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Report on Added Water in Bacon, Ham and Chicken

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A report prepared for the Association of Public Analysts.

The results of analysis of routine samples of bacon, ham and canned ham in natural juices and prepared chickens taken under the provisions of the Food and Drugs Act have indicated that in many cases these articles contain excessive amounts of added water. Public Analysts have expressed their concern that modern methods of processing may have led to excessive adulteration by this means. In order to obtain further information on the extent to which water may be incorporated in these articles a survey was conducted covering the period 1 January to 30 June, 1977, in which analyses carried out in Public Analysts' laboratories were collected; the results are summarised in this paper.

Seventeen Public Analysts' laboratories contributed and results on the following commodities were obtained: canned ham in natural juices, gelatin added; bacon, vacuum packed and open; ham, vacuum packed and open; whole chickens; chicken pieces.

The data are summarised in the accompanying figures and tables, together with explanatory text and critical comment.

In the case of canned ham in natural juices, bacon and ham, the results are reported as percentage equivalent of raw pork.

The figure obtained by deducting the meat content from 100 represents the added water plus any mineral salts and sugars which may have been added during the curing and cooking processes.

The determination of true added water in the articles requires additional analysis and where such analyses have been made, it is found that the true added water is between 2 and 3 per cent. below the difference figure.

We believe that, in the case of cured meats, it is appropriate to set a standard for minimum meat content rather than for added water.

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For chicken, on the other hand, the difference figure should not be seriously in error as an estimate of added water and the results are presented in this fashion. This presentation compares with the method adopted in forthcoming Regulations.

Canned Ham in Natural Juices, Gelatin Added

Analysts were asked to separate the jelly from the meat by simple dissection, weigh the two components and express the weight of jelly as a percentage of the whole. The meat and jelly were then recombined and macerated to produce a homogeneous mass. This was analysed by conventional methods and the raw pork equivalent calculated using the factor, nitrogen = 3·45 per cent.[1] of lean meat without correction for added gelatin, i.e.

$$\text{Total meat (as raw pork)} = \text{fat (per cent.)} + \frac{\text{Total N (per cent.)} \times 100.}{3{\cdot}45}$$

Results for meat content on a total of 292 samples were returned and are summarised in the histogram, Figure 1.

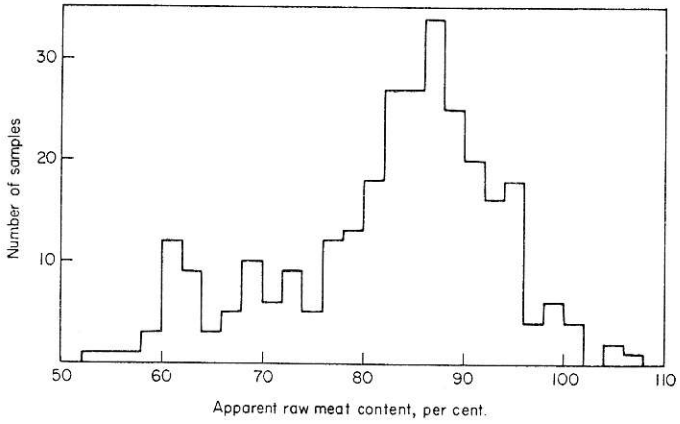

Fig. 1. Apparent meat content of canned ham in natural juices, gelatin added.

The figures, showing a range between 53 and 108 per cent. of total meat, demonstrate a most unsatisfactory situation. Sixty-five samples, or 22 per cent., contained over 20 per cent. of added water. It is tempting to suggest that the histogram shows that two qualities of this commodity are manufactured, and it is Public Analysts' experience that certain brands are consistently better or worse than average. Indeed it is believed that at least two different standards, namely 70 and 90 per cent., are in use by different sections of the trade.

Clearly, it is most unsatisfactory that products sold under the same name should differ so widely in composition.

It might be thought that this difference would be reflected in the proportion of separable jelly. Results were returned for proportion of jelly on 94 samples and these are compared with meat contents in Table I. Inspection shows that there is no correlation between the figures.

TABLE I

CANNED HAM IN NATURAL JUICES, GELATIN ADDED

Jelly, *per cent.*	Apparent raw meat, *per cent.*	Gelatin, *per cent.*	Jelly, *per cent.*	Apparent raw meat, *per cent.*	Gelatin, *per cent.*
20·7	63·6		26	87·2	2·3
20·7	81·9		31	81·8	2·1
17·9	79·7		28	78·1	1·7
30·0	85·5		21	71·1	0·9
26·5	61·6		31	81·6	1·7
10·2	80·8		28	86·4	2·8
6·3	90·6		27	80·2	1·9
27·7	73·6		20	68·0	1·9
25·0	96·0		36·1	93	
37·0	83·7		27·2	89·1	
22·0	91·4	3·0	16·3	82·2	
21·0	91·3	2·8	26·1	86	
20·0	89·9	3·0	16·7	88·8	
25·0	88·8	3·0	21·7	82·1	
25·0	87·2	3·4	24·6	94·8	
16·5	86·0	2·5	23·9	88·7	
21·0	88·2	4·9	18·0	77·4	
20·0	92·3	4·1	21·7	84·6	
23·0	92·5	4·2	25·0	88	3·3
23·0	94·3	2·5	34·5	85	5·4
21·0	105·1	1·5	16·5	64·5	1·0
11·0	98·1	1·2	24·0	89·5	3·8
17·0	107·5	2·8	24·0	94	4·6
17·0	87·7	0·9	29·7	104·3	1·7
12·9	96·6		24·6	81	1·6
10·5	98·6		24·4	86·5	4·5
17·3	98·5		20	89	1·3
17·1	85·9		23	68	2·1
30·0	91·3		31	62	1·7
32·4	95·7		26	85	1·1
24·0	86·1		15	82	1·4
26·9	88·1		18	82	1·9
19·1	84·0		18	76	1·7
33·5	94·5		33	83	3·5
23·1	91·5		14	81	2·2
4·0	80·7		24	84	2·3
5·7	80·0		24	85	2·7
20·0	94·1	1·9	18·5	80·8	
21·0	95·2	2·1	17	100	
21·0	94·6	2·1	14	85	
19·0	93·9	1·2	28·9	85·3	
18·0	86·6	3·0	19·0	86·1	
27·0	84·5	1·7	21·7	79·7	
26·0	78·5	1·7	1·7†	71·5	
26·0	73·0	1·3	23·7	82	
9·7	98·3		24·2	86·8	
22·9	82·7		19·0	86·2	1·0

† 7 lb can with very little separable jelly.

It must be emphasised, however, that the figures for meat content do not include any correction for added gelatin. Some analysts returned figures for total gelatin, also shown in Table I. From these it appears that as much as 3 per cent. of a low setting point gelatin might be added. If correction is not made for such an addition, it inflates the apparent meat content by approximately 15 per cent.

Further work is required to establish the contribution of gelatin added as such, but in our view the poorer quality products are quite unsatisfactory and

the remainder are capable of some improvement. The Regulations define meat content as including cured meat which commences as being about 90 per cent. meat and 10 per cent. water and hence a target figure of 90 per cent. should be set. We believe also that the presentation of the article is important to the consumer and the presence of as much as 30 per cent. of jelly is unacceptable, even if the total meat content is satisfactory.

It should be remembered that the majority of canned hams are designed to be sliced before being served and therefore, even if chilled before carving, much of the jelly is likely to be wasted. It is our opinion that, in addition to a standard for minimum meat content, a tolerance for the maximum proportion of separable jelly should be established. This would avoid the difficulties involved in defining "added gelatin". A standard of 20 per cent. might be an acceptable starting point for discussions.

Bacon

The products sampled were bacon joints and rashers. After mincing and homogenising, each sample was analysed as in the case of canned ham and the equivalent raw meat content calculated as before. One hundred and fifty-one samples were examined and the results are illustrated in the histogram, Figure 2.

Unfortunately, information whether the product was joint or rashers, vacuum packed or open was not available in all cases and no conclusions can be drawn regarding distinction on this basis.

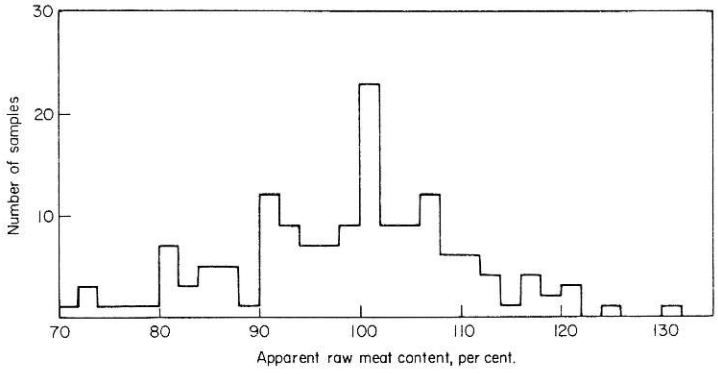

Fig. 2. Apparent meat content of bacon, vacuum packed and open.

The distribution of results shows a maximum at about 100 per cent. The spread, however, is unsatisfactory, as many as 29 samples showing figures below 90 per cent. of meat.

Ham

Ninety-eight samples were examined. They were treated in the same manner as for bacon and the results are illustrated in Figure 3.

Once again, information whether the sample was a joint or sliced, vacuum packed or open was incomplete.

No clear picture emerges from an examination of the results, but it cannot be regarded as satisfactory that 54, or over half, of the samples examined,

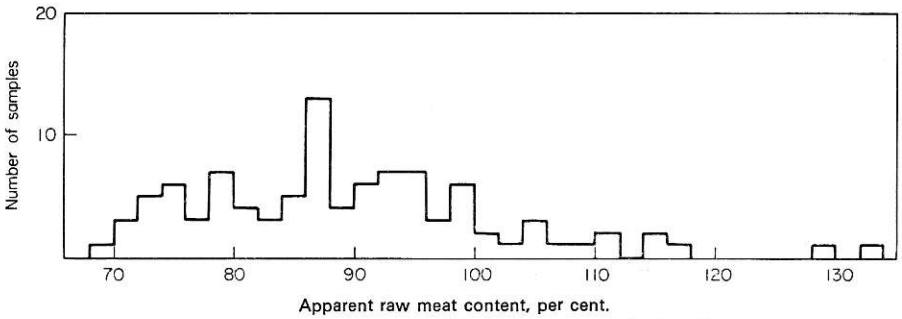

Fig. 3. Apparent meat content of ham, vacuum packed and open.

contained less than 90 per cent. of meat. The type of cure will obviously have some influence on the result and it may be noted that the two exceptionally high results consisted of Parma Ham.

