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Annual Report of the Council for 1984

Presented at the Annual General Meeting of the Association of Public Analysts at Emmanuel College, Cambridge, on Saturday, 30th March 1985 by the Honorary Secretary, Mr. K. T. Chisnall.

This report provides details of those aspects of their duties that public analysts have regarded as of particular importance during the year. A statistical summary illustrates, from the range of samples received, the vital role members of the Association play in providing a scientific service to the community at local and national level.

House of Lords Select Committee

In July the Association was informed that the House of Lords Select Committee on Science and Technology had set up a sub-committee to consider scientific and technical services in and available to Local Government in the United Kingdom. The enquiry was to have two parts: in the first place the review of the scientific and technical functions of Local Government and the facilities available to carry them out, and secondly an assessment of the strengths of Local Government's scientific and technical services.

The full terms of reference remained somewhat vague but the Association responded to the first part with a submission setting out the full range of local authority activities in which public analysts are regularly involved.

The Policy Committee of the Association had already been giving consideration to a detailed review of the Association's policy statement on the "Future of the Public Analysts Scientific Advisory and Analytical Services", produced in 1978, and it was intended that this task should be completed in time to give further evidence in response to the second part of the enquiry.

It was expected that the Select Committee would require this evidence towards the end of the year.

Abolition of the Metropolitan County Councils

The Association's report for 1983 points out that the Government's proposals to abolish the Metropolitan County Councils made no mention of public analysts' laboratories. This was viewed with concern because the present larger laboratory units are found to be more efficient and cost-effective than smaller ones. Experience of the laboratory services set-up by the Metropolitan County Councils reinforced this view and the possible fragmentation of these services due to abolition could lead to a service of reduced efficiency at greater cost.

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During the current year, a number of Government documents have shown that the value of co-ordinated support services has been recognised. For example, district councils have been advised to make provision for sharing specialist skills in connection with waste disposal and joint committees are proposed for the co-ordination of trading standards activities. Public analysts' laboratories are the principal scientific service involved in many activities, including these, yet there are no firm proposals as to how the service is to be organised. The Association contends that the service provided by its members is of sufficient importance to warrant separate organisation. The co-ordination of the needs of all user departments in all districts of a Metropolitan area will be a complex process, which is likely to fail unless given central direction.

Factory Enforcement

The Association submitted a paper on the subject to the Ministry of Agriculture, Fisheries and Food and in addition played an active role in an inter-disciplinary working party of the Local Authorities Co-ordinating Body on Trading Standards, set up to investigate the present and future methods of enforcing food compositional and labelling legislation at all the above stages. This report has produced a number of problems which still need resolving.

It has been suggested that in a revised Food Act there would be provision for more detailed programmes of sampling at the point of food manufacture and that eventually a scheme of "acceptance" sampling based on "average quality" would supersede the "every article must comply" philosophy of the present Act. The following comments must be made at this juncture:

(a) Factory sampling could be a valuable supplement to the present system of enforcement but only if it is carried out by multi-disciplinary teams of properly qualified scientific and inspectorate staff.

(b) If the principles of "average quality" and "acceptance sampling" are to be adopted there will need to be a proper review of standards to ensure that retailed foods are always of acceptable quality. Existing standards could not simply serve as the "average".

(c) A cost-effective approach to enforcement may include inspection of quality control records maintained by manufacturers. It is essential that any such inspections be carried out by properly qualified scientific investigation staff and the inspections must be supported by proper sampling schemes.

(d) The implications of (a), (b) and (c) above are for a much greater expenditure on enforcement than has so far been incurred. The distribution of food manufacturing facilities suggests that this may be disproportionately high for some food authorities.

(e) The retention of adequate retail enforcement provisions is essential.

Royal Commission on Environmental Pollution

Submissions were made by the Association of Public Analysts to the Royal Commission on its study of pollution by waste. These included comments on surface disposal and landfill, incineration, recycling and economic implications. Reference was also made to the problems of land contamination, standardisation of methods of analysis, guideline levels for assessment of data and methods of reclamation.

Surface disposal and landfill, the most widely used form of waste disposal, requires good scientific control to minimise problems, such as contamination of ground and surface water. Monitoring is essential and the public analyst is available to provide this service. The full cost of landfill disposal must include the cost of monitoring of incoming waste and ground water surrounding a site, and also leachate analysis and treatment. Methane production from a waste disposal site can create problems, such as cracking of capping seals and fire and explosion hazard at some distance from the site. The collection and utilisation of methane as a fuel should be undertaken whenever possible.

