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The Detection of Rot in Tomato Products

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A review is made of the use of the Howard Mould Count method for the detection of fungal rot in tomato products. The legal standards for various countries are evaluated and results of the examination of tomato products imported into London are tabulated. Notes are given on the technique, interpretation and significance of the method. The value of the Rot Fragment Count as a confirmation of the Howard Count is discussed and an appraisal is made of the possible detection of mycotoxins in tomato products. Factors which would influence the adoption of a legal standard for Great Britain are assessed.

In view of the increased attention now being focused on fungal deterioration of food and of the obligations imposed upon Public Analysts under Section 8 of the Food and Drugs Act 1955, the Imported Food Regulations 1968 and by the Food Standards (Tomato Ketchup) Order 1949, it is of interest to review the methods available for the detection of unsound, unclean or unwholesome tomatoes in samples of tomato products.

Spoilage of the fruit may arise from a number of different causes. These include fungal, bacterial and viral infections, which may be introduced by airborne or insect-borne organisms, or from a pathological condition of the plant; fly maggots and mites; contamination by pesticides, herbicides and other chemicals, and by dirty extraneous matter. By far the most common cause is fungal rot, and its detection and significance in manufactured tomato products is discussed below.

THE HOWARD MOULD COUNT

As a preliminary test, a general microscopical examination of the product is normally carried out. If the examination reveals much fungus, with hyphae present in every field, the analyst may be able to make an adverse report without further investigation. If only a few hyphae are to be seen they may be regarded as natural to the fruit and unobjectionable. But, if there appears to be rather a high proportion of fungus present, some criterion will be necessary by which to judge whether the product was made from reasonably clean and wholesome tomatoes or from fruits that were partly rotten.

The Howard method,[1] in which the percentage of fields containing mould is counted, was developed in 1908–11 in the U.S. Department of Agriculture for the enforcement of the Food and Drugs Act of 1906. It became a legal Federal

Government standard in 1916 at 66 per cent. fields positive, was reduced to 50 per cent. positive in 1931, and to 40 per cent. in 1940, where it has remained until the present time. In Canada and Australia the limit is 50 per cent. positive. In France there are two legal standards, a 60 per cent. maximum for Standard Quality and a 50 per cent. maximum for Extra Quality purées.

The Netherlands, the Federal Republic of Germany and Denmark do not have legal standards for Mould Counts although the test is commonly used as an indicator of the hygienic condition of tomato products; in Italy the imposition of a legal standard has been under consideration.

In 1958 standards were provisionally approved by the International Permanent Committee on Canned Foods (the C.I.P.C.) for the physical and chemical properties of Standard Quality and Extra Quality Tomato Purées, which included Mould Count limits of 60 per cent. and 40 per cent. positive fields respectively.

A Standard for Processed Tomato Concentrates is under consideration by the Codex Alimentarius Committee on Processed Fruits and Vegetables at the present time, and it is understood that in the Proposed Draft Provisional Standard, now at step three, a maximum Howard Mold Count of 50 per cent. positive fields has been adopted.

In the case of Tomato Juice, the U.S.A. tolerance is 20 per cent. fields positive, the Canadian, 25 per cent., and the French, for both "Standard" and "Extra" Quality, 30 per cent.

In the area controlled by the Port of London Health Authority, merchants were advised in January, 1963, by the Medical Officer of Health that many instances of excessive mould contamination of tomato concentrates had been reported by the Public Analyst and that, after consultation with other analysts and with members of the trade, it was proposed to adopt a maximum limit of a 50 per cent. Howard Mould Count for the purposes of the Imported Food Regulations 1937 and 1948; but that in view of the difficulties which would be experienced by merchants in meeting such a standard at little or no notice, consignments showing a Mould Count of 60 per cent. positive or less would be released for the time being. In cases where samples showed over 60 per cent. of positive fields a letter would be sent to the owner suggesting that such goods should not be imported into this country for sale for human consumption.

Copies of the letter were sent to other Port Health Authorities in U.K., and on 6th September, 1963, a further letter intimating the intention to apply an upper limit of 50 per cent. positive fields was sent to all known tomato product importers.

During 1963 a few consignments of tomato purée showing Mould Counts in excess of 60 per cent. positive were detained by the Port of London Health Authority. From figures supplied by courtesy of H.M. Customs and Excise it was found that the tonnage of tomato purée detained amounted to 0·6 per cent. of the total imported into the Port of London during the relevant months of December, 1962, and January, 1963.

In some quarters, objections were raised to the adoption by the Health Authority of a limit based on the Howard Mould test, and a conference was arranged by the Food Trade Review in London in April, 1963, between the Port of London Health Authority and the trade, at which both sampling and testing procedures were debated at some length[2].

Following this conference, the validity of the Authority's detention notice on a certain consignment of purée showing 72 per cent. fields positive, was challenged by a large firm of tomato merchants. Their action led to a summons under the Imported Food Regulations, 1937, reported elsewhere,[3] in which evidence of the Howard Mould Count was accepted and the view that a purée made from unsound fruit must also be unsound, even though sterilised, was upheld by the Court.

It is of interest to note that this rather fine point of interpretation has now been clarified in the revised Imported Food Regulations, 1968, in which the prohibition on importation of unsound food has been extended to include food in the preparation of which any unsound food has been used.

The limit of 50 per cent. positive is now generally accepted by the trade and is included in the recent Trade Specification for Tomato Purée, dated February, 1967. It is, indeed, understood that the leading British manufacturers have for many years been working to a considerably more stringent standard for their own products.

The test has been applied in the Author's laboratory to retail samples of purée and sauces submitted under the Food & Drugs Act since 1948, but because of the difficulty of attaching the summons to the person responsible for the offence, action has, until last year, been confined to representations to the dealers concerned. In April, 1967, however, a prosecution against an importer under Section 2 of the Food & Drugs Act in respect of tomato purée showing 75 per cent. of fields positive was successfully undertaken at the South Eastern Petty Sessions by the London Borough of Lewisham.[4] A similar prosecution at Kensington Magistrates' Court in September, 1968, against an importer's agent, by the London Borough of Kensington and Chelsea in respect of a Hungarian tomato purée (Howard Mould Count: 75 per cent. positive fields), also resulted in a conviction.

SAMPLES TAKEN UNDER IMPORTED FOOD REGULATIONS

Results of Howard Mould Counts on samples of tomato purée, paste and concentrates, submitted by the Port of London Health Authority, are summarised in Table I.

The above results have been collated from nearly 1000 samples, and to avoid giving a false impression of the frequency of high mould counts due to repeat sampling following each bad report, only one mould count from each invoice, being a mean when more than one sample was submitted, has been included in the table. In other words, if a ship delivered consignments of four different makes of purée at one landing and all were sampled, only four results would be shown above no matter how many samples were examined.

TABLE I

IMPORTS OF TOMATO PURÉE SAMPLED BY THE PORT OF LONDON HEALTH AUTHORITY, 1962–67

Year	Number of Consignments examined	Percentage exceeding 50 per cent. H.M.C.	Average H.M.C. of all consignments *per cent.*
1962 .. .. December	20	35	45
1963			
Jan.-June	94	30	39
July-Dec.	41	17	30
1964	54	7	25
1965	45	2	19
1966	48	10	20
1967	37	3	20

H.M.C.= Howard Mould Count.

The annual imports of tomato purée into the U.K. from Italy alone have been estimated at 35,000 tons[5], and whilst no quantitative conclusions are justified, because of the effect on Mould Counts of adverse climatic conditions in 1963, the results summarised in Table I appear to support the inference that, prior to 1964, substantial quantities of tomato purée of a quality illegal in other countries were being directed into the Port of London.

SAMPLES TAKEN UNDER THE FOOD AND DRUGS ACT

A short survey of tomato purées as sold by retail in the London area may be of interest. Table II shows the Howard Mould Count of ordinary random samples purchased by inspectors in 1965–67. To ascertain if any material difference occurred in the quality of purée filled into different sized cans and tubes, the results have been classified according to size of container.

TABLE II

HOWARD MOULD COUNT TESTS ON RANDOM SAMPLES OF TOMATO PURÉE; 1965–67

	1965		1966		1967			1965/67
	No. of Samples	H.M.C. Average *per cent.*	No. of Samples	H.M.C. Average *per cent.*	No. of Samples	H.M.C. Average *per cent.*	Total Samples	H.M.C. Average *per cent.*
Cans, $2\frac{1}{4}$–$3\frac{1}{2}$ oz. ..	11	16	18	22	14	16	43	19
Cans, 5–8 oz. ..	6	16	6	21	3	13	15	17
Cans, 11–30 oz. ..	2	25	1	10	1	20	4	20
Tubes, $3\frac{1}{3}$ oz.	2	30	4	15	1	35	7	22
Tubes, 5–$6\frac{1}{2}$ oz. ..	5	16	8	28	10	14	23	19
	26	18	37	22	33	16	92	19

As may be seen from the tables, the counts on retail samples show much the same proportion of mould as samples taken on importation; and although the best quality purées were formerly found in the 5 Kg cans imported by the large manufacturers, the results show that small cans and tubes are now also filled with purées of equally low mould counts.

Only two samples in 1966, an informal and a formal of the same brand, in $6\frac{1}{3}$-oz tubes, exceeded 50 per cent. positive. These gave 80 per cent. positive

and 75 per cent. positive respectively and were the subject of the Lewisham prosecution mentioned earlier.

THE HOWARD TECHNIQUE

The counts are carried out precisely as directed in the A.O.A.C., 10th Edition, No. 36.069.* Fuller information about the test, including descriptions of the kinds of rot that affect tomatoes, is given in a booklet entitled "Mold Counting of Tomato Products".[6]

In the U.S.A., special courses of instruction in mould counting are given at Technical Schools sponsored by State Canners Associations. No experienced microscopist should have much difficulty in carrying out the prescribed method, but in the absence of such instruction some minor points have arisen which call for special mention.