Chicken

The analysis of whole chicken carcasses is somewhat complicated by the proposed adoption of the E.E.C. Council Regulation[2] laying down standards for the water content of fowl carcasses. It is understood that the method of analysis laid down in Annex III of this Regulation is likely to be adopted as official in the United Kingdom. Heretofore analysts have always adopted the so-called Stubbs and More[3] calculation for the determination of meat content, the analysis being carried out on the flesh after separation from the skeleton. For the purpose of this survey they were given the choice of using either their normal procedure or the Annex III method, or, if possible, reporting results by both methods. In the event, all the results reported were carried out by the Stubbs and More procedure using the same calculation as in the case of bacon and ham and assuming a nitrogen content of 3·7 per cent. for fat-free chicken meat[4]. The extraneous water content of the carcass was obtained by difference, making an allowance for the weight of the skeleton. The results are illustrated in Figure 4. Ninety-nine results were reported and it will be seen that figures as high as 30 per cent. of extraneous water were obtained. We regard this as quite unsatisfactory and feel that the maximum content of extraneous water in frozen chickens should be not more than 5 per cent.

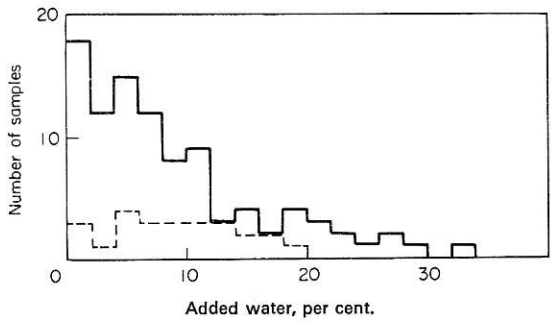

Fig. 4. Added water content of whole chicken (———) and chicken pieces (– – – –).

Chicken Pieces

Twenty-five samples of chicken pieces were reported and the results are illustrated by the dashed line in Figure 4. It would appear that the picture is very similar to that given by the results obtained with whole chickens.

References

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2. Council Regulation (EEC) No. 2967/76 laying down common standards for the water content of chickens, hens and cocks.
3. Stubbs, G., and More, A., *Analyst*, 1919, **44**, 125.
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The Relevance of Reliable Reactions to Analytical Practice

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The substance of a talk given by Dr Stephen at the Annual Conference of the Association of Public Analysts at York on 22 April 1977.

It may well be that a better title would be "The scope of chemical reactions in analytical practice", but the purpose of this talk is to restore some of the imbalance which black-box analysis has brought to analytical chemistry.

The oldest reaction we know which utilises an organic reagent was used by Pliny the Elder in the first century A.D. to establish the adulteration of copper sulphate with iron sulphate. Pliny used, as his reagent, an extract of gall-nuts from the oak, *Quercus infectoria* and impregnated papyrus with a solution of his sample. The result was the first recorded spot test using an organic compound.

The reaction between tannin or gallotannic acid and iron salts is well-known to you all. I like to think of it as the original Stephen's Blue-Black—as writing ink, an article of commerce once advertised on the walls of every little village shop throughout the length and breadth of this country.

This reaction has withstood the greatest test of all, that is, of time. I wish the same could be said of many other chemical reactions which have been used or recommended for analytical purposes. I do not propose to spend any time on ill-conceived or misunderstood chemistry. Instead, in the time available to me, let me show you how the analytical chemist can use the reaction of chemical substances for his own ends, i.e. chemical analysis.

There are many ways of considering the reaction

$$A + B \rightarrow C + D.$$

The analyst looks at this as a means to detect or determine *A*, *B*, *C* or *D*. And it is this simple way of looking at chemical analysis which has provided us with many interesting and useful methods. Let me give one or two examples from the field of masking and demasking, so essential to the improvement of selectivity in analytical processes.

The detection and determination of nickel is best achieved by precipitation with one of the dioximes of vicinal diketones, for example, dimethylglyoxime, which in neutral or alkaline solution gives that characteristic scarlet precipitate first observed by Chugaev in 1905 and containing covalently bound nickel. In the presence of appreciable amounts of cobalt, however, the nickel complex is obscured by the more soluble cobalt complex. The difficulty is easily over-

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come as shown by Feigl and Kapulitzas. Sufficient cyanide is added to form the cyanocomplexes, $Ni(CN)_4^{2-}$ and $Co(CN)_4^{2-}$, followed by hydrogen peroxide to oxidise Co(II) to Co(III). Of course, addition of dimethylglyoxime at this stage gives no precipitate. But if a solution of formaldehyde is now added, the free cyanide necessary to maintain the nickel complex in the tetracyano form is removed as glycolic nitrile as is the cyanide arising from the dissociation of the metal complex. This leads to the production of free nickel ions which react at once with the reagent to give the red precipitate. The much more stable hexacyanocobaltate(III) ion (cobalticyanide) is not affected by the formaldehyde. There is enough chemistry in this overall process to make it the subject of several separate discussions, but as a chemical test for traces of nickel in cobalt salts, it is beautiful in its conception.

The development of the aminopolycarboxylic acids as analytical reagents principally by Schwarzenbach and his co-workers almost 30 years ago has given the analytical chemist a powerful new means of achieving the requisite selectivity in the separation and detection of metal ions. Take the classical example of the alkaline earths. I will not describe in detail the several alternatives for the chemical separation of barium, strontium and calcium. This is a problem to which the Midlands Association for Qualitative Analysis has given much attention over the years. The problem is exacerbated by the possible presence of lead, if all these ions are to be separated as their sparingly soluble sulphates. Consider for present purposes, barium and lead in mixtures as the sulphates. These ions have much in common, and without extensive treatment such as fusion with sodium carbonate, they cannot easily be separated. Traces of one may interfere with the detection of the other in tests using chromate or rhodizonate as reagent. However, both sulphates dissolve readily in ammoniacal EDTA from which barium sulphate only can be reprecipitated by adding a solution of magnesium sulphate. Lead and magnesium EDTA are both much stronger complexes than barium EDTA, which can no longer survive under these conditions and which reforms the sparingly soluble barium sulphate. Addition of dilute sulphuric acid, after removal of the barium sulphate, reprecipitates the lead sulphate. A simple solution to a knotty problem.

Most of you will know of the Malaprade reaction—involving oxidation of vicinal dihydroxy compounds such as glycerol, etc. with periodic acid. The result of such a reaction is that the IO_4^- is reduced to IO_3^-. Under normal circumstances, the excess periodate is determined and is the means for the measurement of the periodate consumed by the organic material. However, this does not allow the more favourable equivalence that the determination of the iodate would confer on the determination. Iodometrically, each iodate ion can produce 6 equivalents of iodine. But, of course, under the same conditions, each periodate ion produces 8 equivalents of iodine. The problem reduces to that of determining iodate in the presence of periodate. Not easy, you say. The ions are so similar. But are they? Periodic acid exists mainly as *para*periodic acid, H_5IO_6, a weak dibasic acid. Like phosphoric acid, it readily forms heteropoly acids with molybdate and tungstate. In fact, the Frenchman, Burnel, described the 6-molybdoperiodic acid, $H_2(H_3IO_6.6MoO_3)$

or $H_2(H_3I(MoO_4)_6)$, in 1965. Such complex acids no longer have the oxidising power of the uncomplexed periodic acid. Thus, in the presence of molybdate ions, iodate can be determined by the advantageous amplification process without interference from periodate. We can go one stage further. Oxalic acid is not oxidised by permanganate in the presence of molybdate ions. This indicates how strongly the oxalate is bound in the molybdeno-oxalic acids. If oxalate is added to the molybdeno-periodic acid, the periodate is released and can be determined iodometrically. Thus, the analysis of a mixture of iodate and periodate is not so difficult to achieve, providing the right reactions are used.

There are numerous other examples one could quote from this subject of masking and demasking in which chemical reactions are used simply and elegantly to achieve particular analytical objectives.

But, having mentioned amplification, I would like to show you how multiplication of reactions can provide more favourable conditions for measurement of particular species.

The classical amplification is that of iodine. Reduced to iodide and oxidised to iodate with bromine water, six times the original iodine is then liberated by reaction with an acid iodide solution:

$$I_2 \xrightarrow{SO_3^{2-}} 2I^- \xrightarrow{Br_2} 2IO_3^- \xrightarrow{I^-,H^+} 6I_2.$$

Vieböck used this type of favourable equivalence in his modified Zeisel alkoxyl determination. The alkyl iodide is reacted with bromine in glacial acetic acid to form iodine bromide. This in turn forms iodate when diluted with water so an iodometric finish to the determination gives 6 equivalents of thiosulphate for every methoxyl group in the organic compound.

A similar situation exists in Untersaucher's method for the direct determination of oxygen in organic compounds. The carbon monoxide formed by pyrolysis of the organic material is led over heated iodine pentoxide and the liberated iodine is oxidised to iodate.

An old but not so well-known method for phosphorus in steel depends on the conversion of the conventional ammonium molybdophosphate precipitate into lead molybdate. As the final weighing form, this is interesting in that it contains none of the element being determined but is about 140 times the weight of the original phosphorus. Each phosphorus atom is equivalent to 12 molecules of lead molybdate.

You are all familiar with cobaltinitrite as a precipitant for potassium. This ion is easily formed from Co(II) ions by oxidation with nitrous acid. If the hexa-amminocobaltic ion is added to it, a precipitate of the cobaltinitrite is formed. This can be filtered off, destroyed with acid to form 2 cobalt ions for each original cobalt ion present. The process can be repeated as often as practicable. Repeating it 10 times would give over 1 gram of cobalt from an original 1 mg.

Well, again these examples serve only to indicate the wealth of chemical reactions which can be classified as amplification reactions.

I should like now to turn to some aspects of trace analysis and to illustrate how a basic chemical reaction can be exploited in several ways. The fact that mercury(II) chloride is only very slightly dissociated has, of course, been used in the mercurimetric determination of the chloride ion. It is not really surprising that organo-mercury halides, such as phenylmercury(II) chloride, are also covalent in character. Not so compounds like phenylmercury(II) nitrate which is completely ionised in aqueous solution. Thus, treatment of a dilute chloride solution with phenylmercury(II) nitrate allows the corresponding chloride to form which can be removed from its aqueous solution by extraction with chloroform. All the chloride is now present in the organic solvent and a variety of methods is now available for its determination. Perhaps the most favoured is gas–liquid chromatography. The thermal stability and volatility of phenylmercury(II) chloride are quite sufficient to allow chromatography to occur at temperatures around 175°C. The sensitivity of the method is dependent on the nature of the compound and the type of detector. With flame ionisation, for example, 10–15 ng of chloride can be detected. With an electron-capture detector this is improved some 10,000 times, when a solvent such as benzene or toluene is used.