Incineration is the only safe option for the destruction of the more toxic and persistent compounds such as pesticides and polychlorinated biphenyls. The incineration of chemicals creates understandable public concern as to whether the surrounding environment is becoming contaminated. Such fears can only be allayed by regular monitoring of air, herbage and soil by local authorities using the expertise of their public analyst.

On the subject of land contamination it is not always recognised at an early enough stage that land may be contaminated and hence pose special problems. It is always more costly to rectify the problem if recognition occurs after development has commenced. One way of dealing with the problem might be by means of a Code of Practice to guide the planner.

When examining contaminated land, the use of standard analytical procedures throughout the UK would be of benefit and the Royal Commission was invited to consider what body might be best made responsible for recommending such methods. It was also asked to consider the role of the Department of the Environment in advising guideline levels, the need for more central guidance on the assessment of investigation data and the mechanism for derivation of guideline levels. The involvement of the public analyst in the development of these methods is essential.

International Activities

The Association has liaison through various members with a number of international bodies such as the International Standards Organisation (ISO), the International Union of Pure and Applied Chemistry (IUPAC), and the Association of Official Analytical Chemists (AOAC). Links are pursued with official analysts in other countries in the European Community, in North America and in the Scandinavian countries. The Association participated in the symposium on the harmonization of

collaborative analytical studies held in Washington this autumn, organised jointly by IUPAC and AOAC.

Meat Products

The Association gives a general welcome to the long awaited Meat Products and Spreadable Fish Products Regulations 1984 effective from November. Products made from meat or fish are very prominent in any Public Analyst's list of food samples with unsatisfactory composition and/or labelling. Previous regulations were by no means comprehensive, so certain foods such as bacon and ham escaped adequate control, and changes in products and manufacturing practices over the years, for example with "burgers" and reformed steaks, increased problems of interpretation and enforcement. Public analysts contributed in depth to the draft proposals for new regulations but, whilst acknowledging numerous improvements in the final issue, regret that on certain points the Regulations fall short on scope, labelling and compositional requirements, to the detriment of consumers.

The intended principle is one of more informative labelling but in reality the declaration of meat content is relegated to the ingredients list which on most packs is small and hidden on the back of the package and purchasers buying meat products for immediate consumption will have no information at all. Bacon may have ten per cent. of added water which can be equivalent to about fifteen per cent. of curing solution, an amount which is well in excess of current practice, before any declaration of additions is required. Certain products have lost compositional protection and standards can be expected to fall, for example with potted meat. The lack of any control on mechanically-recovered meat is also regretted.

Labelling of Food

The EEC Directive on Food Labelling was fully implemented in 1984 by the publication of the new Food Labelling regulations. These regulations, which come into effect in 1986, cover the proper naming of food, the marking of ingredients including additives, instructions for use of the food, indication of origin and date marking. Extensive rules governing claims and misleading descriptions are also contained in the Regulations.

During 1974, the Committee on Medical Aspects of Food Policy issued a report (the COMA report) which set out the effects of diet on cardiovascular disease. Foods specifically discussed included fats, sugar, salt and alcohol and, as a result of the report, it is likely that new and reformulated foods will appear on the market and more nutritional information will be supplied on labels. It is likely that there will be a legal requirement to indicate the content of total, saturated and unsaturated fats on labels and that there will be a voluntary code on the labelling of other nutritional parameters.

A weakness of the Regulations is that there is nothing to prevent the subordination of the statutory or common name for the product to other printed matter. Fancy names are often so dominant on packaging that it is difficult to see what the food actually is.

Water

The Association has been in consultation with the Department of the Environment concerning the application of EEC requirements for the quality of all water intended for human consumption to private water supplies and to all water used in the food processing industry.

Private water supplies with certain exceptions concern all supplies provided by organisations and individuals, other than statutory water undertakings and Government Departments. There are known to be some 80,000 supplies in the UK which require to be considered under these legislative provisions.

The EEC Directive which will come into operation from 15th July, 1985 permits considerable discretion to member states concerning the frequency of sampling and the extent to which the scientific investigation is carried out.

Consultation during the year has centred upon the interpretation of this discretion and in particular the financial implications for local authorities who will be responsible for its enforcement. The Association has been concerned to ensure that the full scientific benefits of the legislation were made available as soon as possible, which could not be achieved without additional cost. On the other hand, Central Government is concerned to keep these to a minimum.

The bacteriological and chemical examinations carried out on private water supplies by local authorities need to be augmented. This would represent a considerable potential increase in both work load and costs to public analysts who would be capable of dealing with both aspects of this work if required. As a consequence of consultation a balance has been achieved between the competing constraints as a result of which it is anticipated that most of the many sources will have been examined within a period of about ten years. Whilst there will be some additional cost to authorities, the Department of the Environment, based on its estimates, considers that this would not represent an undue burden to them.