1. If a binocular microscope is used, or if it is not found possible to adjust the diameter of field to exactly 1·382 mm diameter by means of the draw tube, a circular diaphragm can be cut from a piece of black card with a cork borer and file to give an ocular aperture corresponding to the specified width as measured on a ruled slide. This diaphragm can then be left in the microscope and saves repeated adjustments provided the same ocular and objective are always used.

2. Some moulds, particularly *Colletotrichum*, which causes anthracnose rot, have extremely fine hyphae. Short lengths are easy to miss, sometimes resembling a crease in a cell wall, and it is essential to focus up and down carefully, on four quarters of each field, to make sure hyphae are not missed; and to turn to a higher power when in doubt. Several workers add stains to facilitate recognition of hyphae; methylene blue, cotton blue and crystal violet have been used, but since they also stain tomato tissue their value is questionable.

3. When a field is "borderline" the viewer will be in doubt whether to record it as positive or negative, and the question will arise whether to follow the traditional tendency to give the benefit of the doubt to the product, or to ignore the field altogether and pass on to the next one. Both practices are open to criticism, but in the Author's view the latter is to be preferred, because if a degree of tolerance is introduced into the actual counting, variations will occur between laboratories and it is better to have only one known tolerance to apply for experimental error. On the other hand, at least when a count is near the critical 50 per cent. positive, the result will not be much affected by ignoring a few borderline fields.

4. Occasionally one may find a small colony of compact, short-branched mycelium, measuring in width less than a sixth of the diameter of the field. No directions are prescribed in the official method but it is suggested that the field should be regarded as positive, because if the hyphae were disentangled and straightened out they would measure more than the specified sixth.

* Howard cells are available from Watson & Sons Ltd., Barnet, Herts or from Hawksley & Sons Ltd. of 17 New Cavendish Street, W.1.

5. Possibly the most important part of the procedure is to ensure that the aliquot spread out on the disc is really representative of the whole of the suspension. The Author has found difficulty in securing this by the method prescribed in the A.O.A.C., which consists in taking a portion of the suspension on a knife blade or scalpel and spreading it evenly over the disc to give uniform distribution; the difficulty lies in removing a small volume of a suspension containing solid fibrous matter and an aqueous liquid on a scalpel, and again in transferring a portion of this to a slide, without permitting any separation of liquid from the solid. The use of a dissecting needle to remove the sample portion from the scalpel to the mount has been suggested, but a simple and efficient method is to use a straight sided tube of 3 to 3·5 mm bore and, removing the finger from the top momentarily, allowing a large drop to fall on to the disc. This drop carries down the cellular matter without separation of liquid, and it should then be spread evenly over the disc with a needle before the coverslip is placed in position.

INTERPRETATION OF THE RESULTS

The Trade Specification for Tomato Purée, referred to above, lays down a Sampling Procedure and a statistical method for Treatment of Results. A minimum of 10 cans is suggested from a consignment of 100 tons and the number of fields to be counted on each sample is 50. The True Mean Value for the consignment, with a 95 per cent. probability, is then calculated from the arithmetic mean and the standard error.

In the Author's experience, a count based upon only 50 fields may be very different from the mean of say 500 fields; and whilst the method of the Trade Specification is designed to provide the most reliable and economical means of ascertaining the Howard Mould Count of a sizable consignment of purée, it cannot usually be adapted to the practice of a Public Analyst.

Under the Food & Drugs Act the sale of any one article of unsound or defective quality constitutes a presumptive offence, and a Public Analyst may be required to issue a certificate on one small sample of purée. Generally speaking samples are taken informally in the first instance, an adverse report being followed up by a formal sample. Hence, before legal action was taken, more than one sample would have been examined, and in the case of 2–3 oz. cans, perhaps six might have been purchased and mixed together for the formal sample.

If a sample taken under the Imported Food Regulations were found to be defective, a few more samples would normally be taken to see if the whole consignment is similar. If these showed significant differences, further samples would be drawn, but under present circumstances, and within the limits of the financial and laboratory facilities available, it would not be practicable for Public Health Authorities to carry out the regular sampling of consignments upon the scale recommended in the Trade Specification.

There is, however, nothing to stop the importer taking as many samples as he wishes, and, in the event of disagreement between his analyst and the Port

analyst, a formal sample could be drawn in the presence of his representatives, mixed and divided into three or four parts to enable independent tests to be made if necessary. Post-sampling mould growth can be prevented by addition of formalin and refrigeration.

The Public Analyst has therefore to take greater care to ensure the correctness of his count than is envisaged in the Trade method. This he can do by counting more than 50 fields and thus reducing the standard error of his mean. He will not usually be very concerned to determine the exact count when it is low, and in view of the number of other tests to be applied to manufactured tomato products, it may be suggested that for routine laboratory purposes one count, *i.e.* 25 fields, might be sufficient if the result is below 30 per cent. positive; 50 fields if above 30 and below 40 per cent. positive; 100 fields if above 40 and below 50 per cent. positive; but that if the count is 50 per cent. positive, or whatever the currently accepted limit may be, or over, counting should be continued until further counts of 25 fields do not significantly alter the mean of preceding counts. At least two suspensions of the purée should be made, by different operators, and it will usually be found sufficient if each operator counts one hundred fields on each suspension, making 400 fields in all. Some purées yield very variable counts, however, and if such a sample is encountered still more counts may be necessary, occasionally as many as 800 fields.

The above suggestions are based solely upon experience obtained in one laboratory; they are not part of the official procedure and it may well be that in other laboratories experience may indicate a preference for some other procedure or method of interpretation.

SIGNIFICANCE OF THE HOWARD MOULD COUNT

No attempt is made when counting to differentiate between the types of mould that may be present. Unless characteristic sporulation can be found this would indeed be impossible; the product is sterile and no cultures can be grown. The only value of the count is as a guide to the extent of fungal rot in the tomatoes used.

Several investigators have studied the relationship between the amount of rot in the raw tomatoes and the Mould Count of the purée, and Howard's original data are of interest.[7] His averaged results from 179 determinations of visible cut-out rot and 235 mould counts from 17 factories, are summarised in Table III.

Unfortunately, different types of mould have different effects on the mould count[8,22]. This has been well shown by Eisenberg[8], who determined the mould counts of purées made from tomatoes affected by specific moulds. Reference should be made to his paper for fuller information, but briefly it may be inferred that, although the averages of his counts accorded with Howard's data, individual samples showed widely scattered results, sometimes exceeding $\pm$ 50 per cent from the mean. Also of interest in this paper is the relative area of the rot. A cut-out rot of 10 per cent. by weight, with a mould count of about 68 per cent. positive, corresponds roughly to an area of visible rot of $1\frac{1}{2}$ inches diameter in

TABLE III
VARIATION OF MOULD COUNTS WITH VISIBLE ROT

Percentage of Rot by weight	Mould Count of pulp
0·2	5
0·6	10
1	15
2	25
3	33
4	40
5	46
6	51
7	56
8	61
10	68
12	75
15	80
20	87
30	92

an average size tomato; but, again, variations due to the kind of rot were very great.

One of the difficulties in relating the mould count to the percentage of rot is that some portions of rot are soft and completely broken up in the extraction but others are relatively hard and may be discharged with the cores and skins. Hence the presence of a high proportion of visible rot can nevertheless give a low mould count.

After thorough studies by the U.S.A. Department of Agriculture it was concluded that in properly sorted stock the extent of decay should not exceed 1 per cent.[9] J. C. Dakin states[10]:

> "Whatever the deficiencies of the Howard technique may be as a means of providing a true indication of the state of the fruit and manufacturing conditions, it is the best available for indicating contamination by mould and all that this implies."

The general inference, as originally expressed by Howard and Stephenson[7], appears to be universally accepted:—

> "A low mould count does not necessarily indicate sound stock, but a high mould count always indicates bad stock or improper handling."

This is the real value of the test to the Public Analyst.

In addition to the dependence of the mould count on the species of mould, criticism has been made of the accuracy of the technique itself. Only small quantities of sample are taken for each count and, as emphasised in the Trade Specification,

> ". . . it must be recognised that any mould present in tomato purée is very unlikely to be uniformly distributed throughout the consignment, or even throughout a given sample."

H. R. Smith[9] states that it is quite possible for properly qualified analysts to obtain satisfactory checks on the same sample and quotes a trial in which eight analysts representing different organisations found no significant difference

between their counts; but a recent collaborative trial between eight analysts in independent laboratories in this country showed very wide differences. The procedure in the latter trial, however, specified 50 counts, and as already indicated, far more counts are necessary to obtain a reliable figure. A statistical examination of other collaborative tests in this country has also been carried out by Vas *et al.*[21], and a standard deviation of 7·4 is reported for a single observer on one sample.

A further criticism of the Howard method is that, since it is bsaed on the recognition of mould filaments, rots which are caused by bacteria, yeasts, viruses and physiological causes unaccompanied by mould are not detected[8]. All evidence of spoilage should, however, be taken into account in considering whether a product is sound or unsound; and a Mould Count of less than 50 per cent. positive, if accompanied by excessive bacterial growth, might afford evidence for condemnation.

The taste, preferably after dilution in the case of a purée, may also be of evidential value; but not always, because the degree of mustiness depends upon the type of infection, a high mould count sometimes being found when the flavour is normal and vice versa. A musty taint may also be caused by certain pesticides.

Furthermore, the mould count does not differentiate between bad stock and improper handling. Conditions in the factory may be conducive to deterioration even after the tomatoes have been sorted, cleaned and any visible rot cut out. Any accumulation of tomato slime, inadequate cleansing of equipment or delay before sterilisation might cause a high mould count.