The great advantage of gas–liquid chromatography is that useful separations can also be achieved. Mixtures of chloride, bromide and iodide can be resolved and simultaneously determined.

Solution spectrophotometry can also be used for the final measurement. The chloroform solution of phenylmercury(II) chloride can be treated with a solution of sodium diethyldithiocarbamate and the resultant phenylmercury complex absorbs strongly at 297 nm. This procedure will not differentiate between chloride, bromide and iodide and gives only the total halide present. A third method requires the phenylmercury(II) halide to be removed from the aqueous solution with a non-halogen solvent; propyl acetate is quite effective. The organic solution is then vaporised in an atomic absorption machine and the mercury content thus determined.

This type of solvent extraction-gas chromatography process can be applied also to the determination of metals in trace amounts. The environmentally significant metals such as beryllium and chromium can be determined in amounts of 10^{-12} gramme or even lower. In 1920, Morgan and Drew in a paper on certain acetylacetonates stated rather figuratively that "acetylacetone has given wings to the metals, for certain of these compounds are volatile without decomposition". In this same paper, the authors suggest the term "chelate" to describe the caliper-like groups which lead to the binding of a central metal atom in a stable complex ring structure. These chelate compounds find very wide application in analytical chemistry through the participation of organic reagents with metal ions. The β-diketones, of which acetylacetone is the best known example, readily form metal complexes of a chelate character, molecules with no overall charge and with largely covalent properties. Beryllium and chromium acetylacetonates can be distilled without decomposition, such is their volatility, but enhanced characteristics can be imparted to the resultant metal chelates by using appropriately substituted β-diketones. Thus, trifluoro- and hexafluoroacetylacetone give more volatile chelates and thus are more

suited to gas chromatographic methods than the parent acetylacetonates. As with the halides, separations and individual determinations are possible. The conventional β-diketones are not so useful for divalent transition metals for various reasons. But the coordination chemistry of these ions suggests that ligands containing sulphur may produce more useful chelate structures. Monothioacetylacetone, in which one of the keto-oxygens of the parent β-diketone is replaced by sulphur, is a more effective ligand and monothiotrifluoroacetylacetone is better still. Thus, nickel, palladium and platinum can easily be separated on a column heated to little more than 180°C. The determination of nickel is possible in a variety of materials, such as alloy steels, fats and oils, and instant tea powder. The last mentioned is interesting in that the tea plant tends to concentrate metal ions in the young growing leaves. Foreign ions such as nickel may be assimilated by the plant from fungicidal sprays containing nickel dithiocarbamates. Analysis of several samples has shown them to contain about 12 p.p.m. of nickel. The gas chromatographic method seems preferable to the atomic absorption method (AAS), in giving a lower standard deviation.

The exploitation of chemical reactions in this way promises much in the way of useful analytical methods, particularly for the analysis of troublesome combinations of metals, such as potassium and rubidium, niobium and tantalum, zirconium and hafnium and much work is being done to provide more effective ligands for these particular mixtures of metals.

I want to conclude this rather brief account of reaction chemistry with a comment on the usefulness of nephelometric methods. Precipitation reactions especially as applied to conventional gravimetric analysis are considered as rather a bore. Gravimetry is tedious, troublesome and, above all, demands a high degree of experimental ability. Although most precipitants used in gravimetry are of sufficiently low solubility to allow quantitative analysis, solubility effects can be serious especially during the isolation and purification of the precipitate. The determination of the sulphate ion as barium sulphate is a good example. Tractable precipitates are obtained only by adequate digestion and ageing of the precipitate in the mother liquor. If, however, one wishes to determine small amounts of sulphate ion by nephelometry, that is, by measurement of the light scattering of the suspended particles of barium sulphate, then quite different considerations apply in the formation of the precipitate. It needs to be as uniform as possible with a particle size of about 0·5–1·0 microns in diameter. The precipitate should not age rapidly or aggregate readily. It should be the ideal suspension and in very dilute solution this is sometimes difficult to obtain, especially with purely inorganic ionic precipitates.

But there are many advantages to the use of precipitation reactions in this way. It is no longer necessary to remove, isolate and purify the precipitate. Precipitation is generally favourably influenced by the continued presence of the excess of precipitant—the Common Ion Effect—and the full sensitivity of the precipitation reaction can be utilised in a way which is difficult with the gravimetric operation.

Organic precipitants generally give precipitates which are often easier to

handle, and two reagents, amino-chlorodiphenyl and 2-aminoperimidine, show the ease with which nephelometric measurements of low sulphate concentrations can be made.

An interesting method for the determination of traces of mercury in various materials, but particularly in laboratory dusts, depends on a conventional wet digestion of the sample followed by reduction, and volatilisation of the mercury in a stream of air. This is absorbed in dilute nitric acid and a new reaction is used to convert the mercury into a suitable form of suspension for nephelometric measurement. In aqueous solution, mercury(II) ions readily form diphenylmercury, $C_6H_5HgC_6H_5$, on reaction with an aqueous solution of phenylboric acid, $C_6H_5B(OH)_2$. The finely-divided organo-mercury compound is insoluble in water and forms an ideal type of suspension. The method is somewhat less sensitive than AAS but is simple and easily adapted for routine purposes at the p.p.m. level.

Other environmentally significant metals such as zinc, cadmium and lead are equally amenable to this sort of nephelometric process, thus providing useful alternatives to the more costly flame spectrometric methods.

I have tried in the brief time at my disposal to emphasise the role of chemical reactions in the continuing development of sound analytical methods. There are many who say that there is little or no chemistry remaining in modern analytical practice. I am one who thinks it will be a very sad day if and when that happens. In my own work I shall continue to pursue the fascinating subject of analytical reaction chemistry.

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New Approaches to Carbohydrate Analysis in Food

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A paper presented at a symposium on New Approaches to Carbohydrate Analysis in Food, organised by the Association of Public Analysts, at Heskin Hall, near Chorley, Lancashire on 1 April, 1977.

Man analyses food with his taste-sense with respect to sweetness, acidity and bitterness. Fundamentally, evolution has truly pointed the way with respect to the future of food analysis in that the response time of man's taste buds is immediate, no extracellular reagents are required and rapid changes can be detected in a self-sampling mode. Perfection is not achieved in three aspects of the analysis. Firstly, it is difficult to distinguish between two compounds that are both sweet such as sucrose and saccharin, particularly in mixtures. The sensing mechanism is thus limited, based on a selective non-specific sensor. Secondly, it is not quantitative in absolute terms but can be made so by comparison with standardised known solutions. Its strength is its ability to distinguish between two solutions of only slightly differing sweetness. Its third weakness is that other compounds that are not sweet may bind to the taste buds and obscure the senses; indeed, this can happen with some very sweet proteins recently discovered. In total, man's senses with respect to food analysis are of a limited number but are self-renewing and mobile.

What then will be the future basis of man's instruments for performing his food analysis? Logically they should be based on a limited number of sensors made specific for each component of food by the addition of a cheap component such as a disc that converts a widely based sensor (e.g. a thermistor) into a specific sensor. Since every component of man's food has been made by a specific enzyme and often can be modified by a series of types of enzymes (e.g. oxidases, hydrolases) in a specific way, it is evident that there exists in nature a vast range of protein enzymes that will interact either absolutely specifically with a food carbohydrate (e.g. glucose oxidase with D-glucose) or less specifically with a related group of compounds, thus permitting both specific or group analysis. Enzymes will act both on small and large substrates. Other proteins exist in nature that will interact specifically with carbohydrate components of food. Many seeds contain special proteins called lectins that interact with carbohydrate components but these, like antibodies, are more applicable to the analysis of carbohydrates in a macromolecular form and further have no catalytic activity themselves. However, they are useful in their immobilised form on

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supports like agarose as tools for separating carbohydrate-containing entities of food prior to analysis by a sensor. In short, our analysis would be more reliable if separation accompanied sensing by a specific enzyme.

Enzymes can now be presented in a formidable number of ways, each one of which may be suitable for a particular mode of analysis. Thus tubes of glucose oxidase immobilised on the inside of nylon tubes can be purchased from one manufacturer (Miles Laboratories) and used as reaction tubes in an Autoanalyser. This saves one reagent line but the Autoanalyser still has an insatiable thirst for other reagents, illustrating all that is bad in the current analytical approach. Another manufacturer (Leeds & Northrup) has immobilised the glucose oxidase on porous glass beads and sensed the resultant hydrogen peroxide produced by this enzyme with glucose by using a three-electrode amperometric cell. This represents a major step forward. The instrument assays 0–50 p.p.m. of glucose and gives 98 per cent. of its response in less than two minutes. The accuracy is ± 2 per cent. of instrument range and reproducibility is $\pm$ 1 per cent. of instrument range. The flow though the column is 5 ml/minute nominal at $35 \pm 0{\cdot}1$°C. Normal maintenance procedures call for flushing the instrument for 15–20 minutes once every 24 hours with 50 p.p.m. of H_2O_2. The instrument remains within calibration specifications for two weeks before there is a drop in response without this maintenance. The upper limit for glucose concentration is set by the solubility of oxygen in the buffer required for oxidation of the glucose to gluconolactone. The concentration of oxygen in equilibrium with air in the buffer is 8 p.p.m.

An alternative approach to sensing an enzyme reaction is the enzyme thermistor developed by Mosbach, Danielsson, Bergerud and Scott[1]. The thermistor is put in direct contact by placing it in the bed of a microcolumn (0·8–1 ml) of immobilised enzyme and letting the reactants flow over the thermistor tip. The technique was applied to the determination of D-glucose, penicillin G and urea, using glass-bound glucose oxidase, penicillinase and urease respectively. Linear relationships between Δt measured and concentration of substrate present were observed. Solutions were pumped through at 60 ml/hr. and the system was allowed to equilibrate for 1–2 min. Reproducibility was ± 2 per cent. and the Δt recorded was as high as 50 per cent. of the theoretical value.