From the professional point of view, the Association would have preferred a more rapid introduction of these provisions. On the other hand it acknowledges that if fully applied these arrangements make a positive if hesitant step forward from the present position.

Standards and Methods Committee

It will be apparent from this report that the Association, despite its relatively small size, is required by virtue of its ever-widening responsibilities to the community on scientific matters, to make its advice available to Central Government and to numerous other organisations including the local authorities.

The administration of such a service, which is undertaken without the benefit of a full time secretariat, poses considerable logistical problems which are not easily resolved on a voluntary basis.

The Association's Standards and Methods Committee is the principal Committee which carries the main responsibility for advising the Council on

technical matters and providing a response on scientific issues on behalf of the Association.

During 1984 the method of operation of this Committee has been extensively revised with the objective of improving the speed of response to enquires and in particular redistribution of the work load to a number of sub-committees with redefined terms of reference enabling the more active involvement of members in the advisory process.

Collaborative Trials and Quality Assurance

For many years each individual public analyst's laboratory has relied upon "in-house" systems of analytical quality assurance, and many have participated in different external collaborative trials organised by the Ministry of Agriculture, Fisheries and Food, the Association of Official Analytical Chemists, the International Union of Pure and Applied Chemistry, etc.

Whilst this no doubt provided reliable data on a day-to-day basis, it failed to make proper collective use of data. Recognising this deficiency, the Association this year approved a formal analytical quality assurance system for use in all laboratories.

The essential features of the common analytical quality assurance protocol are that:

(1) Each method of analysis must be documented in a defined manner and the laboratory must have a system of work to ensure its proper application.
(2) Each method of analysis must have a defined performance in terms of limit of detection, precision and bias.
(3) Each method of analysis must have associated with it a within laboratory quality control procedure such that not less than 10 per cent. of analytical results are accompanied by a performance check.

The current gradual change to a common system will assist in a coordination of effort and help ensure that maximum benefit is derived from data produced throughout public analysts' laboratories.

Training of Public Analysts

Evidence from other industrialised nations as well as our own indicates that proper training of employees within an industry results in a motivated and satisfied work force, greater efficiency and more competitive products.

There is a need within the laboratories for skilled and experienced scientists who can cope with the extra demands now made upon them. These range from evaluating water quality to the determination of pesticides and toxic metals in foodstuffs at levels of parts in one hundred million. Public analysts must have a knowledge of chemistry, microbiology, statistics, pharmacology, toxicology, food technology and microscopy, quite apart from an understanding of legislation governing food, agricultural materials, waters, cosmetics and many other products to which consumer protection and trades description legislation relates.

In the past it was possible for a trainee analyst to acquire his expertise solely within his own laboratory but the required amount of theoretical knowledge and practical experience is now so vast that it is unlikely to be gained without the assistance of outside specialist courses.

The Association is actively pursuing the setting up of courses suitable for trainee analysts and it is hoped that funding for the courses will be arranged with the Local Government Training Board.

Road Traffic Act

In 1983 the new procedure was introduced which required provision of a breath sample rather than a blood sample by motorists suspected of driving with excess alcohol. This enables a result to be obtained at the police station and avoids the delay involved in the laboratory analyses of large numbers of blood and urine samples.

It was always realised that safeguards would be necessary to ensure that this new procedure could not give rise to injustices and it was inevitable that attempts would be made to throw doubts on the validity of the results. After considerable criticism of the procedure, it was agreed that for a limited period motorists would be offered the opportunity to provide a sample for laboratory analysis in every case, not only borderline cases as before.

The Royal Society of Chemistry felt it was appropriate to set up a Working Party to investigate the reliability of the instruments and to compare the results so produced with the results of chemical analysis of blood samples taken from the suspects on the same occasions. In view of the many years of involvement of public analysts in this field, it was appropriate that the expert committee should be chaired by a Past President of this Association and include other members of our profession. The collection of data is proceeding and the findings will be published in due course.