Although the final product might seem equally objectionable to a consumer, whatever the origin of the mould, it would be useful for the purposes of the Food Standards (Tomato Ketchup) Order, 1949, to be able to ascertain if a sample of ketchup has in fact been "made from clean and wholesome tomatoes, or from tomato purée made from clean and wholesome tomatoes." In this connection the "Rot Fragment Count" may be of use.

ROT FRAGMENTS

A method for the estimation of Rot Fragments in Tomato Products (Method M13D) published by the National Canners Association of U.S.A. in 1943, was introduced into the A.O.A.C. in 1945, omitted in 1950, reintroduced in 1955, and appears as an "Official, First Action" method No. 36.072 in the current 10th Edition, 1965. No British papers have come to light on the test and, although some experimental work on the method has been published in U.S.A., no recommended limits for rot fragments in tomato products have been discovered. H. R. Smith[9] has attempted to show the relationship between the percentage of visible rot and the rot fragment count of the purée, but the results varied too much to form a basis for quality assessment. The samples tested were, however, drawn from the trimmed tomato input, and the cyclone juice output at six factories over several days under ordinary working conditions where accurate sampling was impossible; and further tests under laboratory-

controlled conditions would be desirable to show a truer relationship and the natural variation of the rot fragment count for a known percentage of rot.

There are, unfortunately, two ambiguities in the A.O.A.C. directions which may affect the results considerably. A rot fragment is defined as "tomato tissue to which mould filaments are attached"; but in several of the photomicrographs of rot fragments illustrated in the A.O.A.C. no tomato tissue can be recognised, and the question arises whether mould colonies without obvious tomato tissue should be included. In McCormack's definition[11], "a rot fragment is a clump of opaque or semi-opaque material with a periphery of mould filaments"—which would apparently include colonies if they were dense but perhaps not if they were light and open. Since about half the clumps of fungus one finds do not show identifiable tomato tissue the question cannot be ignored and it is necessary to decide which to count.

The second point is that the minimum size of rot fragment to be counted is not specified. This may be because the area or length of a fragment with a fluffy edge defies definition, or it may have been assumed that the size would be controlled by the method, all fragments of less than 0·25 mm in width passing through the No. 60 sieve. In practice however much fine material fails to pass through the sieve, being retained by the coarser material, and it becomes necessary to decide upon a minimum, however rough the definition may be.

Conversely, Olsen and Stoner[12] have found that a considerable number of rot fragments pass through the sieve, indeed, many more than are retained by it; the implication being that to obtain a correct count the sample should be diluted, stained and examined without being screened. To count unscreened aliquots, however, would take a great deal of time owing to the high dilution necessary (ten times as great as in the A.O.A.C. method), and it is suggested that their findings reflect the importance of defining the size of the fragments to be included.

In the Author's laboratory, McCormack's definition[11] of a rot fragment, which seems to accord best with the illustrations in the A.O.A.C., has been adopted; and all fragments exceeding 0·3 mm in longest measurement, including about a third of the sparse peripheral filaments in the measurement in the case of dense colonies, have been counted. This is an arbitrary procedure, but in the absence of special instruction, or more detailed directions in the method, it is necessary for each analyst to decide upon his technique and to acquire his own experience before drawing any inference from a rot fragment count.

A stereo microscope ($\times 40$ magnification), fitted with a mechanical stage is most suitable for counting fragments, counts being recorded by a counter of the cyclometer type. Ordinary cleaned slides are used, taking 0·15 to 0·2 ml of the suspension, measured from an inverted, straight-sided, graduated 1-ml pipette, spread with a needle over an area of about $3 \times 1{\cdot}5$ cm on the slide. As the method has only been used up to the present time as a general confirmation of the presence of rot, only two slides have been counted (about 0·3 ml ex 10 ml prepared from 2 g of purée, with glycerin as

stabiliser), unless they showed a wide difference when four slides would have been counted. (The quantities taken were based on an earlier edition of the A.O.A.C., now modified).

The method provides a very practical confirmation of the presence of rot in a pureé or other tomato product. The appearance presented by pieces of tomato tissue with a periphery of mould is quite convincing and makes an effective subject for photography if permanent evidence is required. Slime from a tomato factory, and fungal development in a purée after the container has been opened, usually seem to consist of *Oospora sp.*, a feathery growth with tapering filaments, which will be observed in the liquid phase, in between particles of tomato tissue rather than emanating from them. In the case of an infected

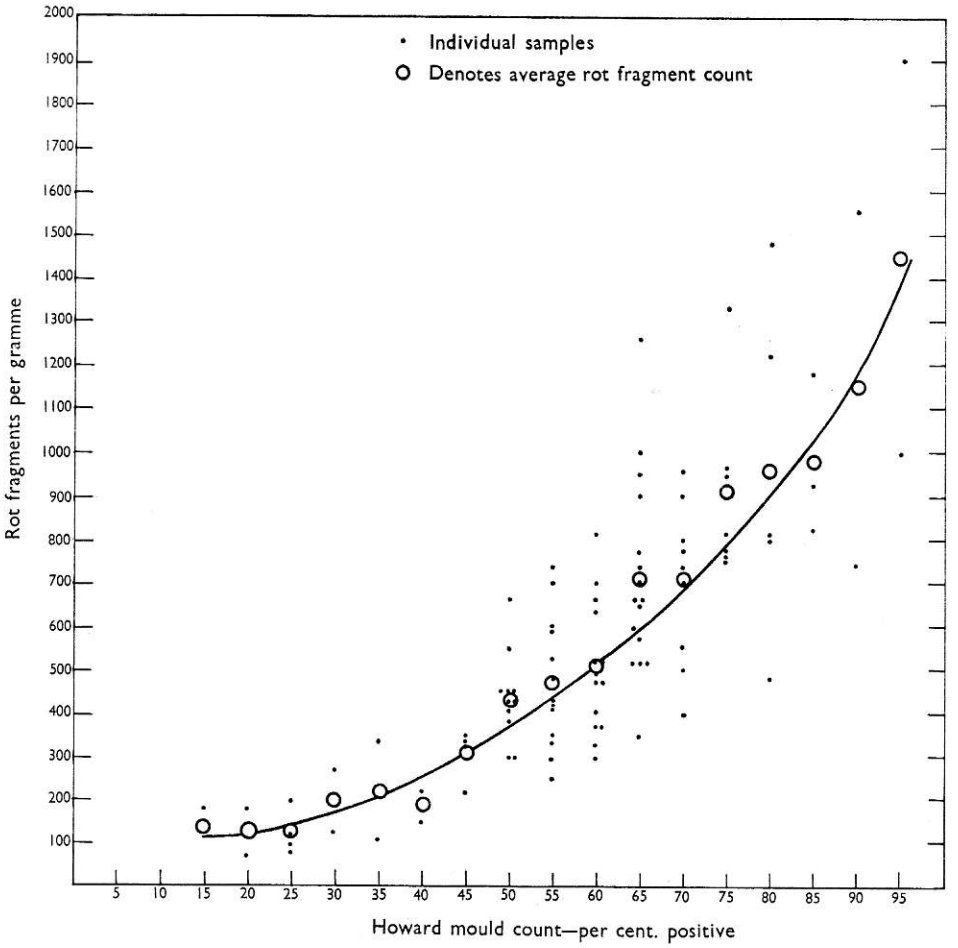

Fig. 1. Relationship between Mould Counts and the incidence of Rot Fragments in Tomato purée.

product due to a faulty closure, or delay after sampling, a copious surface growth develops first and is sufficiently obvious to render any further examination unnecessary.

In case they might be of interest to other workers of limited experience, the results obtained in this laboratory over the past five years, mostly on purées of high mould counts, have been collated and are shown against the respective mould counts in the accompanying figure.

Wide variations will be observed between rot fragment counts on samples of similar mould count. Such variations may be ascribed partly to experimental error, arising from the application of a high factor to a small count, and partly to variations of assessment by four different operators over the period. It will, however, also be observed that, if the results for all samples of similar mould count are averaged, a distinct relationship appears between the two methods.

It is suggested, therefore, that if the procedure of the Rot Fragment Count were amplified so as to obtain more uniform counts, the result might afford a useful confirmation of the Howard Mould Count; and that if the relationship showed any marked departure from normal, it would indicate conditions in the purée that might affect the conclusion to be drawn from the Mould Count.

FLY EGGS, MAGGOTS, MITES

For further evidence of the use of damaged fruit the A.O.A.C., First Action method 36.073, is worthwhile and often provides interesting results. Coulter's modification[13] provides a cleaner residue by digesting the tomato tissue with sodium phosphate. A quantity of sample weighing 100 g is suitably diluted and digested, with trisodium phosphate added to give a pH of 7·5–8·5. The mixture is boiled for 5–10 minutes, then washed, first through a No. 10 sieve if seeds are present, and secondly through a No. 80 sieve until there is no further reduction in the volume of the residue, which is then transferred to a cloth or filter for counting.

OTHER TOMATO PRODUCTS

For tomato sauce, it has been the Author's practice to examine a portion of the sample directly in a Howard cell. Occasionally, difficulty is encountered in recognising all the mould filaments, particularly in products containing gelatinised starch as a thickening or stabilising agent, and in such cases it may be advantageous to hydrolise the starch before counting, *e.g.* by adding 1 ml of NaOH solution (50 per cent. w/w) to 10 ml of the sauce.

In the 1965 changes in Official Methods of Analysis of the A.O.A.C., to facilitate identification of hyphae, it is directed that the catsup is mixed with an equal volume of sodium carboxymethyl cellulose solution before being mounted in the Howard cell. For predicting mould counts at various dilutions, the Poisson distribution equation has been found to apply, using a graph to find the relationship between the mould count and the number of countable elements per field.[14] No directions are given in the official method for calculating the result back to the undiluted catsup, nor for relating it to the tomato content; and it is not clear what limit would be applicable.