Having combined a sensor with a specific immobilised enzyme, it is now quite feasible[2] to interpose between the two a separation device such as a dialysis membrane. It is even feasible to immobilise the enzyme on the membrane itself, though if the Autoanalyser dialysis membrane is used it is wise to coat this already set up in its holder and attach the requisite enzyme thereto *in situ*. We have attached dextranase to such a membrane for the analysis of dextran, a common, often unsuspected, impurity in commercial sugar. The carbohydrates produced from the dextran are small enough to diffuse through the membrane and there be assayed. Starch impurities can be assayed in a similar way using immobilised glucamylase. In practice, it is often easiest to keep the dialysis membrane and immobilised enzyme separate. A good all-purpose method of coating dialysis membranes can be based on the method described by Gray, Livingstone, James and Barker[3].

A multistage enzyme process using up to four enzymes in sequence to produce

a product that can be assayed is quite achievable. Thus, we have immobilised the four enzymes required to convert glycogen to fructose so that they will all operate simultaneously at the same pH and temperature. It was necessary to immobilise the enzymes in their separate micro-environments which acted as insoluble buffering agents permitting remarkable changes in the pH optimum of the enzymes by up to two pH units. Indeed, we have thus defined the limitation of the applicability of immobilised enzymes separately or in sensors as that of the physiological range of pH and temperature, which fortunately covers the extremes to be encountered in different foods from vinegar to alkaline media. In order to achieve the multistage sequence from glycogen to fructose it was necessary to use membranes to keep the products and substrates of some of the enzymes apart. One we found very suitable for separating ionic (e.g. sugar phosphates) and non-ionic species (e.g. sugars) was a conventional dialysis membrane on which polyacrylic acid had been grafted by giving the membrane a dose of radiation in the dry state to initiate radical formation and then immersing it in acrylic acid. The conversion of glycogen or starch to fructose used (a) phosphorylase in the presence of phosphate to convert these polymers to glucose-1-phosphate, (b) phosphoglucomutase to catalyse the conversion of the latter to glucose-6-phosphate which then became the substrate of (c) phosphoglucoseisomerase to yield fructose-6-phosphate which is broken down by (d) alkaline phosphatase to fructose. Such high technology should now be replaceable in some cases by the use of immobilised cells (see Gist-Brocades[4]) which can even be obtained in a state where they respire and operate like cells in suspension. It would be necessary to operate the immobilised cells with an inhibitor to cut out the metabolic pathway required. We are very near now to man's use of his own taste-buds in the form of whole cells—membranes, concerted reaction sequences and a sensing mechanism.

The wide-ranging sensor required for food analysis of carbohydrates is an oxygen electrode mated with a specific immobilised carbohydrate oxidase (e.g. glucose oxidase) in a true enzyme electrode. The first commercial instrument of this type (Yellow Springs Instrument Co., U.S.A.) became available this year. Although it can be done, it is quite unnecessary to fix the enzyme to the polythene membrane of the oxygen electrode; close proximity (merely contact) will suffice. It is essential, however, for fast response times to have easy open access between substrate glucose, enzyme glucose oxidase and polythene membrane. We have patented a disc of woven glass fibres (curtain material is ideal) to which is chelated the enzyme. Our "cement" is wholly inorganic (Emery, Novais and Barker[5]) and can be based on prior treatment of the glass with titanium tetrachloride, drying, followed by a water wash and mere contact with the enzyme which then chelates to the glass via a permitted food additive! A linear response to glucose is given by our enzyme electrode and the "magic" disc can be used repeatedly for up to 18 months, although in practice this would never be attempted. By the same chelating procedure we can simultaneously immobilise both invertase and glucose oxidase so that now we have a specific sensor for sucrose, as the product of the first enzyme becomes the substrate of the second. Again there is a linear response to sucrose and, remarkably, the response time is almost the same as the two enzymes gear together. In the same manner, by

immobilising glucamylase and glucose oxidase simultaneously on the one disc, we have made a starch sensor. Since there are at least 50 known enzymes that oxidise substrates with consumption of oxygen that can be sensed by the oxygen electrode and hundreds of enzymes that can be coupled with the fifty, the whole field of analysis of food is opened up. Much of this can be done by buying a cheap robust oxygen electrode and making similar discs by the many methods for immobilising enzymes now available.

The "Tomorrow's World" of food analysis, depicted above, in reality only covers half the area of food analysis. Such methods are excellent for beverages, homogenates, jams and extracts just as current methods are in clinical chemistry for serum and urine. New methods for examining food cellular material, preferably of a quantitative nature, are urgently required. The methods of quantitative histochemistry may well be the answer, particularly in the use of "guided" enzymes, conjugates of a direction-finder protein (Bowen and Barker[6]) such as concanavalin attached to marker enzyme. Similar conjugates based on antibody-enzyme conjugates would also be applicable. Films of food material immobilised by our titanium chelation method would immobilise the cellular matter in a surface film that thereafter could be treated with the conjugate which would adhere very specifically to particular macromolecular components. After suitable treatment the "immobilised" enzyme in the surface film could be assayed.

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The Composition of Rapeseed Meals, Part I: The Determination of the *Iso*thiocyanate Present in *Eruca sativa*, a Cruciferous Seed, Present in Some Rapeseed Meals

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The determination of the total volatile oil of mustard in rapeseed meals by the distillation-silver method, and the G.L.C. method, adding the allyl, butenyl and pentenyl isothiocyanates together, shows good agreement in many commercial samples, but bad agreement in others. This difference, which is very substantial in some rapeseed meals, was found to be due to the presence of the cruciferous seed *Eruca sativa* that contains a glucosinolate which on enzymatic hydrolysis yields 4-methyl thiobutyl *iso*thiocyanate. A G.L.C. method for the determination of this *iso*thiocyanate is described, together with the proportions normally found in *Eruca sativa*.

For many years, rapeseed meals and cakes have been one of the protein concentrates used in the preparation of some compound animal feedingstuffs, and since the early days it has been realised that these meals may contain substances which may be toxic to livestock. Although the possible toxic substances now include some four different groups of compounds the one on which most attention has been focused is the glucosinolates.

Under the action of enzymes these compounds yield *iso*thiocyanates which constitute the so-called "volatile oil of mustard". *Iso*thiocyanates are present in most species of *Brassica* or rape, but it is the allyl *iso*thiocyanate present in the mustard species of *Brassica*, particularly *Brassica juncea*, which causes most concern. The problem arises because the rape grown in some parts of the world includes *B. juncea*.

By the early twenties it had been established that the mustards *B. juncea* and *B. nigra* yielded mainly allyl *iso*thiocyanate while other *Brassicas*, *napus* and *campestris* etc., yielded only the butenyl and pentenyl compounds.

Toxicity tests carried out at the time showed that the allyl compound was approximately four times as toxic as the butenyl and it became desirable, therefore, to avoid feeding rapeseed meals which yielded substantial amounts of the allyl compound or in which the allyl compound predominated.

A determination of total "volatile oil of mustard" was the first step towards an analytical assessment of the possible toxicity of rapeseed meals. In this method, the meal was mixed with white mustard, in case it was lacking in the enzyme myrosinase, mixed with water and the whole digested at blood heat for

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two hours. At the end of the digestion the *iso*thiocyanates thus liberated were distilled into alcohol and ammonia, and finally determined by silver titration.

This method gave a measure of the total *iso*thiocyanates present, but failed to differentiate between the allyl and butenyl compounds. Jorgensen devised the next step by a method based on the determination of the nitrogen content of the thioureas (or, as they were called at the time, thiosinnamines) produced when the volatile oil of mustard was allowed to react with alcohol and ammonia. He recommended that if the nitrogen content of the mixed thiosinnamines exceeded a certain percentage then the rapeseed meal was unsuitable for animal feeding; this method was used for some years to grade rapeseed meals which contained a proportion of *Brassica juncea*.

A major advance was made in 1967 when Youngs and Wetter[1] described a gas liquid chromatographic method which enabled the individual *iso*thiocyanates to be determined.

In this method, the dry, defatted rapeseed meal was mixed with a buffer solution, myrosinase and methylene chloride, and shaken at room temperature for two hours; at the end of this time a known volume of the methylene chloride was examined by G.L.C. Butyl *iso*thiocyanate was used as an internal standard.

Comparison of Methods

A modified form of the G.L.C. method[2] was used in our laboratory for the examination of a number of samples of rapeseed meal from the Indian subcontinent, and the results obtained were compared with the total volatile oil of mustard determined by the official distillation-silver method[3].

The modified G.L.C. method was as follows.

EXTRACTION

Two grammes of dry defatted meal, 15 ml of buffer solution (pH 7), 0·2 g of white mustard and 10 ml of chloroform containing 0·05 per cent. of butyl *iso*thiocyanate were mixed in a centrifuge tube and shaken for 2 hours; the tube was centrifuged and 2 μl of chloroform layer injected into the G.L.C. column.

CHROMATOGRAPHY

Gas Chromatograph, with flame ionisation detector. Column: 1·5 m $\times$ 4 mm glass column packed with 5 per cent. Poly (neopentyl glycol succinate) (Hi Eff 3BP) on Diatomite C-AW-DCMS 80-100 mesh (177–149 microns); column temperature, 85°C; nitrogen carrier gas-flow, 40 ml/min.

Some of the typical results which have been obtained by the two methods are shown in Table I. They show that in some of the samples the total *iso*thiocyanates determined by the G.L.C. method agreed well with the total volatile oil of mustard determined by distillation (the results in each method being expressed in terms of allyl *iso*thiocyanate). In others, on the other hand, a wide difference between the two methods was found, the total volatile oil of mustard determined by chemical means being in some samples more than double the figure obtained by the gas liquid chromatographic technique. This fact indicated that other volatile sulphur compounds or possibly another

TABLE I
*ISO*THIOCYANATES†

Sample	G.L.C. method: Allyl, *per cent.*	Butenyl, *per cent.*	Propenyl, *per cent.*	Total expressed as allyl, *per cent.*	Distillation method, total as allyl, *per cent.*	Difference between the methods as allyl, *per cent.*
P51	0·17	0·49	nil	0·60	1·16	+0·56
I61	0·21	0·67	nil	0·80	0·75	—0·05
I14	0·15	0·54	nil	0·62	0·59	—0·03
I45	0·06	0·12	nil	0·17	1·01	+0·84
P14	0·11	0·35	nil	0·42	0·72	+0·30
I69	0·20	0·62	nil	0·75	0·84	+0·09

† Expressed on the dry defatted meals.

*iso*thiocyanate was present apart from the allyl, butenyl and pentenyl compounds normally present in rapes and mustards.

