or unpleasant. In this concentration, orange oil has a slightly bitter taste, and these persons obviously found that the salt in series D counteracted this taste, because they all found the latter series more pleasant. Salt apparently enhanced the sweetness, flavour and acceptability of sucrose solutions in an earlier experiment. In the present experiment, sweetness and flavour were apparently increased by the presence of salt, but these solutions were not acceptable. This

may be attributable to the slight bitterness associated with oil of orange in the concentration used in series C and D.

In the two previous experiments, 25% concentrations of sucrose were the most popular. For this reason, 25% solutions of sucrose containing 0·05 ml. of vanilla essence per 100 ml. were subdivided as follows: A, 0·005 g. of salt; B, 1·125 ml. of rennet; C, 1·125 ml. of fresh lemon juice; D, 0·2 g. of salt per 100 ml.; E, no addition (Table IX). A panel of 20 reported on these solutions.

Table IX

	A 0·005 g. salt	B 1·125 ml. rennet	C 1·125 ml. lemon juice	D 0·2 g. salt	E no addition
Sweetest	6	6	1	2	2
Most acceptable	10	1	2	1	0
Most unpleasant	1	4	0	0	0
Least sweet	0	4	2	0	0

Sample B, containing rennet, was criticized for a salty flavour, but sample D, containing salt, was not. No one remarked upon or identified vanilla. It is noteworthy that no one found the solution of sucrose flavoured only with vanilla essence to be as pleasant as solutions containing additional substances, and that 0·005 g. of salt per 100 ml. of solution apparently increased the acceptability of this solution to a very marked degree.

In earlier experiments the panel found that 0·2 g. of salt per 100 ml. increased the acceptability of sucrose solutions flavoured with oil of lemon. It would be interesting to learn whether 0·005 g. of salt, instead of 0·2 g., would increase the popularity of these solutions as much as it did those flavoured with vanilla. Further, it might be found that a higher concentration of vanilla essence increased the palatability of solutions containing 0·2 g. of salt per 100 ml. It was shown that 0·2 ml. of oil of lemon and 0·2 g. of salt did not. The same might well be true of other flavouring agents.

Conclusions

This work is unfinished, but what has been completed enables some conclusions to be drawn. The opinion of a tasting panel on many sugar solutions was so divided that there is obviously a wide divergence of taste on the subject of sugars. On occasion, members of the panel identified flavours in solutions which did not contain them and claimed to have differentiated between identical solutions.

In so far as organoleptic tests are considered admissible, the present experiments have shown that sucrose is the most acceptable sugar and maltose the least acceptable. Fructose is the sweetest sugar, sucrose the next sweetest and lactose the least sweet. Fructose is, apparently, approximately 5% sweeter than sucrose, and lactose is more than 10% less sweet than sucrose. The most popular concentration of fructose is 15% and of sucrose is 20 or 25%, but with added flavouring oils a 25% solution proved the most popular.

The addition of flavouring oils to sugar solutions resulted in a higher concentration proving acceptable to the panel, although a wider range of sweetness received individual votes. More uniformity of opinion amongst members of the panel was usually obtained when flavoured solutions were tasted. The different flavouring agents employed obviously varied in their appeal to members of the panel. Stronger flavours masked the taste of salt whereas weaker ones did not. Further, at least half the panel considered that the flavour of orange and of lemon was more marked in higher concentrations of sugar than in solutions which were less sweet but contained the same amount of oil. The addition of small amounts of salt to flavoured sugar solutions apparently increases the strength of the added flavour, the sweetness of the solution and, sometimes, its acceptability. Acceptability appears to depend upon the amount of flavouring added to the solution and upon the amount of salt, more salt being preferred in strongly flavoured solutions.

Household Arts Department
Queen Elizabeth College
Campden Hill Road
London, W.8

Received 18 March, 1953

THE ISOLATION OF LAURIC ACID FROM BUTTER FAT

By R. P. HANSEN and N. J. COOKE

Although lauric acid has generally been assumed to be a minor constituent of butter, it has not hitherto been isolated from this fat. The present work reports the isolation and identification of lauric acid from a representative sample of butter fat.