Tomato Juice is examined as received, without dilution or concentration. It will be noted that in this case the count will be based upon an average soluble tomato solids of 5·5 per cent., as compared with 8·4 for purées, and that the U.S.A. limit is considerably lower for juice than for purées.

Dehydrated tomato powder may be diluted with water to give a refractive index of 1·3447 to 1·3460, or with a stabilising solution to 8·4 per cent. tomato solids, and then counted. If difficulty arises with other products such as tomato soup, or beans, spaghetti or fish with tomato sauce, the operator may be referred to the method of the A.O.A.C. para. 36.075, duly noting that in such products the count is expressed on the original volume of the soup or sauce.

MYCOTOXINS

In view of the large number of highly toxic metabolites recently identified from fungi of relatively common genera, it is evident that the occurrence of moulds in foods and feeding stuffs requires a new and much more serious appraisal.

Instead of any question of poisoning being restricted to a few notorious fungi such as the Amanitas and Claviceps, many common saprophytes are now incriminated and the decomposition of food can no longer be regarded merely as an unhygienic but basically harmless nuisance. Consequently, it becomes necessary to consider whether a mould count of less than 50 per cent. positive fields can be regarded as unimportant if the fungal species are unknown.

Of the more than fifty species of fungi that thrive on cereals and other foods of vegetable origin, and which are known to produce toxins, the following call for prior consideration. In most instances the mycotoxins have now been identified; they show wide differences of chemical constitution and physiological action, and in some instances their toxicity to animals far exceeds that of the traditional chemical poisons.

The known pathogenic metabolites include Hepatotoxins (Ochratoxin from *A. ochraceus*; Islanditoxin and Luteoskyrin from *P. islandicum*; Rubratoxin from *P. rubrum*; Xanthocillin from *P. notatum* and *A. chevalieri*); Nephrotoxins (Citrinin and Citreomycetin from *P. citrinum* and other *Penicillia*); Neurotoxins (Patulin from *P. urticae* and other species of *Penicillium* and *Aspergillus*; Maltoryzine from *A. oryzae microsporus*; Citreoviridin from *P. toxicarium* and *P. citreoviride*); Haemotoxins (Fusariogenin and the Cladosporic acids from *F. sporotrichioides* and other species of *Fusaria*, and a toxin from *Stachybotrys atra*); Dermatoxins (Methoxy- and Trimethyl-psoralen from *Sclerotinia sclerotiorum*); Oestrogens (Zearalenone from *Gibberella zeae*, or *Fusarium roseum*; and a metabolite from *Monascus papulospora*); Carcinogens (Aflatoxin from *A. flavus*, Sterigmatocystin from *A. versicolor* and possibly Ochratoxin).

Many of the mycotoxins mentioned above do not appear to be affected by boiling. Since the species of fungi present in a sterilised product cannot usually be identified, any attempt to ascertain by analysis whether a purée containing fungus has become injurious to health (Section I of the Act) must depend upon identification of the mycotoxins.

With recent advances, this problem is not quite so intractable as might be thought. Most of the toxigenic fungi are highly coloured and many of the mycotoxins are intensely fluorescent under u.v. light, including aflatoxin B & G, ochratoxin A, xanthocillin, zearalenone, citreoviridin and sterigmatocystin. Patulin gives a fluorescent compound if the lactone ring is ruptured, *e.g.* by exposing the chromatoplate to ammonia, and it is probable that the anthraquinone derivatives and some other toxins could also be recognised by u.v. fluorescence after separation by chromatography.

Screening methods for mycotoxins are at present under trial in U.S.A. and a method for the detection of aflatoxin, ochratoxin and zearalenone in various commodities has recently been published.[18] The mycotoxins are extracted with chloroform and the extracts passed through a silica gel column. After they have been cleaned up with hexane and benzene, the adsorbed zearalenone is eluted with acetone/benzene, the aflatoxins with methanol/chloroform, and the ochratoxins with acetic acid/benzene mixtures. Aliquots of each eluate are developed alongside standards on TLC plates and compared under u.v. light.

Several samples of tomato purée containing mould have been tested for aflatoxin, with negative results, but no information is available regarding tests for other mycotoxins.

As regards the general question of the probability of toxigenic moulds occurring in rotting tomatoes it should be said at once that enquiries so far have elicited no specific grounds for apprehension. The principal moulds encountered in over-ripe tomatoes[6,19,22] mainly introduced by pest or airborne infection, are represented by species of *Colletotrichum*, *Oospora*, *Mucor*, *Alternaria*, *Rhizopus*, *Botrytis* and *Phytophthora*, which do not appear yet to have been incriminated. Nor have the fungal parasites to which the growing plant is subject[20], and which are also liable to occur to some extent in the fruit, so far come under suspicion. The commonest of these are *Fusarium oxysporum*, *Verticillium alboatrum* and *V. dahliae*, which cause wilt; *Pythium*, *Phytophthora*, *Colletotrichum*, *Thielaviopsis* and *Rhizoctonia* species, which cause root and foot rots; *Botrytis cineria* and *Didymella lycopersici*, which cause stem rots, and *Cladosporium fulvum*, the common leaf mildew. *Sclerotinia sclerotiorum* does not usually affect tomatoes but could arise from intercropping plants. As the fungus of "pink rot", it causes dermatitic lesions if handled and would consequently be undesirable in food.

A potential danger might arise, however, from the use of fallen tomatoes, the ripe fruit providing an ideal medium for many soil organisms, especially such genera as *Aspergillus*, *Penicillium*, *Fusarium*, and *Cladosporium*, to which many toxigenic species belong.

POSSIBLE LEGAL STANDARDS

The question has been raised whether a maximum permissible Howard Mould Count for tomato products in United Kingdom should be prescribed by Regulation.

In favour of such a standard it is argued that both the trade and the enforcing Authorities would then know exactly where they stood in this respect; that the standard would be enforceable, not only on retail sale, but at Ports of entry throughout the country. This would eliminate the dodging from Port to Port which is believed to have occurred in the past. The analyst's task would be simplified—one official test for soundness, yea or nay, with perhaps a latitude of 5 per cent.; any injustice to the supplier would be unlikely, since a high count always indicates bad stock or improper handling, although a low count does not always indicate sound stock. Finally, limits for mould in tomato products have already been adopted in other countries and proved workable.

Against adopting the Howard Mould Count as a legal standard are some of the other points mentioned above, *viz.*

1. *Factors affecting the Count.* Variations in samples and in the aliquots examined. Variations in the training of analysts, in factory methods, of comminution, straining, etc. Perhaps above all, the method lacks the precision desirable in any procedure intended for the enforcement of Food Standards Orders and Regulations.

2. *Factors affecting its significance.* There is a lack of a reasonably constant relationship between mould count and percentage of rot. The method takes no account of rot, disease or unwholesomeness caused by other factors. Its foundations are based upon American data, not necessarily applicable to European conditions where different fungal flora may predominate.[10] There would be no latitude for exceptional climatic influences. The mould count, if too high, could be reduced in the factory by finer straining and by addition of pulp from unripe tomatoes, with consequent reduction in the quality of the product. In certain growing districts where climate favours rapid fungal growth there might be a temptation either to pick the fruit before it was ripe or to apply excessive treatment by fungicides. Finally, under present law, a Public Analyst may base his opinion as to the soundness of a sample of food upon any tests that may be desirable, and in the event of a prosecution, it is open to the defence to challenge his certificate or testimony on any relevant grounds. If the legal test for soundness were based solely upon a Howard Mould Count, the field of cross examination open to the defence would be restricted to one figure, the significance of which could not be questioned.

The above arguments will be sufficient to show that, whilst the test is of undoubted value as an indication of soundness, and suitable for incorporation in a trade specification or a Code of Practice, any proposal to lay down a legal standard for the Howard Mould Count of Tomato Products as a basis for criminal proceedings would require very careful consideration.

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Drug Testing Under the Food and Drugs Act during 1967

The Report of the Drugs Sub-Committee of the Association of Public Analysts

A survey of the sampling and analysis of drugs submitted under the Food and Drugs Act, 1955 has been carried out by the Drugs Sub-Committee, under the supervision of the Council of the Association of Public Analysts. It covers the period from 1st January, 1967 to 31st December, 1967, and is a summary of the work of 29 laboratories in England and Wales, serving a population of approximately 24 million.

During the twelve months under review, 5,467 samples of drugs were examined, and of these 226 (4·2 per cent.) were reported as unsatisfactory compared with 4·2 per cent., 5·7 per cent. and 4·2 per cent. in 1964–65, 1965–66, and 1966–67 respectively. Of the drugs sampled, 539 (9·9 per cent.) were available to the public only on prescription, as compared with 4·5 per cent., 7·8 per cent. and 8·3 per cent, in 1964–65, 1965–66 and 1966–67 respectively.

The classes of "adulteration" were as follows:—

1.	Labelling irregularities	67
2.	Deterioration on Storage	66
3.	Misleading Claims	11
4.	Compositional irregularities	74
5.	Miscellaneous irregularities	8
		226

Having regard to the number of drugs of all kinds which have been sampled, the quality of pharmaceutical preparations is considered to be good.

The Examination of Toys for Celluloid

by V. HUSBANDS, J. W. CLOONAN AND G. KEEN
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The testing of the plastic for nitrate, the extraction and identification of camphor and the use of manner and time in which the plastic burns as tests for celluloid are described.

The Toys (Safety) Regulations, 1967, S.I. No. 1157*, decreed that after the 31st October, 1967, a toy shall not, either wholly or in part, be made of or impregnated with cellulose nitrate.