HIGHER COLUMN TEMPERATURE

The column temperature used for the determination of the allyl, butenyl and pentenyl *iso*thiocyanates was 85°C; approaching that recommended by Youngs and Wetter, and as a first step towards identification of the unknown volatile sulphur compound a higher column temperature of 170°C was employed. This was tried since the previous workers had reported finding additional peaks at this elevated temperature. Accordingly, extracts from the rapeseed meals which gave widely differing results were examined at this temperature. Under these conditions, the allyl, butenyl and pentenyl compounds appeared almost immediately after the solvent peak, but two new peaks, one a large one and the other very much smaller, were obtained, as shown in Figure 1. Extracts from

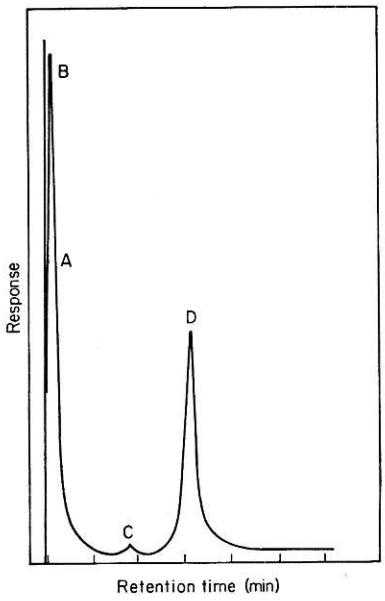

Fig. 1. Rapeseed meal, column temperature 170°C. A, allyl; B, butenyl; C, unknown; D, 4-methyl thiobutyl.

rapeseed meals showing good agreement by the two methods gave no additional peaks at this higher temperature.

Examination of extracts from authentic specimens of *B. nigra, juncea, napus, campestris* and *dichotoma* after enzymatic hydrolysis showed no evidence of peaks at this elevated temperature. The peaks at high temperature, therefore, were a clue to the identity of the material causing the discordant results.

Moreover, this large peak was given by all the samples which showed a marked difference between the two methods and it was found that there was a constant ratio between the peak heights and the difference between the determination by the different methods.

Identity of New Peak

The first question to be answered was, were the compounds giving the new peaks *iso*thiocyanates?

Remembering Jorgensen's thiourea or thiosinnamine test, we converted the allyl, butenyl and pentenyl *iso*thiocyanates into the corresponding thioureas, which are considerably less volatile, by treating with ammonia and alcohol in the following manner.

To 3 ml of the chloroform extract, obtained after enzymatic hydrolysis, add 1 ml of ethanol, and 1 ml of 0·88 ammonia and shake the mixture at intervals for 4 hours. Allow the chloroform layer to separate and chromatograph at 85°C.

Working in this way no peaks were obtained, indicating that all three *iso*thiocyanates had been converted under these conditons to thioureas. Furthermore, the extracts gave no peaks at 170°C. Extracts from rapeseed meals containing the unknown compound were similarly treated, and the major peak no longer appeared at 170°C; the very small peak, however, remained unchanged. This was strong evidence, therefore, that it was an *iso*thiocyanate which was responsible for the difference in the results.

***Eruca Sativa*, Possible Origin of New Peak**

The samples showing this new *iso*thiocyanate were then examined microscopically, and all were found to contain substantial amounts of *Eruca sativa*; whereas samples in which agreement between the two methods was good were found to contain no appreciable amount of this seed.

Eruca sativa is a cruciferous plant which is grown in the northern provinces of the Indian subcontinent together with rape and *Brassica juncea*. At one time, *Eruca sativa* was grown as a single crop, like rape, for its oil content and the cake, after expression of the oil, was used as a feedingstuff for animals.

Pure *Eruca sativa* was difficult to obtain in quantity and after a number of unsuccessful attempts, small samples of pure seed were finally supplied by the Indian Agricultural Research Institute, and by Dr Vaughan of Queen Elizabeth College, London. Our examination of this material showed that on treatment with white mustard and distillation it yielded 0·98 per cent. of volatile oil of mustard expressed as allyl isothiocyanate stated on the dry fat free material. Moreover, when examined by the G.L.C. method, no allyl,

butenyl or pentenyl compounds were found at 85°C, but at 170°C the same large peak was obtained as had been found in the rapeseed meals containing the "unknown". The ratio of peak height to volatile oil of mustard was also the same as that found in the unknowns.

In 1909, it was stated by Hals and Gram[4] that *Eruca sativa* contained mustard oil and Mohammad and Ahmad, in 1945[5], assumed that allyl *iso*thiocyanate was present in the seeds. The former workers were correct in asserting that a mustard oil was present, but in none of the samples we have examined has any evidence of allyl *iso*thiocyanate been obtained.

Apart from the different behaviour when examined by gas liquid chromatography, the low values obtained for nitrogen in the mixed thioureas obtained from the volatile oils yielded by rapeseed meals containing *Eruca sativa* suggested the presence of an *iso*thiocyanate having a higher molecular weight than either the allyl, butenyl or pentenyl compounds.

For example, the mixed thioureas obtained from sample P51 in Table I contained only 17·9 per cent. of nitrogen, whereas if the *iso*thiocyanate present had all been the butenyl compound, the thiourea would have contained 21·5 per cent. of nitrogen.

Our attempts to identify the unknown *iso*thiocyanate were severely restricted owing to the small amounts of pure seeds at our disposal. The chloroform extract obtained from the defatted material after enzymatic hydrolysis was examined by infra-red analysis and this gave the following information.

1. The presence of the *iso*thiocyanate radical was confirmed; the response was very strong and could have masked other possible groups.
2. The compound contained no unsaturated ethylene groups, CH = CH, thus differing significantly from the *iso*thiocyanates present in brassica seeds.
3. Weak responses indicating possible saturated aliphatic groups were shown.

A literature search of *iso*thiocyanates was then carried out and it was found that Kjaer and Gmelin, in 1953[6], had reported the presence of 4-methyl thiobutyl *iso*thiocyanate in seeds of *Eruca sativa* which had been grown in Denmark, and furthermore, they suggested that the glucoside should be called glucoerucin.

We obtained a quantity of Pakistan *Eruca sativa* through the help of Dr Vaughan. From this seed we obtained a substantial quantity of the thiourea which, after purification, gave the following results when subjected to elemental analysis: carbon 40·59 per cent., hydrogen 7·79 per cent., nitrogen 15·96 per cent. and sulphur 35·75 per cent. The theoretical figures for 4-methyl thiobutyl thiourea are: carbon 40·45 per cent., hydrogen 7·87 per cent., nitrogen 15·73 per cent. and sulphur 35·96 per cent. The weak responses obtained on infra-red analysis would be consistent with methyl and butyl groups.

These results confirm the earlier work of Kjaer and Gmelin[6], but it should be pointed out that these workers made no report on the proportion of sulphur in the thiourea. The structure, however, was confirmed by subsequent synthesis.

DETERMINATION OF 4-METHYL THIOBUTYL *ISO*THIOCYANATE

The amount of 4-methyl thiobutyl *iso*thiocyanate present in a rapeseed cake, and consequently the amount of *Eruca sativa*, may readily be determined by G.L.C., using the conditions which lead to its recognition. The major problem is the need to obtain a pure specimen of the *iso*thiocyanate for comparison purposes; this may be overcome by the use of an internal standard. In the initial tests that were made diphenyl was found to make an ideal internal standard by employing a 0·01 per cent. solution in chloroform for extraction after enzymatic hydrolysis. When a relationship, however, had been established between diphenyl and the *iso*thiocyanate it was realised that the diphenyl peak coincided with the very small second peak given by *Eruca sativa*, and this could lead to significant errors if the peak size varied widely from sample to sample. Diphenyl was, therefore, abandoned and fluorene used in its place as the internal standard, although a higher column temperature was required.

In order to compare the retention times and the responses given by fluorene and 4-methyl thiobutyl *iso*thiocyanate, a specimen of the latter compound which assayed 102·8 per cent. was prepared from *Eruca sativa*.

The experimental conditions were as follows.

REAGENTS

1. *Buffer solution*, pH 7·0. Dissolve 5·88 g of disodium hydrogen phosphate dodecahydrate, 0·37 g of citric acid monohydrate in water and make up to 100 ml. Adjust to pH 7·0 if necessary.
2. *4-Methyl thiobutyl isothiocyanate standard.* 0·0375 g dissolved in 10 ml of chloroform containing 0·25 per cent. of fluorene.
3. *Chloroform* containing 0·25 per cent. of fluorene.

EXTRACTION

To 2 g of dry defatted rapeseed meal add 15 ml of buffer solution, 0·5 g of white mustard, and 10 ml of chloroform containing 0·25 per cent. of fluorene, shake for 2 hours and centrifuge. Inject 1 μl.

GAS CHROMATOGRAPHY

Column	1·8 m × 2 mm i.d. borosilicate glass.
Packing	7 per cent. neopentylglycolsuccinate (Hi-Eff-3BP) on 100–120 mesh AW CELITE 545.
Column temperature	194°C isothermal (detector and injection port heating were not employed).
Gas flow	40 ml/min nitrogen.
Detection	Flame ionisation.

The column was conditioned (with nitrogen flow) at 210°C for 48 hours prior to use.

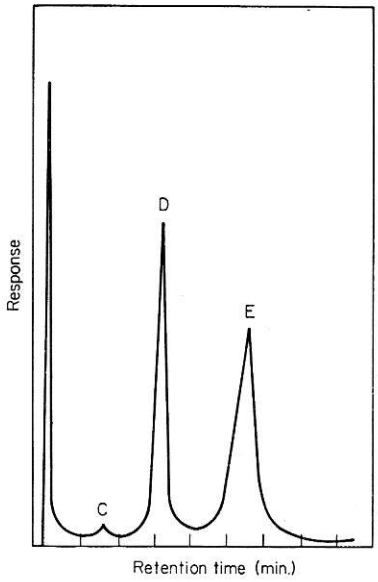

Fig. 2. *Eruca sativa*, column temperature 194°C. C, unknown; D, 4-methyl thiobutyl; E, fluorene.