In the many published analyses of the fatty acid composition of butter fat, lauric acid is invariably shown as being present in minor amounts.[1–7] Examination of these papers reveals that in all cases lauric acid is assumed to be a constituent and its content has been determined by calculations based on the saponification equivalents and iodine values of fractions. There appears to be no evidence of lauric acid's having been isolated from butter fat and identified. Indeed Hilditch[6] in 1937 stated: 'This acid (lauric acid) is usually included in the list of milk-fat acids, but the evidence for its presence is not altogether convincing; up to the present no definite identification of lauric acid in the mixed acids of butter appears to have been put on record.' A search of the literature published subsequent to 1937 has similarly failed to disclose any evidence of the isolation of this acid from butter fat.

In the course of investigations of the minor constituents of hydrogenated butter fat, the authors of this paper isolated lauric acid. The amount separated exceeded the quantity which could have resulted from hydrogenation of the C_{12} unsaturated component (Δ^9-dodecenoic acid). However, in order to confirm its presence in butter fat which had not been hydrogenated, the work now reported was undertaken.

Results and discussion

Three methyl ester fractions (D/86,1, L5; D/86,3, L4; and D/86,4, L4) separated from butter fat in the process of triplicate fatty acid composition analyses of sample D/86,[8] and calculated to contain a preponderance of methyl laurate, were bulked together and denoted N. Characteristics of these ester fractions were as follows:

D/86,1 L5	Wt.	6·52 g.,	sap. equiv.	218·5,	iodine value	9·5
D/86,3 L4	„	7·86 g.,	„	„	213·3,	„	„	8·8
D/86,4 L4	„	11·08 g.,	„	„	212·6	„	„	8·9

The methyl esters of fraction N (13·55 g.) were then fractionated in a 50 × 1·8-cm. column (Column E[9]), the results being as shown in Table I.

Table I

Fractionation of 13·55 g. of methyl esters N

Fraction	Wt., g.	Sap. equiv.	Iodine value	M.p.
NL1	1·11	189·6	12·6	—12·8°
NL2	4·68	209·5	7·1	2·8°
NL3	2·44	213·6	5·6	5·5°
NL4	2·10	215·9	6·1	3·7°
NLR	2·93	245·6	15·9	

Fractions NL2, NL3 and NL4 were bulked together, converted into fatty acids (m.p. 39·2°) and denoted N1. The N1 fatty acids were then subjected to a series of low-temperature crystallizations from light petroleum, methanol, ether, and acetone respectively (see Table II). Fatty acid fractions N1S6S, N1S6LS and N1S6L2S were bulked together, denoted N2, and crystallized from ether and from light petroleum respectively at low temperature (see Table III).

The characteristics of fatty acid fraction N2S2S were: Wt. 4·66 g., m.p. 44·0° (recorded values 44·2°,[10] 44·0°,[11] 43·75°[12] setting pt.); mixed m.p. with pure lauric acid (m.p. 44·0°) gave m.p. 43·2°; saponification equivalent 200·4 (theoretical for $C_{12}H_{24}O_2$, 200·3). Combustion analysis:* C 72·24%, H 12·13% (calc. for $C_{12}H_{24}O_2$: C 71·96%, H 12·08%); iodine value 0·0; *X*-ray long spacing 27·5 Å† (recorded values 27·18 Å,[13] 27·4 Å,[14]); m.p. of anilide 76·8° (recorded value 78·0°[15]). The complex formed with *S*-benzylthiuronium chloride gave m.p. 132·2° (corresponding complex made with pure lauric acid gave m.p. 133·8°).

* Analysis by Drs. G. Weiler & F. B. Strauss, Oxford

† *X*-ray measurement by Mr. L. D. Swindale, Soil Bureau, Department of Scientific and Industrial Research, Wellington, New Zealand