When used in toys, cellulose nitrate is plasticised with camphor to form celluloid, the proportion of camphor varying from 24 to 30 per cent. Other plasticisers have been used in the manufacture of celluloid but they are unlikely to be met with in toys.

A rapid, simple method is described for the examination of plastics for the presence of celluloid when infra-red spectroscopy or pyrolytic gas chromatography are not available. The procedure involves the qualitative testing of the plastic for nitrate using diphenylamine/sulphuric acid reagent, the extraction and subsequent identification of the camphor by gas-liquid chromatography, and the examination of the manner and time in which the plastic burns.

Apparatus

1. Gas Chromatograph: Varian Aerograph, model 1522/1B.
2. Recorder: by Leeds and Northrup, with 1 mV full-scale deflection (Chart speed 15 inches/hour).
3. Flame ionisation detector.
4. Columns: *a.* 5 ft. × $\frac{1}{8}$ in. o.d., stainless steel column, packed with 10 per cent. Carbowax 6000 + 5 per cent. potassium hydroxide, on Celite (100 to 120 mesh).
   b. 5 ft. × $\frac{1}{8}$ in. o.d., stainless steel column packed with 5 per cent. silicone SE.30 on Chromosorb W. (50 to 60 mesh).

Reagents

1. *Diphenylamine:* AR grade.
2. *Sulphuric acid:* concentrated, AR grade.
3. *Stannous chloride Soln.:* 10 per cent. w/v.
4. *Chloroform:* AR grade (redistilled).

* Obtainable from H.M. Stationery Office, London.

Procedure

EXTRACTION AND IDENTIFICATION OF CAMPHOR

For the rapid detection of the presence of camphor, mix about 0·2 g of the plastic with chloroform, and evaporate the chloroform to low bulk, when the odour of camphor, if present, can be readily recognised.

For the GLC identification of camphor, place 0·2 g of finely-chopped plastic in a glass-stoppered, 10-ml standard flask and make to volume with chloroform. Shake the mixture and allow it to stand for two hours. Inject 4 microlitres on to the GLC (Carbowax) column under the conditions given below.

Flow Rates

Carrier gas (nitrogen)	30 ml per min.
Hydrogen	20 ml per min.
Air	300 ml per min.

Equipment Data

Column 170° C: Injector 250°C: Detector 210°C. Range 10; attenuation 32 (equivalent to $9{\cdot}6 \times 10^{-9}$ amps.).

NITRATE

Place a small piece (10 sq. mm) of the plastic in a test tube and wet it with one drop of distilled water. Add 3 ml of a solution of 25 mg of diphenylamine in 25 ml concentrated sulphuric acid. In the presence of nitrate, a blue colour rapidly develops, diffusing away from the plastic. Shake the tube and allow it to stand for five minutes. If the solution is blue, divide it into two parts. To one portion add 10 ml of distilled water. If the blue colour remains or a purple colour develops, carefully add to the second portion a few drops of stannous chloride solution. Mix and dilute the solution with 5 ml of water. After about 20 minutes a yellow or orange precipitate develops in the presence of nitrate.

CELLULOID

Cut a strip of the plastic, about 1 cm wide, to weigh one gramme. Hold the strip horizontally, ignite one end and record the time required to burn the strip. Observe the manner in which it burns and the nature of the residue.

For further evidence of the presence of celluloid, take 0·2 g of suspected celluloidal plastic and dissolve it in 5 ml of acetone by gentle warming. Pour the solution into 10 ml of distilled water in a porcelain dish, and dry the mixture on a water-bath. Transfer the residue to an extraction thimble and extract it for two hours with chloroform. Evaporate the solution to about 1 ml, and inject 4 microlitres on to a 5 per cent. silicone SE.30, GLC column under the conditions used for the Carbowax column (but using range = 1 and attenuation = 4).

Results and Discussion

Camphor: Of the solvents used, chloroform was found to achieve the most efficient extraction of the camphor. To avoid loss of camphor, heating should not be used in the extraction stage. Some typical peaks obtained for camphor on the Carbowax column are shown in Figure 1.

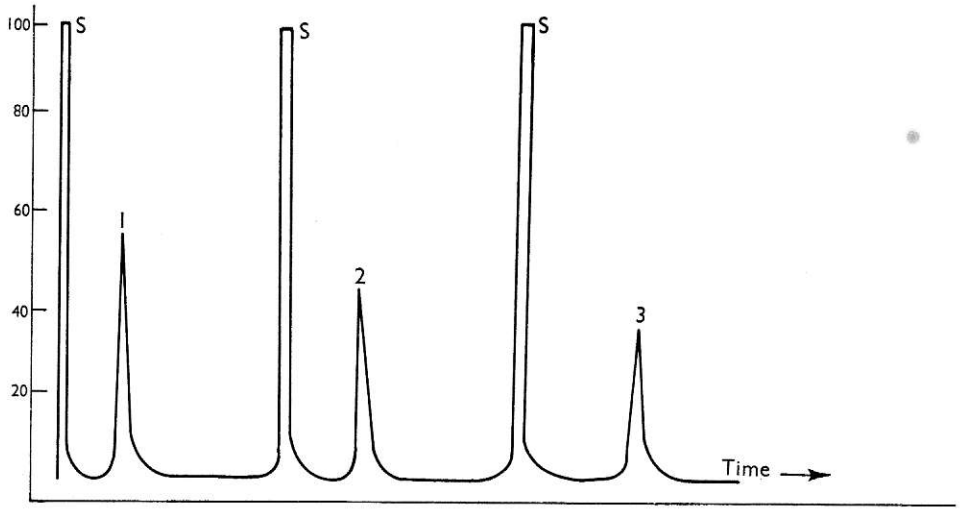

Fig. 1. Characteristic peaks obtained by GLC from solutions of celluloid.
S Solvent peak
1. Standard camphor peak
2. Ping-pong ball extract
3. Extract from doll suspected of containing celluloid

The retention time for camphor on this column is 1·33 minutes. The low sensitivity and high concentration of camphor are used to give a better separation of the camphor and solvent peaks. An approximate quantitative estimation of celluloid is possible but because the efficiency of the extraction cannot be determined at present, it is impossible to judge the accuracy of any figures obtained.

A variety of other plastics including polypropylene, polyethylene, perspex, fluon, P.V.C., bakelite and co-polymer of propylene and ethylene were extracted and examined under identical conditions. The results showed the absence of camphor and no interfering peaks.

Nitrate: The development of the blue colour is not specific for nitrate. The colour will develop in the presence of such oxidising agents as chromates, etc., which may be present in pigments used to colour the plastic. However, the use of stannous chloride solution followed by dilution will distinguish between the colour due to other oxidising agents and that due to the presence of nitrate.

The diphenylamine reagent should be freshly prepared before use since it readily develops a blue colour from atmospheric oxidation.

Celluloid: The rate of burning and the manner of burning, may, with experience, be used as a means of distinguishing celluloid from other plastics. Celluloid burns very vigorously (less than 20 seconds for 1 g) to give a very fine "thread-like" ash. The majority of the other plastics examined require much longer to burn (more than 90 seconds) and drip while burning, to leave a wax-like residue.

The type of peak patterns obtained when extracts of toys are examined by GLC with the silicone column are shown in Figure. 2.

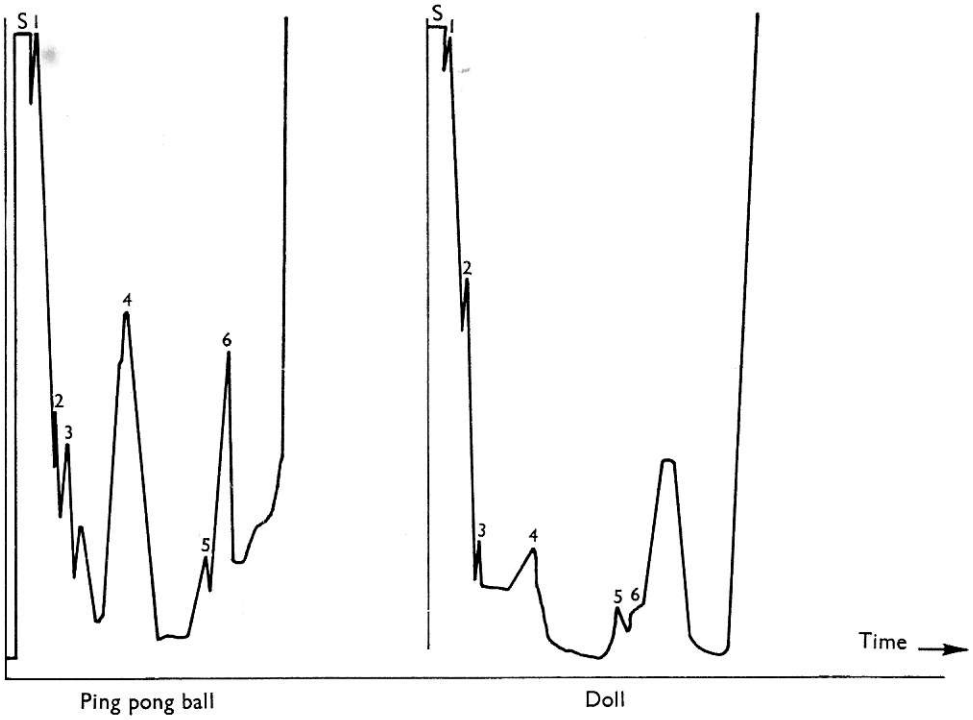

Fig. 2. Characteristic peaks obtained by GLC from toys suspected of containing celluloid.