The retention times and responses working under these conditions are shown in Figure 2. It is found that for these conditions:

$$\frac{\text{area of } \textit{iso}\text{thiocyanate peak } (I)}{\text{area of fluorene peak } (F)} = 0{\cdot}918.$$

For rapeseed meal following the conditions specified above we have:
percentage of 4-methyl thiobutyl *iso*thiocyanate in sample =

$$\frac{\text{area } I}{\text{area } F} \times \frac{\text{per cent. fluorene in internal standard}}{\text{mass taken} \times C} \times \text{vol. of internal standard}$$

$$= \frac{\text{area } I}{\text{area } F} \times \frac{0{\cdot}25}{2} \times \frac{10}{0{\cdot}612} = \frac{\text{area } I}{\text{area } F} \times 2{\cdot}04.$$

C = correction factor = $I/F \times 0{\cdot}25/$(per cent. 4-methyl thiobutyl *iso*thiocyanate

$$= \frac{(0{\cdot}918 \times 0{\cdot}25)}{0{\cdot}375} = 0{\cdot}612.$$

Having established the cause of the marked discrepancy between the distillation and the original G.L.C. methods for the determination of *iso*thiocyanates in some rapeseed meals, the new technique for the determination of the 4-methyl thiobutyl compound was applied to three of the samples showing this marked discrepancy between the results. Table II shows the results obtained, and Figure 1 shows the general type of chromatograph obtained.

TABLE II

*ISO*THIOCYANATES IN RAPESEED MEALS EXPRESSED ON DRY DEFATTED MEAL

Sample	Total by G.L.C. at 85°C allyl + butenyl expressed as allyl, *per cent.*	Total by distillation as allyl, *per cent.*	4-Methyl thiobutyl by G.L.C. at 194°C as allyl, *per cent.*
P58	0·41	0·76	0·36
I46	0·49	0·95	0·51
P51	0·60	1·16	0·59

These results show that where discrepant results are obtained in rapeseed meals, owing to the presence of *Eruca sativa*, the difference is accounted for by the presence of the 4-methyl thiobutyl compound.

Nearly all the samples of rapeseed meal which also contain *Eruca sativa* have shown evidence of the second very small peak in the 194°C chromatogram. This peak we have not identified, but we have established that it is not an *iso*-thiocyanate and that it constitutes only a small part of the total volatile sulphur compounds liberated from *Eruca sativa*.

Youngs and Wetter[1] refer to the finding of 4-methyl thiobutyl and 5-methyl thiopentyl *iso*thiocyanates in samples of rape, but these were not detected in the specimens which we examined.

Pure *Eruca sativa*

The proportion of volatile *iso*thiocyanates yielded by different samples of *Eruca sativa* varies considerably as shown in the Table III.

TABLE III

TOTAL *ISO*THIOCYANATES, EXPRESSED AS PER CENT. OF DRY DEFATTED *ERUCA SATIVA* SEED

Sample	By distillation stated as allyl, *per cent.*	By G.L.C. at 194°C	
		As allyl, *per cent.*	As 4-methyl thio butyl, *per cent.*
Pakistan, supplied by Dr Vaughan	1·57	1·55	2·53
Indian, supplied by Indian Agricultural Research Institute	0·98		
Eruca sativa or *rocket* supplied by Messrs Sutton Seeds Ltd	1·09	1·06	1·73

The presence of this new *iso*thiocyanate raises important issues when considering the suitability of rapeseed meals containing it for inclusion in animal feeding stuffs.

The present method of assessment laid down in the Fertiliser and Feeding Stuffs Amendment Regulations, 1976, which is based on total volatile oil of mustard, or *iso*thiocyanates expressed as allyl, may give an unfair picture of the

toxicity of the meal if the 4-methyl thiobutyl compound of *Eruca sativa* is present.

We can find no records of *Eruca sativa* cake or meal causing poisoning in animals and it is clear that it lacks the pungency which is characteristic of *Brassica juncea*, and is even less pungent than *B. napus*. On the other hand, if it is judged on the basis of volatile oil of mustard expressed as allyl, it could be regarded as being on a par with *B. juncea*.

The authors thank Dr E. A. H. Hall for making the infra-red analysis and Dr Vaughan of Queen Elizabeth College, London and the Indian Agricultural Research Institute for supplies of pure *Eruca sativa*.

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A Comparison of Three Methods for the Determination of Sulphur Dioxide in Food and Drink

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Three methods for the determination of sulphur dioxide in foods have been examined and the results compared. The Tanner method, involving distillation in the presence of methanol and phosphoric acid, followed by oxidation of the distillate with peroxide and titration with standard alkali, was found to be applicable to most types of samples. An electrochemical technique using a gas sensing probe was suitable for certain types of liquid samples only. The third method using an automated colorimetric procedure with pararosaniline was preferred for the rapid analysis of large numbers of liquid samples.

Sulphur dioxide has long been known for its sanitizing and preservative properties and reports of the use of burning sulphur as a fumigant go back to Roman times[1]. The incorporation of sulphites into meat products in America and Germany was reported during the nineteenth century; the use of sulphur dioxide in the production of wine is also of long standing and its presence in that beverage has been controlled in France since 1892[2]. Specific regulations for the control of sulphur dioxide in foodstuffs have been in existence in the United Kingdom since 1927[3] and its use is at present controlled by the Preservatives in Food Regulations, 1975. Some typical permitted levels taken from these regulations are given in Table I, and illustrate both the differing levels and the wide range of products likely to be encountered by the food chemist.

TABLE I

PERMITTED LEVELS OF SULPHUR DIOXIDE IN FOODSTUFFS, TAKEN FROM THE PRESERVATIVES IN FOODS REGULATIONS, 1975

Specified foods	Sulphur dioxide content, *mg/kg*, must not exceed:
Dehydrated vegetables (other than potatoes)	2500
Dried fruit	2000
Dehydrated potato powder	550
Wine, sausages and hamburgers	450
Soft drinks (for dilution), flavouring syrups and fruit juices	350
Cider	200
Jams, pickles and most sauces	100
Beer, vinegar and soft drinks (for consumption without dilution)	70
Yoghurt	60
Coconut, frozen mushrooms and raw peeled potatoes	50

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On addition to foods, some of the sulphur dioxide reacts with free aldehydic and ketonic groups present in the food as well as with other constituents such as proteins and nucleic acids[4]. Therefore, both "free" and "bound" forms of sulphur dioxide may exist. Bisulphite addition compounds are readily hydrolysed by alkali and the sulphur dioxide is released on subsequent distillation with acid. Other bound forms, e.g. sulphonic acid derivatives, are less readily hydrolysed and are not normally determined. However, it is important to distinguish between free- and total-sulphur dioxide since it is only the free and undissociated form which is thought to possess preservative action[5], although the Regulations do not distinguish between free and bound sulphur dioxide. Furthermore, manufacturers may need to know the sulphur dioxide binding power of their product in order to estimate the dose required to produce a given level of free sulphur dioxide for preservative action in the final product.

In addition to its antimicrobial action, sulphur dioxide prevents both enzymic and non-enzymic browning during food processing[1]. Stafford *et al.*[6] have shown that sulphur dioxide helps to preserve both the ascorbic acid and carotene contents of dried apricots as well as inhibiting the browning which occurs during drying and storage. On the other hand, it is known that sulphur dioxide destroys thiamine activity[7]. A comprehensive account of the chemistry of sulphur dioxide, and its use as a food preservative, has been prepared by Green[5].

Despite the long-established use of sulphur dioxide in food preservation, concern over possible hazards to health has been expressed. Animal feeding studies have established a "no-effect level" for the rat of at least 72 mg of SO_2/kg/day[8]. This, and other work, has led to the adoption by the WHO/FAO Joint Expert Committee on Food Additives of an Acceptable Daily Intake level of 0·70 mg/kg body weight/day, or around 50 mg/day for a 70-kg person[9]. Whilst dietary intake represents only one part of man's exposure to sulphur dioxide, it is important that unnecessary dietary consumption should be avoided. In view of the wide use of sulphites in food preservation, a survey of foods was commissioned by the Ministry of Agriculture, Fisheries and Food in which several brands of each specified food were analysed in this Laboratory for their sulphur dioxide content. The results of the survey, and its implications for total dietary intake, will be published elsewhere. As some 700 samples of a wide variety of foods had to be analysed, the performance of three different analytical methods was first assessed to establish not only the reliability of differing analytical techniques but also the feasibility of using automatic procedures, involving the minimum of sample pretreatment, for the analysis of large numbers of different products. The following three methods were selected for detailed study.

1. The Tanner Method[10]—a modification of the long established Monier–Williams distillation procedure[11].
2. An electrochemical method using the EIL sulphur dioxide gas-sensing probe[12].
3. An automated colorimetric procedure using pararosaniline reagent with a Technicon Auto-Analyser system modified and assembled in these laboratories.

Each method was assessed with respect to applicability, reproducibility, recovery tests and convenience of handling large numbers of samples differing in both composition and sulphur dioxide content.

Experimental

1. THE TANNER METHOD[10]

Many workers have used some variation of the original distillation procedure of Monier–Williams[11]. However, the modifications first proposed by Tanner have, over the years, found wide acceptance. This method involves distillation of the sample in a medium of water, methanol and phosphoric acid in a specially designed apparatus. Methanol is added to lower the boiling point of the mixture and so reduce interference from other volatile substances. Phosphoric acid is less volatile than hydrochloric acid and does not pass over into the distillate. A stream of nitrogen is passed through the distillation flask to facilitate removal of the sulphur dioxide, which is then oxidised by hydrogen peroxide to sulphuric acid in the receiver and titrated with standard alkali using screened methyl red as indicator. The use of a stream of nitrogen also ensures the removal of dissolved carbon dioxide from the distillate and, hence, avoids the problem of interference with the colour change of the indicator. To ensure maximum recovery the distillation was continued for a total time of 30 minutes. Full experimental details of the method are given in Appendix A.

Taking a 50 g sample and using 0·01 M sodium hydroxide solution as titrant, a sensitivity of 1 mg/kg is readily achieved. Tests using standard metabisulphite solution, and also samples to which metabisulphite solution had been added, showed that recoveries were better than 90 per cent. (Table II), with a coefficient of variation of 2 per cent. The repeatability of 12 separate determinations for one sample of dried fruit containing high residual levels of sulphur dioxide (mean 1580 mg/kg) is shown by a standard deviation of 83·2, equivalent to a coefficient of variation of 5·2 per cent. For marmalade containing much lower levels of sulphur dioxide (mean 42 mg/kg) the standard deviation given by 11 determinations is 2·2 with a coefficient of variation of 3·5 per cent.