Table II

Low-temperature crystallization of fatty acid fraction N1

Fraction	Conditions of crystallization	Soluble			Insoluble		
		Fraction	Wt., g.	M.p.	Fraction	Wt., g.	M.p.
N1	Light petroleum, 40 vol. at −70°	N1L	0·64	7·0°	N1S	7·22	41·2°
N1S ..	„ „ „ „ „ „	N1SL	0·33	15·0°	N1SS	6·88	42·4°
N1SS ..	„ „ „ „ „ „	N1S2L	0·15	16·3°	N1S2S	6·70	42·4°
N1S2S ..	Methanol, 40 vol. at −70°	N1S3L	0·24	29·3°	N1S3S	6·45	43·2°
N1S3S ..	„ „ „ „ „	N1S4L	0·17	38·2°	N1S4S	6·25	44·0°
N1S4S ..	„ „ „ „ „	N1S5L	0·17	40·0°	N1S5S	6·06	43·7°
N1S5S ..	Ether 40 vol. at −40°	N1S6L	4·87	44·0°	N1S6S	1·09	44·2°
N1S6L ..	„ 20 „ „ „	N1S6LL	1·80	42·4°	N1S6LS	3·04	44·0°
N1S6LL ..	Acetone, 20 vol. at −40°	N1S6L2L	0·13	39·0°	N1S6L2S	1·65	44·0°

Table III

Low-temperature crystallization of fatty acid fraction N2

Fraction	Conditions of crystallization	Soluble			Insoluble		
		Fraction	Wt., g.	M.p.	Fraction	Wt., g.	M.p.
N2	Ether, 20 vol. at −50°	N2L	0·99	43·0°	N2S	4·76	44·0°
N2S ..	Light petroleum, 20 vol. at −50°	N2SL	0·03	39·0°	N2SS	4·73	44·2°
N2SS ..	„ „ „ „ „ −40°	N2S2L	0·05	41·0°	N2S2S	4·66	44·0°
						Sap. equiv. 200·4	
						Iodine value nil	

The *X*-ray diffraction measurement reported in this paper was made by means of a Philips Geiger *X*-ray spectrometer. The sample was melted on a glass slide and quickly cooled. All melting points were determined in closed capillaries and are uncorrected.

The chemical and physical characteristics presented above establish fraction N2S2S as being lauric acid (*n*-dodecanoic acid).

In the triplicate fatty acid composition analyses of butter fat sample D/86[8] the mean content of lauric acid was calculated from the saponification equivalents and iodine values of the appropriate methyl ester fractions to be 3·6 mol.-%. In this present investigation the weights and the melting points of the recorded acid fractions indicate that the proportion of lauric acid is of the same order as that derived by calculation from the ester-fractionation data.

Acknowledgment

The authors are indebted to Dr. F. B. Shorland, Director of the Fats Research Laboratory, for his advice and interest.

Fats Research Laboratory
Department of Scientific & Industrial Research
Wellington
New Zealand

Received 2 March, 1953

References

[1] Browne, C. A., *J. Amer. chem. Soc.*, 1899, **21**, 807
[2] Crowther, C. & Hynd, A., *Biochem. J.*, 1917, **11**, 139
[3] Hilditch, T. P. & Jones, E. E., *Analyst*, 1929, **54**, 75
[4] Hilditch, T. P. & Sleightholme, J. S., *Biochem. J.*, 1930, **24**, 1098
[5] Hilditch, T. P. & Paul, H., *Biochem. J.*, 1936, **30**, 1905
[6] Hilditch, T. P., *Analyst*, 1937, **62**, 250
[7] Hilditch, T. P., 'The Chemical Constitution of Natural Fats', 1947, 2nd ed. (London: Chapman & Hall)
[8] Hansen, R. P. & Shorland, F. B., *Biochem. J.*, 1952, **52**, 207
[9] Shorland, F. B., *J. appl. Chem.*, 1952, **2**, 438
[10] Schuette, H. A. & Vogel, H. A., *Oil & Soap*, 1939, **16**, 209
[11] Ralston, A. W., 'Fatty Acids & their Derivatives', 1948 (New York: John Wiley & Sons, Inc.)
[12] Francis, F. & Piper, S. H., *J. Amer. chem. Soc.*, 1939, **61**, 577
[13] Slagle, F. B. & Ott, E., *J. Amer. chem. Soc.*, 1933, **55**, 4396
[14] Francis, F., Piper, S. H. & Malkin, T., *Proc. roy. Soc.*, 1930, [A] **128**, 214
[15] Robertson, P. W., *J. chem. Soc.*, 1919, **115**, 1210

J. Sci. Food Agric., 4, July, 1953

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

CONTENTS

Annual Subscription to the *Journal of the Science of Food and Agriculture*, £2 10s. post free, single copies 5s. post free