Peaks 1 to 6 in Figure 2 were obtained from a ping-pong ball and a doll suspected of containing celluloid. In a similar examination of other plastics it was found that they did not dissolve in acetone and no corresponding peaks were observed.

The retention time of camphor on this column is less than 0·25 min. and hence it is difficult to separate it from the solvent peak.

The Semi-Micro Determination of Butter Fat in Fat Mixtures by Gas Chromatography

by A. R. PHILLIPS AND B. J. SANDERS
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A gas-chromatographic method for determining butter fat in other fats is described. Saponification of the fat is followed by acidification and filtration to isolate the water soluble fatty acids. Direct isothermal analysis by gas chromatography to assay the butyric acid content of this aqueous solution makes it possible to estimate butter fat content at low levels with only 0·1-g quantities of fat.

The amount of butter fat in fat mixtures is readily determined by the Reichert-Polenske-Kirschner process. However, the method is time-consuming, and needs a 5-g sample of the fat. A small-scale version of the method is useful but still requires 1 g of the fat, which is often more than is available.

The well-known Hydroxamic Acid Index method (H.A.I.)[1] can be carried out with only 0·1 g of fat, but is empirical and is often unreliable when the proportion of butter fat is less than 20 per cent. The method described here requires only 0·1 g of the fat, is simple and accurate, and can detect as little as 1 per cent. of butter fat in admixture with other fats.

Since butter fat differs from most other fats in that it contains the glycerides of butyric acid, the gas-chromatographic assay of butyric acid, either as such, or after conversion to the methyl or ethyl ester, can be used to determine the butter fat content of a fat. Transesterification methods have been described in which losses of the relatively volatile methyl or ethyl butyrate have been minimised by sealed tube[2] or reflux procedures[3]. In such cases, however, the butyrate peak, on which the analysis is based, is followed by a complex pattern of higher fatty acid esters which must be eluted from the column at an elevated temperature before the next injection can be made.

A simpler procedure is to saponify the fat, acidify in aqueous solution and filter. The filtrate contains only the water-soluble fatty acids, predominantly butyric and caproic, and the content of butyric acid may then be directly determined isothermally using a suitable gas chromatographic column.

Apparatus

Gas chromatograph: Pye 104, model 24. Dual column, with flame ionisation detectors (hydrogen flow 50 ml per min., air 500 ml per min.).
Attenuation: 100 (equivalent to 10^{-10} amps full scale).
Operating temperature: 125° C.
Carrier gas: Nitrogen (flow rate 50 ml per min.).
Columns: 5 ft. × $\frac{1}{4}$ in. o.d. (glass).

Column packing: 5 per cent. Carbowax 15,000 + 0·5 per cent. terephthalic acid on 100–120 mesh, acid-washed Supasorb (B.D.H.) (*Prepare as follows*:—Reflux 100 ml of ethanol with 4 g of Carbowax 15,000 (Griffin & George Ltd.) and 0·4 g terephthalic acid until dissolved. Add 20 g of Supasorb and boil under reflux to remove air. Filter rapidly at the pump (approximately 25 ml of the solution is retained by the Supasorb) and dry the residue under vacuum. After packing the columns, purge with nitrogen at 220° C for 24 hours).

Reagents

1. *Alcoholic KOH Soln.:* 0·5 N solution of KOH in ethanol.
2. *o-Phosphoric acid Soln.:* 5 per cent. solution in water.
3. *n-Butyric acid Soln. (Standard):* a solution of 400 parts of redistilled butyric acid per million in water.
4. *n-Valeric acid Soln.:* a solution of 1000 parts of valeric acid per million in water. The solution should be free of butyric acid.

Method

Weigh accurately, into a 50-ml beaker, between 0·1 and 0·15 g of the fat, and add 3 ml of alcoholic KOH solution. Cover the beaker with a watch glass and heat on a steam bath for 10 minutes. Remove the watch glass and continue heating until the ethanol has completely evaporated. Allow the beaker to cool, add 5·0 ml of water and cover with the watch glass. Swirl gently until the soap has dissolved. Add 5·0 ml of phosphoric acid solution, and swirl gently to coagulate the precipitated fatty acids. Filter through a 9-cm, No. 1 Whatman filter paper into a test tube.

To 5·0 ml of the filtrate add 2·0 ml of the valeric acid solution, mix well, and inject 1 microlitre on to the gas chromatographic column. *n*-Butyric acid is eluted first in approximately $4\frac{1}{2}$ minutes, followed by *n*-valeric and caproic acids in approximately 7 and $12\frac{1}{2}$ minutes respectively. A typical chromatogram is shown in Figure 1. The first peak, which goes off the scale, is due to a residual trace of ethanol which has not been completely eliminated by evaporation on the steam bath.

On to the column inject 1 microlitre of a standard solution made by mixing 5·0 ml of standard butyric acid solution with 2·0 ml of the valeric acid solution. Calculate the peak height ratios of butyric acid to valeric acid for both the sample (R_1) and the standard (R_2).

$$\text{The percentage butyric acid content of the fat} = \frac{R_1}{R_2} \times \frac{400}{10{,}000} \times \frac{10}{W}$$

$$= \frac{0{\cdot}4\,R_1}{R_2 W}$$

where W is the weight in grammes of fat taken for analysis.

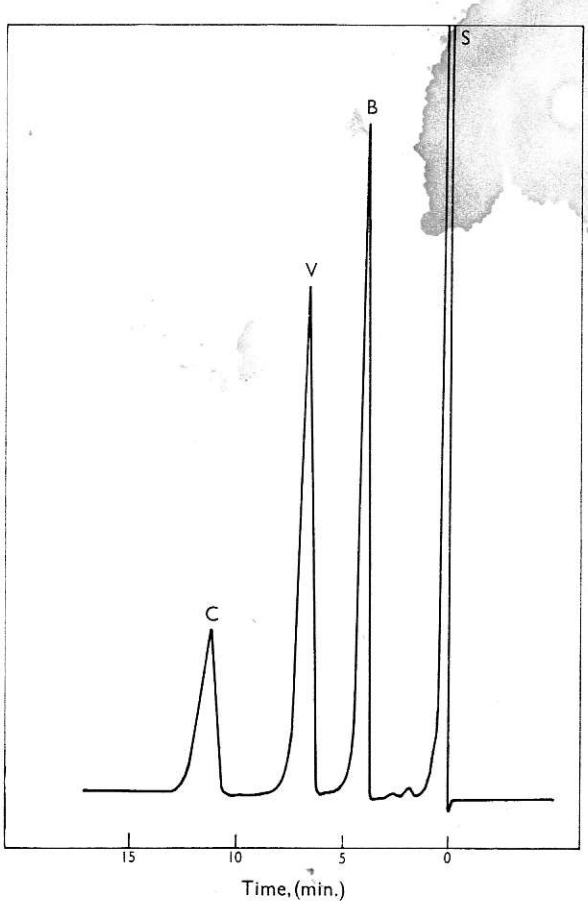

Fig. 1. Chromatogram of genuine butter fat examined in accordance with the method in the text.
S. Residual solvent
B. Butyric acid
V. Valeric acid
C. Caproic acid

The percentage of butter fat in the mixture can then be calculated on the basis of the average butyric acid content of butter fat as determined by this method (*viz.* 3·6 per cent.)
(*Note*: *Flush the micro-syringe out well with distilled water after use to prevent possible corrosion of the plunger by residual traces of phosphoric acid*).

Calibration

Prepare butyric acid solutions at concentrations of 400, 300, 200, 100, 50, 25, and 10 p.p.m. in water. To 5·0 ml of each solution add 2·0 ml of the valeric acid solution, mix and inject 1 microlitre aliquots as above. Calculate the peak height ratios of butyric acid to valeric acid and plot these values against the concentration of the butyric acid standards used. A typical calibration graph

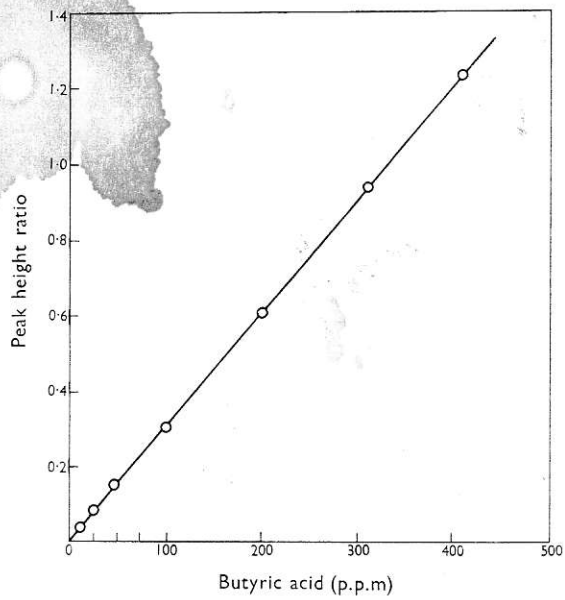

Fig. 2. Variation of peak height ratio (butyric:valeric acid) with butyric acid concentration.

is shown in Figure 2. Although results of sample analysis may be referred to the calibration graph, in practice, variations in gas chromatographic operating conditions make it preferable to examine sample and standard at the same time. Since the calibration graph shows that response is linear, it is sufficient to use a single standard, choosing its concentration to give a peak height similar to that in the sample.

Experimental

The direct analysis of the lower fatty acids, dissolved in organic solvents, has been successfully carried out by gas chromatography[4]. Analysis of aqueous solutions of such acids appears to be more difficult and initial experiments, using 10 per cent. of diethylene glycol succinate and 2 per cent. phosphoric acid on crushed firebrick (Silocel C22) were not particularly satisfactory. When aqueous solutions of butyric and valeric acids at a level of 400 p.p.m. were injected on to this column, good peaks and clear separations were obtained, but severe base line draft occurred, presumably because of excessive column bleed caused by the water injected.