2. THE SULPHUR DIOXIDE GAS SENSING PROBE[12]

The EIL probe, model No. 8010–2 was used in conjunction with an EIL model 7050 expanded-scale pH meter. When the probe is immersed in an acidic solution containing sulphur dioxide, the gas diffuses through the permeable silicone-rubber membrane into a thin film of electrolyte containing bisulphite ions at a rate dependent on the concentration of gas in solution. This produces a fall in the pH value of the electrolyte solution and the change is monitored with a glass electrode/reference electrode system built into the probe body. In solutions containing more than 100 μg/ml of sulphur dioxide, the equilibrium (and, hence, the response time of the probe) is established in about 0·5 min. The response time increases at lower concentrations to about 2 to 3 minutes at 30 mg/l and to 7 to 10 minutes at 1 to 2 mg/l.

Free sulphur dioxide is first determined by inserting the probe into a continuously-stirred solution acidified to pH 1 and diluted if necessary to bring the

TABLE II

RECOVERIES OF SULPHUR DIOXIDE FROM PURE SOLUTIONS AND SPIKED SAMPLES (ADDED AS SODIUM METABISULPHITE) BY THE TANNER METHOD

Sample		Sample size, *ml* or *g*	SO_2 in unspiked sample, *μg*	SO_2 added, *μg*	Total SO_2 as determined, *μg*	Amount of added SO_2 recovered, *μg*	Recovery, *per cent.*
Pure solutions of sodium metabisulphite			—	5000	4710	4710	94
			—	5000	4590	4590	92
			—	5000	4780	4780	96
			—	5000	4650	4650	93
			—	5000	4740	4740	95
			—	2000	1880	1880	94
			—	1000	910	910	91
Spiked Samples							
Freeze dried peas	1	6	2540	5000	7080	4540	91
	2	5	1390	5000	6090	4700	94
Lemon juice conc.	1	10	1860	5000	6590	4730	95
	2	10	2140	5000	6970	4830	97
Rosé wine		10	1660	5000	6560	4900	98
White wine		10	1280	5000	6170	4890	98
Red wine		10	1570	5000	6370	4800	96
Orange juice conc.	1	20	800	2000	2700	1900	95
	2	20	640	2000	2530	1890	95
Beer	1	50	950	1000	1910	960	96
	2	50	350	1000	1280	930	93

Sample	Standard deviation	Coefficient of variation, *per cent.*
Pure solutions	1·72	1·8
Spiked samples	2·10	2·2

concentration within the calibration range. Total sulphur dioxide is then determined on a separate aliquot to which 2 M sodium hydroxide solution has been added to raise the pH above 13·0. After 15 minutes the solution is then acidified with 2 M sulphuric acid to bring the pH to 1·0. The final volume in each case is adjusted to a simple multiple of the original volume and the probe is inserted into the stirred solution and a reading taken at equilibrium. Table III shows total- and free-sulphur dioxide contents of certain wines and soft

TABLE III

DETERMINATION OF FREE, TOTAL AND BOUND (BY DIFFERENCE) SULPHUR DIOXIDE CONCENTRATIONS OF SOME BEVERAGES USING THE GAS SENSING PROBE

Sample	Free SO_2 content, *mg/l*	Total SO_2 content, *mg/l*	Bound SO_2 content, *mg/l*
Fruit syrup concentrate	250	260	10
Orange juice concentrate	30	40	10
White wine	30	200	170
Sherry	2	120	118

drinks illustrating the variation in possible ratios between the free and combined forms depending on other constituents of the sample. Full experimental details are given in the handbook which accompanies the electrode.

A study of the effect of sample dilution on the reproducibility of the probe (Table IV) shows that sample volumes below 10 ml may give erroneous results

TABLE IV

EFFECT OF DILUTION ON THE DETERMINATION BY GAS SENSING PROBE OF FREE SULPHUR DIOXIDE IN BEVERAGES

Sample volume, *ml*	Free SO_2 concentration, *mg/l*		
	Orange squash	Fruit syrup	White wine
40	30	—	28
35	30	255	30
30	30	240	30
25	27	250	—
20	28	250	29
15	27	220	27
10	28	210	29
8	—	—	31
6	—	—	36
5	31	220	—
4	—	—	44
2	—	220	75
1	—	220	115
Standard deviation	1·40	19·00	3·63 (1·07)†
Coefficient of variation, *per cent.*	4·9	8·0	12·0 (3·7)†

† Figures for sample volumes less than 10 ml have been omitted.

since the resulting solution after making up to volume (50 ml) then contains concentrations of sulphur dioxide outside the optimum working range of the instrument. However, this is not the only effect since errors were also observed with high dilutions of the fruit syrup containing much higher levels of sulphur dioxide. Bailey and Riley[13] have postulated that osmotic effects are also important. Alternatively, it is possible that at high dilutions and acidities some hydrolysis of bound forms of sulphur dioxide occurs. At high dilutions the response of the probe becomes slow and erratic. Hence, the ideal working range, after dilution if necessary, is 20 to 100 mg/l.

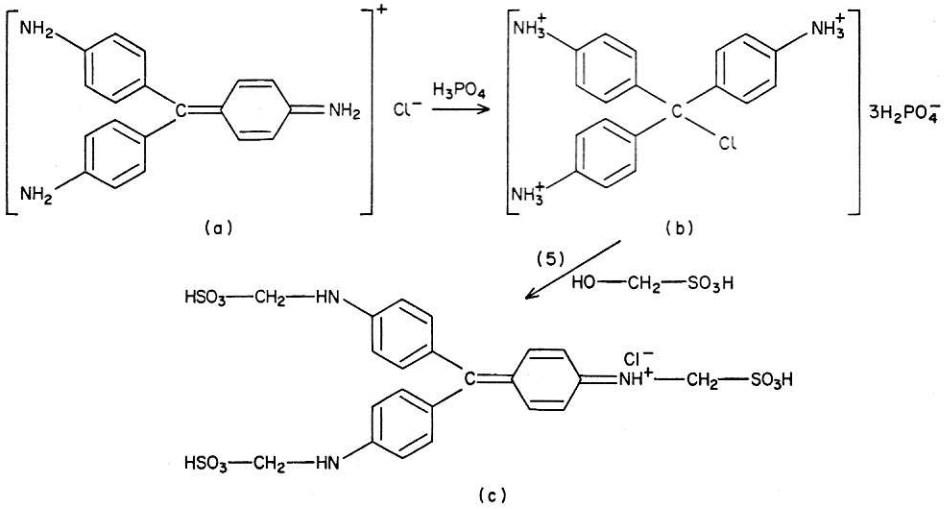
Alcoholic samples (especially red wines) were found to have a deleterious effect on the probe membrane. In this study, the membrane was renewed and allowed to stabilise for a period of 18 hours after each series of measurements on any one type of sample containing alcohol. Generally, for non-alcoholic samples, it is necessary to renew the membrane every two weeks and to replace the internal filling solution at weekly intervals.

3. AUTOMATIC COLORIMETRIC METHOD[14]

The principle of the method is as follows: a portion of the sample is acidified with phosphoric acid and transferred to a flash-distillation unit[15], from which the liberated sulphur dioxide is carried by a stream of nitrogen and absorbed

in potassium tetrachloromercurate solution. A portion of this solution is mixed with *p*-rosaniline, formaldehyde and sulphamic acid. The colour of the bleached *p*-rosaniline is restored on reaction with sulphur dioxide and the absorbance of the solution is measured at 560 nm. The chemical reactions are shown sequentially below.

(1) $HgCl_2 + 2KCl \longrightarrow 2K^+ + [HgCl_4]^{2-}$;
(2) $[HgCl_4]^{2-} + SO_2 + H_2O \longrightarrow [HgCl_2SO_3]^{2-} + 2Cl^- + 2H^+$;
(3) $[HgCl_2SO_3]^{2-} + HCHO + 2H^+ \longrightarrow HgCl_2 + HO\text{–}CH_2\text{–}SO_3H$;
(4)



Reaction (4) shows the following: (a) λ_{max} at 538 nm; (b) λ_{max} at 538 nm reduced; (c) λ_{max} at 560 nm.

The method gives a linear response over the range 2 to 14 mg/l of sulphur dioxide and samples can be analysed at a rate of 12 per hour. One difficulty is that samples falling outside the linear range need to be re-examined after adjustment of the dilution factor. Table V shows the results of replicate determinations of the sulphur dioxide content of samples of red and white wines obtained during a single run. Details of the method, including the preparation of the reagents and the flow diagram are given in Appendix B.

Results

LOSS OF SULPHUR DIOXIDE FROM SAMPLES DURING STORAGE

Sulphur dioxide is usually added to foods as sulphites and is only temporarily fixed; its concentration is thus expected to fall on storage. This effect was studied by determination of the sulphur dioxide content of samples of dried peas and lemon juice during storage at laboratory temperatures as follows. After opening the containers and determining the sulphur dioxide content on day zero, the samples were stored in sealed containers and sub-samples for further determinations withdrawn at intervals. A steady fall in the sulphur dioxide content was observed, as shown by the results in Table VI.

TABLE V

REPRODUCIBILITY OF THE AUTOMATIC METHOD FOR THE SULPHUR DIOXIDE CONTENT OF WINES

	Total SO_2 concentration in sample, *µg/ml*		
	White wine	Red wine 1	Red wine 2
	310	135	110
	305	135	110
	300	135	110
	295	135	110
	310	135	115
	310	135	110
	290	140	110
	290	135	110
	310	140	115
	305	140	115
Dilution	1:40	1:20	1:10
Standard deviation	8·92	2·20	0·97
Coefficient of variation, *per cent.*	2·9	1·6	0·9

TABLE VI

LOSS OF SULPHUR DIOXIDE FROM STORED SAMPLES OF DRIED PEAS AND LEMON JUICE

	Total SO_2 concentration in sample	
Number of days opened	Dried peas, *mg/kg*	Lemon juice, *mg/l*
0	425	255
1	—	195
2	—	185
9	375	170
11	330	165
15	285	145
16	—	145
17	275	—

All other samples were analysed as soon as possible after opening the container.

COMPARISON OF THE THREE METHODS FOR THE DETERMINATION OF SULPHUR DIOXIDE

A number of locally purchased foods were examined simultaneously by each of the three methods and the results are given in Table VII. In some cases it was only possible to analyse the samples by two methods; these results are presented in Table VIII. Generally there is a satisfactory correlation between the results obtained using the Tanner method and those obtained by the auto-analyser technique. The electro-chemical method gave more variable figures

TABLE VII

THE DETERMINATION OF TOTAL SULPHUR DIOXIDE CONCENTRATION IN SAMPLES OF FOOD BY THREE METHODS

Sample	Total SO_2 concentration, *mg/l*		
	Tanner's method	Probe method	Automatic method
White wine A	205	180	220
White wine B	130	125	130
Red wine A	85	115	75
Red wine B	155	65	165
Lemon juice A	215	250	175
Lemon juice B	85	115	85
Beer A	5	10	5
Beer B	<1	5	<1
Cider A	85	90	90
Cider B	115	105	120
Sherry A	125	120	135
Grapefruit juice A	95	130	110
Orange perry	50	45	50
Orange juice A	40	35	45
Fruit syrup	90	100	95

TABLE VIII

THE DETERMINATION OF TOTAL SULPHUR DIOXIDE CONCENTRATION IN FOOD SAMPLES BY TWO OF THE THREE METHODS

Sample	Total SO_2 concentration *mg/l* († as *mg/kg*)		
	Tanner's method	Probe method	Automatic method
Dried peas A†	1390	1450	—
Dried peas B†	425	415	—
Fruit syrup	265	—	255
White wine C	225	235	—
White wine D	—	250	265
White wine E	—	215	210
White wine F	105	—	110
White wine G	155	—	160
Champagne A	145	135	—
Champagne B	40	35	—
Champagne C	—	140	140
Champagne D	40	—	35
Rosé wine	255	240	—
Red wine C	155	160	—
Red wine D	70	—	70
Red wine E	160	—	155
Sherry B	100	100	—
Port	25	—	25
Cider C	80	80	—
Cider D	—	85	90
Cider E	115	—	120
Grapefruit juice B	105	100	—
Barley water	205	200	—
Lemon juice C	80	80	—
Lemon juice D	90	—	95
Blackcurrant juice	80	—	80
Orange juice B	55	55	—
Pineapple juice	<1	—	<1
Ginger ale	—	15	20
Wine vinegar	5	—	5
Dried potato†	190	180	—

by comparison with the other methods but its use is recommended, however, for those types of sample where no interference from other constituents has been demonstrated.

None of the samples contained levels of sulphur dioxide greater than those permitted in the Regulations.

We thank the Government Chemist for permission to publish this paper.

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Appendix A: The Tanner Method

REAGENTS

1. *Nitrogen*, from cylinder.
2. *Phosphoric acid*, 88 per cent. ($d = 1{\cdot}75$).
3. *Hydrogen peroxide solution*, 0·2 per cent. w/v. Dilute 0·7 ml of 100 vol. hydrogen peroxide to 100 ml. Prepare freshly daily.
4. *Sodium hydroxide solution*, 0·01 N. Standardise against potassium hydrogen phthalate, dried at 110°C.
5. *Methanol*, AR.
6. *Mixed indicator solution:* mix 50 ml of 0·03 per cent. ethanolic solution of methyl red with 50 ml of 0·05 per cent. ethanolic solution of methylene blue and filter.

METHOD

Weigh, or pipette, a quantity of sample into the distillation flask (Figure 1) as indicated by the table below:

Expected SO_2 content, *mg/kg*	Quantity of sample to be taken, *g* or *ml*	Vol. of distilled water to be added, *ml*
<10	40–50	20
10–100	20–25	30
>100	5–10	40

Add distilled water to the flask as indicated. Add 50 ml of methanol and mix. Introduce into the distillation receiver 10 ml of hydrogen peroxide, 60 ml of distilled water and a few drops of mixed indicator solution. Add a few drops of 0·01 N sodium hydroxide solution to produce a green colour. Add a similar quantity of neutralised hydrogen peroxide solution to the guard wash bottle.

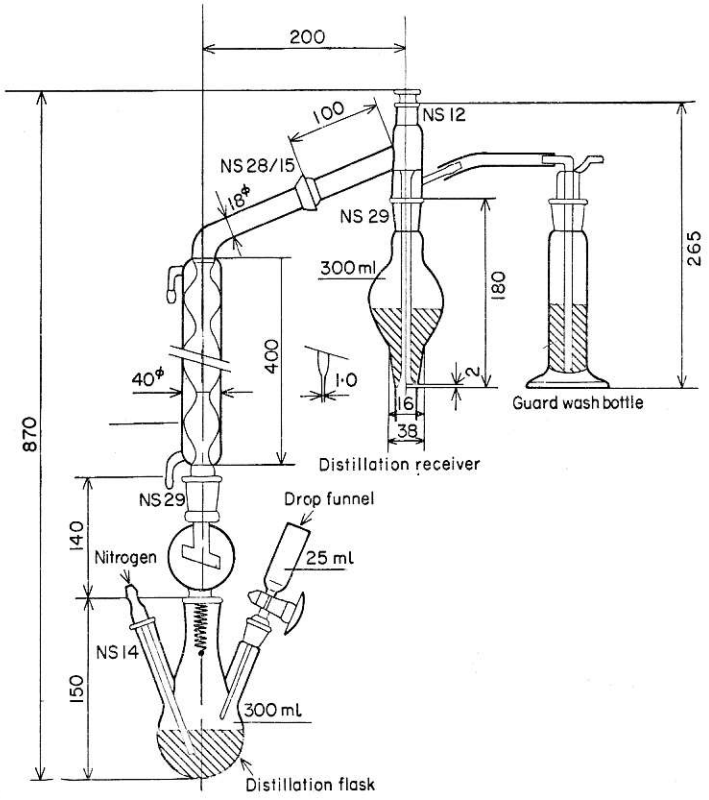

Fig. 1. Distillation apparatus for the determination of sulphur dioxide (Tanner). All dimensions in mm.

Connect up the apparatus and adjust the nitrogen flow to approximately 60 bubbles per minute. Add 15 ml of phosphoric acid to the funnel and run it into the distillation flask. Heat rapidly to boil the mixture and then simmer gently for a total period of 30 minutes. Detach the receiver from the distillation apparatus and rinse the tube. Titrate the sulphuric acid present with 0·01 N sodium hydroxide solution until the indicator turns green.

CALCULATION

$$\text{Sulphur dioxide content (mg/kg or mg/l)} = \frac{a \times N \times 32 \times 1000}{Q}$$

where a = volume (ml) of sodium hydroxide solution, N = normality of sodium hydroxide solution, and Q = weight of sample in g or volume of sample in ml.

Appendix B: Automatic Method

APPARATUS

See Figures 2 and 3.

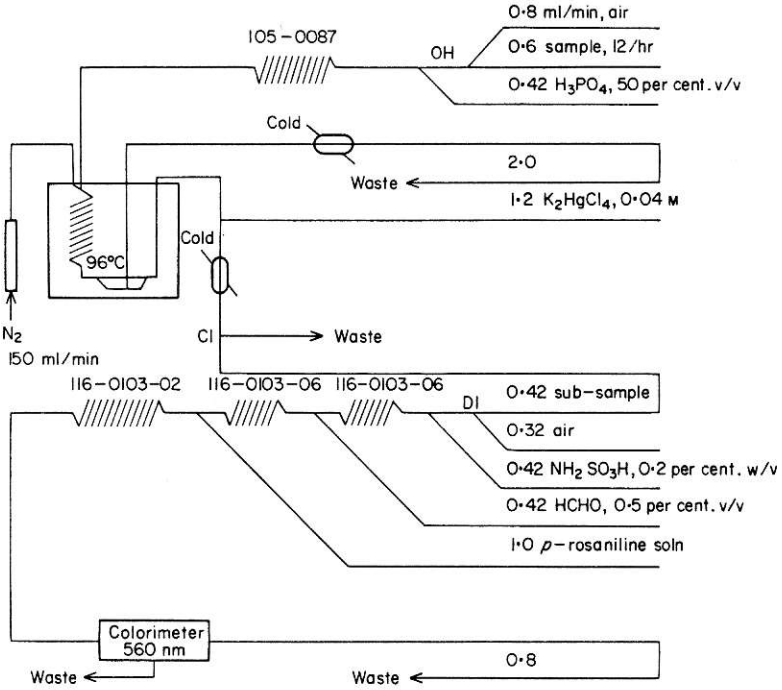

Fig. 2. Manifold diagram for the automatic determination of SO_2.

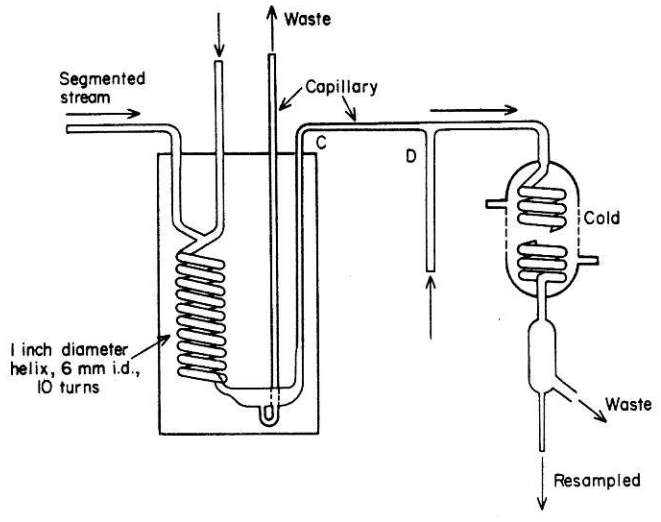

Fig. 3. Improved distillation unit.

REAGENTS

1. *Potassium tetrachloromercurate.* Dissolve 10·9 g of mercuric chloride and 6·0 of of potassium chloride in about 600 ml of distilled water. Dilute to 1 litre and store in an amber glass bottle.
2. *Phosphoric acid,* 50 per cent. v/v. Dilute phosphoric acid (88 per cent.) with water: 1 + 1.
3. *Sulphamic acid,* 0·2 per cent. w/v. Store in an amber glass bottle.
4. *p-Rosaniline, stock solution.* Dissolve 2 g of *p*-rosaniline hydrochloride in 1 litre of distilled water. Allow to stand for 3 days, filter and store in an amber glass bottle.
5. *p-Rosaniline, working solution.* Add 125 ml of concentrated phosphoric acid to 200 ml of *p*-rosaniline stock solution with stirring. Allow to stand for 5 minutes and dilute to 1 litre with distilled water.
6. *Formaldehyde,* 0·5 per cent. v/v. Add 5·0 ml of formaldehyde solution to about 800 ml of distilled water. Dilute to 1 litre and mix. Prepare freshly each week.
7. *Sulphur dioxide stock solution,* 1000 mg/litre. Dissolve 1·649 g of sodium metabisulphite in about 400 ml of distilled water and make up to 1 litre. Store in a tightly stoppered glass container in a refrigerator.
8. *Sulphur dioxide standard solutions.* Prepare by dilution of the stock solution a series of standards in the range 2 to 14 mg of SO_2 per litre.