Some experiments were therefore made with a polyglycol/terephthalic acid phase. Terephthalic acid has a low volatility and water solubility and has even been proposed as a support material for the analysis of strongly polar substances including aqueous solutions, the particles being coated with various liquid phases including polyglycols.[5] In combination with Carbowax 20M, it has

also been used as a stationary phase. Using Carbowax 15,000 (obtained from Griffin & George Ltd., and similar to Carbowax 20M) and a proportion of terephthalic acid which was considerably in excess of the amount required to esterify the free hydroxyl groups of the polyethyleneglycol, an acid washed Celite-type support (Supasorb) was coated as indicated under column preparation. This column, after conditioning at 220°C, gave well-shaped peaks, good separations and a stable base line using aqueous injections of the fatty acids. There was no evidence of adsorption of butyric acid on the column, the variation of response with concentration being linear from 400 p.p.m. down to the limit of detection.

The *n*-butyric acid used as a standard was distilled and its purity was 99·9 per cent. (by titration). Gas chromatography showed no evidence of other fatty acids in the butyric acid thus purified. *n*-Valeric acid was chosen as the internal standard because it is eluted between butyric and caproic acids, is clearly separated from both, and does not occur naturally in common oils or fats.

Filtration and direct analysis of the aqueous solution of water-soluble fatty acids from the saponified sample was chosen in order to keep the method as simple as possible. Distillation and solvent extraction could have been used, but would have introduced further steps, with the possibility of incomplete recoveries. The removal of ethanol subsequent to saponification was necessary to prevent solution of higher fatty acids. Furthermore, ethanol could not be eluted satisfactorily from the chromatographic column used. The incorporation of a calcium carbide plug at the end of the column showed that the water from aqueous injections was eluted rapidly under the conditions used, and was clear from the column before butyric acid was eluted.

Results

The percentage of butyric acid in 19 different samples of butter fat was determined by this method. These had been submitted as routine samples under the Food & Drugs Act from various sources, and had been judged genuine using classical procedures. They included 3 New Zealand, 1 Australian, 1 Danish, 1 Irish and 1 Welsh, the remainder being of unknown origin. The mean content of butyric acid found in this group of samples was 3·62 per cent. The individual percentage results were as follows:—

3·38, 3·42, 3·44, 3·45, 3·47, 3·48, 3·52, 3·52, 3·58, 3·58, 3·60, 3·65, 3·70, 3·78, 3·79, 3·83, 3·87, 3·90, and 4·00.

The average figure found appears to be in reasonable agreement with the figure in the literature. According to Richmond[6] the usual variation likely to be found in the butyric acid content of butter fat is from 3·0 to 4·5 per cent., although market butter would be unlikely to show such wide variations.

The figure of 3·6 per cent. has been used subsequently to calculate the amount of butter fat in unknown mixtures. It is not likely that estimates based on this average value would be in error by much more than 10 per cent.

A series of mixtures of butter fat and corn oil were prepared accurately by weight and were then analysed on two separate occasions by this method using 0·1 gramme of the fat. The results obtained are given in Table I.

TABLE I

DETERMINATION OF BUTTERFAT IN CORN OIL BY GLC

	Butyric acid content *per cent.*	Butter fat present *per cent.*	Equivalent butter fat found *per cent.*
Butterfat	3·65	100	—
Corn Oil	0	0	0
Mixture A	1·86	49·8	51·1
	1·84		50·4
B	0·95	25·1	26·1
	0·94		25·7
C	0·34	9·7	9·3
	0·34		9·3
D	0·17	4·7	4·7
	0·18		5·0

Five samples of margarine (two containing no butter and three containing 10 per cent. of butter) were examined by the proposed method and also by the H.A.I. procedure with the results shown in Table II.

TABLE II

DETERMINATION OF BUTTERFAT IN MARGARINE

	Butter fat found	
	Proposed method *per cent.*	H.A.I. method *per cent.*
Margarine A (Kosher)	0	9
Margarine B	0	5
Margarine C (10 per cent. butter)	10·5	20
Margarine D (10 per cent. butter)	10·0	14
Margarine E (10 per cent. butter)	8·4	14

The data indicate that misleading results may be obtained when the H.A.I. is used to assess butter fat contents as low as 10 per cent., whereas the gas chromatographic method provided realistic results.

As an example of the use of the method when the amount of available fat is somewhat limited, the fats extracted from the milk chocolate coatings of three different samples of biscuits were analysed by gas chromatography using 0·1 grammes of fat, and also by the semi-micro Reichert-Meissl method using one gramme. The results showed reasonable agreement between the two methods and are given in Table III.

TABLE III

DETERMINATION OF BUTTER FAT IN MILK CHOCOLATE

	Butter fat found	
	Proposed method *per cent.*	Reichert-Meissl *per cent.*
Milk chocolate A	12·0	14·7
Milk chocolate B	12·5	12·5
Milk chocolate C	17·3	18·7

Summary

The method presented has been found reliable for the examination of butter, margarine and of butter fat mixtures. It has been used for routine examination of fat extracted from foodstuffs, *e.g.* cream soups containing butter or milk as ingredients, fish and meat pastes with butter, biscuits claiming the presence of butter or milk, and butter sugar confectionery. It has proved of particular value when the amount of fat available for analysis is limited, and the proportion of butter fat in the total fat is low. The procedure is simple and analysis of a number of samples can be completed in rapid succession, since only sufficient time (about 12 minutes) for caproic acid to be eluted from the column is necessary before injecting the next sample.

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A Note on the Comparative Composition of Some Scottish Shortbread and Shortcake Biscuits

by D. PEARSON AND F. GOUSSOUS*

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Compositional data and values obtained on the extracted fat of shortbread and shortcake biscuits are tabulated. Whilst there were only slight differences in the basic components of the samples analysed, major variations were found in the composition of the fats extracted. There is evidence that shortbread contains mostly butter fat but none was found in the samples of shortcake biscuits examined.

Sampling

Three brands of Scottish shortbread and two brands of shortcake biscuits were purchased from local retail shops and replicate analyses made to obtain the figures listed in the tables.

Methods of Analysis

Moisture was determined by drying at 100°C, protein (N × 5·7) by the macro-Kjeldahl method, sugars by the Lane and Eynon method[1,2], and the ash by incineration at 500–550°C. Salt was determined on the ash by water extraction and direct titration with silver nitrate solution, using potassium chromate as indicator. Fat was extracted with light petroleum ether (b.p. 40–60°C) in a Soxhlet apparatus. The extracted fat was examined for iodine value (by Wijs's method), for the hydroxamic acid index (HAI)[3], and by the semi-micro Reichert-Polenske-Kirschner process[4,5].

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Discussion

McCance and Widdowson[6] have quoted data obtained on one sample of shortbread (prepared from a domestic recipe comprising flour, butter and castor sugar) as:—water 5·0 per cent., sugars (as invert sugar) 16·1 per cent., starch and dextrins (as glucose) 48·8 per cent., protein 6·1 per cent., fat 27·2 per cent., available carbohydrate (as mono-saccharides) 64·9 per cent., chloride 141 mg per 100 g.

TABLE I

COMPOSITION OF SOME BRANDS OF SHORTBREAD AND SHORTCAKE BISCUITS

	Shortbread			Shortcake Biscuits	
	A	B	C	D	E
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Moisture	2·6	3·1	2·4	2·4	2·1
Protein (N × 5·7)	4·0	4·5	3·8	5·1	5·1
Total Sugars (as sucrose)	20·4	20·5	17·7	19·8	19·5
Total Ash	1·1	1·1	0·9	1·1	0·9
Salt (NaCl)	0·43	0·52	0·51	0·40	0·36
Fat	26·8	24·7	28·1	23·0	23·9

TABLE II

CHARACTERISTICS OF FAT EXTRACTED FROM SHORTCAKE AND SHORTBREAD SAMPLES

	Shortbread			Shortcake Biscuits	
	A	B	C	D	E
Iodine Value	41	36	40	44	75
Reichert Value	22	28	23	2	1
Polenske Value	2·3	2·1	2·7	0·6	0
Kirschner Value	19	23	17	—	—
Hydroxamic Acid Index	8·7	10·3	8·0	0	0

The Authors' analytical results on three brands of Scottish shortbread and two of shortcake biscuits are quoted in Tables I and II. These show only slight differences in the basic components in the five samples. The shortbreads contained a little more fat and slightly more moisture and salt. The higher protein content in the shortcake biscuits is compatable with a higher flour content. Since genuine butter fat gives a mean Reichert value of about 30, corresponding to a Kirschner value of 25 and an average hydroxamic acid index of approximately 11·0, the fat in shortbreads would appear to contain 80 to 95 per cent. of butter fat. The low HAI and Reichert values obtained on the fat of the shortcake biscuits examined indicate that no butter fat was used in their manufacture. The variation in iodine values of the shortcake biscuits indicates the use of quite different fats in their preparation.

The Authors thank Miss J. Riddiford for carrying out some of the analytical work.

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The Determination of Cyclamates in Sweetening Tablets

by G. S. MEADOWS
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The sulphate formed by the action of nitrous acid on cyclamates is determined by titration with standard alcoholic barium perchlorate solution after purification by ion exchange. The method has been applied successfully to determining cyclamate in mixed sweetening tablets, and saccharin does not interfere.

Cyclamate has been determined by direct titration with sodium nitrite solution using starch—iodide as external indicator[1]. This technique was improved by Richardson and Luton who detected the end point electrometrically[2]. Most of the methods, however, are based on the determination of one of the products of the nitrous acid—cyclamate reaction. Rees[3], and Richardson and Luton[4], measure by gas chromatography the cyclohexene produced in this reaction whilst the official method of the Association of Agricultural Chemists[5], and that of Davies[6], measure the sulphate produced, the former by the usual gravimetric procedure and the latter by titration of excess barium with E.D.T.A.

The semi-micro method of Fritz and Yamamura[7] is a very useful one for the direct volumetric determination of sulphate and it was thought that this might form the basis of a suitable method for cyclamates as an alternative to those already mentioned. In this method, interfering cations are removed from solution by passage through an ion-exchange column and a portion of the eluate is titrated with barium perchlorate solution using thorin as indicator. The sodium derived from the sodium nitrite was found to be satisfactorily removed by ion exchange but the nitrite seriously interfered with the titration. However, by boiling the eluate, the nitrite was readily destroyed and satisfactory titrations resulted.

Because of the importance of the ion exchange procedure and the relatively high concentration of cations in solution, it was necessary to ensure that the column was always sufficiently activated before use.

The method is equally applicable to calcium cyclamate, sodium cyclamate and cyclamic acid and, using the procedure described, it is possible to determine small quantities of these compounds conveniently and accurately. Satisfactory results were obtained when the method was applied to sweetening tablets containing both cyclamate and saccharin. Preliminary trials have shown that the method promises to be satisfactory also for determining cyclamates in soft drinks, and results of application of the method to this field are being collated.

Apparatus

1. Ion exchange column: Approximately 1·5 cm internal diameter, 20 cm long, with a tap, and containing Amberlite resin 1R—120(H) to a depth of 5 cm. The column is regenerated by treatment with 100 ml of 3 N HCl and washing with water, after each experiment.
2. Burette: 10 ml capacity with subdivisions of 0·02 ml.

Reagents

1. *Alcoholic barium perchlorate Soln.:* A 0·005 M solution. Dissolve 1·7 g of barium perchlorate in 200 ml of distilled water and dilute to 1 litre with Industrial Methylated Spirits (99 per cent.)
2. *Thorin Indicator:* Dissolve 0·2 g of 1-(*o*-arsonophenylazo)-2-naphthol-3:6-disulphonic acid, sodium salt (thorin) in water, and dilute to 100 ml.
3. *Sodium nitrite Soln.:* 10 per cent. in water.
4. *Sulphuric acid:* 0·01 N H_2SO_4 solution.

Method

STANDARDISATION OF THE BARIUM PERCHLORATE SOLUTION

Pipette 5·0 ml of 0·01 N sulphuric acid solution into a 100-ml conical flask. Add 20 ml of industrial methylated spirits (99 per cent.) and two drops of indicator. Titrate with the barium perchlorate solution, swirling the flask and contents vigorously throughout the titration. The end point is a delicate, but quite definite, change from yellow to pink and is seen best in good natural light; bright light and artificial light should be avoided.

From the titration result, calculate the factor F (mg per ml) for the perchlorate solution as follows:—

$$F = \frac{5{\cdot}0}{\text{titre}} \times 0{\cdot}896 \text{ for cyclamic acid}$$

$$\text{or} \begin{cases} \times\ 1{\cdot}006 \text{ for sodium cyclamate} \\ \times\ 1{\cdot}0815 \text{ for calcium cyclamate} \end{cases}$$

PROCEDURE FOR SWEETENING TABLETS

Dissolve an accurately-weighed quantity of the powdered tablets, equivalent to about 0·15 g of cyclamate, in distilled water. Add a small piece of litmus paper, and if necessary, dilute hydrochloric acid dropwise until the solution is just acid, and make up to 100 ml. Pipette 5 ml of this solution into a 100-ml, narrow-necked, conical flask, dilute with water to about 30 ml, and add 1 ml of sodium nitrite solution. Cover the neck of the flask with a watch glass, and boil the mixture gently for $1\frac{1}{2}$ minutes. Cool the solution, transfer it quantitatively to a 50-ml calibrated flask and dilute to the mark with water. Pass this solution through the ion exchange column, discarding the first 2×10-ml of eluate. Transfer 10 ml of the remaining eluate by pipette into a 100-ml, conical flask, and boil gently for $1\frac{1}{2}$ minutes taking care to avoid loss of contents by bumping. Cool the solution, add 40 ml of industrial methylated spirits (99 per cent.), two drops of indicator and titrate with the barium perchlorate solution as described under 'standardisation'. Record the titration (A ml).

Carry out a blank determination by repeating the above procedure using 5 ml of water in place of the 5 ml of prepared cyclamate solution. (B ml).

Using the factor found in the standardisation procedure, calculate the amount of cyclamate per tablet from the formula:—

$$\text{Cyclamate per tablet (mg)} = \frac{100\ F\ (A-B) \times W}{w}$$

where w = weight of sample taken,
and W = average weight of a tablet.

Control experiments

Solutions containing varying amounts of sodium cyclamate, saccharin sodium and sodium bicarbonate were prepared as follows:

Solution A: 0·2 g of sodium cyclamate in 100 ml of solution.

Solution B: 0·24 g of sodium cyclamate, 0·024 g of saccharin sodium, and 0·096 g of sodium bicarbonate in 100 ml of solution.

Solution C: 0·144 g of sodium cyclamate, 0·120 g of saccharin sodium and 0·096 g of sodium bicarbonate in 100 ml of solution.

Aliquots, each of 5 ml of these solutions, were taken for assay as above, and the results are shown in Table 1 on the basis of the equivalent amount of cyclamate present in the final solution which was titrated. The mean percentage recovery of these 12 results is 99·88 and the standard deviation 0·79. The results for solution B and C show that saccharin does not interfere with the determination.

TABLE I
RECOVERY OF CYCLAMATE FROM MIXED SWEETENERS

Solution	Quantity of Cyclamate present *milligrammes*	Quantity of Cyclamate found *milligrammes*	Recovery *per cent.*
A	2·0	1·97	98·7
	2·0	1·99	99·5
	2·0	1·99	99·5
	2·0	2·01	100·5
B	2·4	2·42	101·1
	2·4	2·34	97·5
	2·4	2·40	100·0
	2·4	2·41	100·5
C	1·44	1·45	100·8
	1·44	1·44	100·2
	1·44	1·43	99·5
	1·44	1·45	100·8

Blank values were determined, and in all cases were within the range 0·07 to 0·10 ml. For routine purposes, therefore, it should not be necessary to carry out a blank determination for each assay but an average value of 0·08 ml can be used, periodically checking that this is satisfactory.

Table II shows the results obtained on typical mixed sweetening tablets:

TABLE II
DETERMINATION OF CYCLAMATE IN SWEETENING TABLETS

Quantity stated *mg per tablet*		Quantity of Cyclamate found
Sodium cyclamate	Saccharin	*mg per tablet*
40	4	38·3
		38·7
50	5	48·9
		49·6
		48·3

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Book Review

GAS CHROMATOGRAPHIC ANALYSIS OF DRUGS AND PESTICIDES. By BENJAMIN J. GUDZINOWICZ. Pp. 616 + ix. London. Edward Arnold Ltd., 1968. Price £13.

This book, first published in America in 1967, is Volume 2 in a series of monographs on Chromatographic Science. It is divided into two parts (1) the theoretical approach to Gas-Liquid Chromatography and (2) the analytical methods and technique as applied to drugs and pesticides.

The three chapters in Part 1 deal with *a.* the fundamentals of gas-liquid chromatography, general theory and principles, column efficiency, separating power and resolution, *b.* detectors—operating principles and theory, hydrogen flame ionization, argon ionisation, electron affinity and coulometric detectors, and *c.* qualitative and quantitative methods of analysis.

Part 2 concerns the application of gas-liquid chromatography to the analysis of drugs and pesticides. Eight chapters are devoted to drugs and pharmaceuticals. The subtitles of each chapter indicate the range of drugs examined:—phenothiazines and barbiturates, phenylethylamine-type and tryptamine-indole base alkaloids, morphine, nicotine, and pyrrolizidine-related alkaloids and marihuana cannabinols, antihistamines, high boiling amine anaesthetics and vitamins and a chapter on miscellaneous drugs and pharmaceuticals.

The final chapter in Part 2, comprising approximately one third of the volume, presents a wide coverage of the examination and analysis of pesticides, herbicides and related compounds including carbamates and triazines.

In the Analytical Section the Author has presented the results of numerous investigations carried out by many researchers in the fields of drugs and pesticides, from a non-theoretical approach and has included throughout the section a very comprehensive collection of data tables, and numerous illustrations of actual chromatograms.

Whilst the price of the book is rather high by average standards, the reviewer considers it to be an invaluable basic reference work with the added advantage that it is backed by a wealth of practical information. As far as Public Analysts are concerned, whether they are already involved in the application of gas-liquid chromatography to drugs and pesticides or are contemplating the purchase of this very versatile instrument, this book can be recommended as the analysts' Book of the Month.

(MISS) A. COOK.

THE LAW OF WEIGHTS AND MEASURES (SUPPLEMENT) By JOHN A. O'KEEFE. Pp. 80 + x. London; Butterworth and Co., 1967. Price 25s.

An extract from the Preface suitably describes the purpose of this volume, and reads—

"Since the *Law of Weights and Measures* was published in June 1966, there have been some new statutory provisions, interpretations by the High Court which affect the law in various ways and administrative directives from the Board of Trade which have added new procedures and clarified the actions to be taken in various contingencies.

"None of these particular matters is in itself of major importance, yet taken together the changes that they make in the law as stated in the main volume are of sufficient importance to make it necessary that it should be brought up-to-date. This the present supplement seeks to do and its aim is to correct and add to the principal volume so that together they present the position as at the 1st September 1967.

"The subject is one of constant concern to traders, administrators and the public and is particularly subject, in its flexibility, to changes in content and the elucidation of judicial decisions".

J. H. E. MARSHALL.