Asbestos

The public concern regarding asbestos remains at a high level with many laboratories finding this area of work continuing to expand. The year 1984 has been notable for those involved, with the introduction of a new, more comprehensive Guidance Note EH 10, The Asbestos (Licensing) Regulations, and the modified Regular Interlaboratory Counting Exchanges (RICE) scheme. EH 10 formally introduces the European Reference Method for use in determining personal exposure to asbestos for the purpose of assessing compliance with the control limits which have, once again, been lowered. The introduction of the European Reference Method is not universally welcomed as it requires a far more hazardous method of mounting filters for counting, involving the use of boiling acetone and is generally unsuitable for use in the important area of post-stripping monitoring thus necessitating the use of two different protocols for similar types of work. The mark 2 RICE scheme is self financing involving a significant additional expense for the participants. The scheme now ranks laboratories according to the quality of their results and laboratories seeking

National Testing Laboratory Accreditation Scheme accreditation for this work will be required to maintain a high standard in the Regular Interlaboratory Counting Exchanges. Public analysts' laboratories are now participating in the RICE scheme which is an indication of the importance they attach to quality control.

Identification of bulk samples forms a large part of many public analysts' involvement with asbestos. This must be carried out by a competent microscopist with suitable equipment but the results obtained are, as always, only as good as the samples received. Many analysts prefer to take their own samples and the Asbestos Regulations specifically exempt these operations from the licensing requirements. As the public analyst is frequently called upon to advise on procedures to be adopted on sampling, identification and removal of asbestos-containing materials, the safety procedures employed must be beyond reproach. This has entailed increasingly rigorous safety precautions being taken which adds to the cost and time of both sampling and analysis.

Toys and Other Consumer Goods

Over the year the Association has been consulted by the Department of Trade (Consumer Safety Unit) upon a number of topics relating to proposed consumer safety legislation or problems in relation to consumer safety. These have included consultations upon possible safety standards in respect of the water contained in water filled toys (e.g. "water snakes" and "snowstorms"); proposals for extending the provisions of the Nightdresses (Safety) Regulations with a view to strengthening the protection afforded by these regulations with regard to fire hazard and extending this protection to a wider range of nightwear; proposed extensions to the Novelties (Safety) Regulations in order to control potential dangers associated with the use of joke sneezing powders; and proposals for Asbestos (Safety) Regulations designed, primarily, to prohibit the supply of products containing either blue or brown asbestos and require warning labelling with regard to products containing white asbestos. Additionally the Association's comments have been sought upon the Government's White Paper relating to the "Safety of Goods" which introduced some new basic concepts in relation to consumer safety. These include an overall "General Safety Duty" requiring suppliers of consumer goods to achieve an acceptable standard of safety; and obligations are placed on first time suppliers (manufacturers or importers) to have adequate safety checks carried out. The Association is particularly concerned that any such requirement for safety checks by first time suppliers should not lead to a reduction in enforcement action, by way of sampling and examination, particularly at the retail stage of supply to the ultimate consumer.

During the year Public Analysts throughout the country have participated in a survey by the Local Authorities Co-ordinating Body on Trading Standards (LACOTS) into the incidence of lead in consumer goods not currently covered by specific legislation. This involved the analytical

examination of various classes of goods including childrens' painting kits, water transfers, household paints and nursery furniture. This survey is now being finalised and a LACOTS report on the matter is anticipated in about the spring of 1985.

Conclusion

This report shows the wide range of services and scientific advice that are provided by the Public Analyst in pursuit of local authority responsibilities. The service must receive additional support in order to meet the challenges presented by technological change and increasing demands.

Statistical Summary

Foods (including complaint samples and those submitted under the Imported Food Regulations)	89319
Milks (including those examined for antibiotics and those submitted under the Milk (Special Designation) Regulations)	20330
Drinking Waters	22325
Environmental Pollution (Waters) (Trade effluents, tip leachates, ground waters etc.)	41401
Environmental Pollution (other samples)	34352
Feeding Stuffs (Agriculture Act, Medicines Act) and Feed Supplements	4503
Fertilisers (all kinds)	2755
Consumer Protection and Trades Description Act samples	7087
Miscellaneous (HM Coroners, Health Authorities, other Local Authority Departments)	44479
Health and Safety at Work Act	26258
Swimming Pool Waters	5985
Total	298794

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The Effect of Seed Removal on the Inorganic Composition of Raspberries for Jam

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Seventeen samples of raspberries were analysed for ash, potassium, nitrogen and phosphorus. Samples included frozen as well as sulphited fruit, and were tested both whole and after the seeds had been sieved out. It was found that the seeds contained much higher concentrations of minerals than the fruit flesh. Overall, although the mean yield of pulp was more than 95 per cent. of the weight of the fruit, the seedless pulps contained, on average, only 59 per cent. of the ash, 76 per cent. of the potassium, 70 per cent. of the nitrogen and 63 per cent. of the phosphorus present in the whole fruit. The analytical data are presented in a variety of tabulations, and the significance of the results in the estimation of fruit content is discussed. It is concluded that potassium, nitrogen and phosphorus concentrations are poor indicators of fruit content. If such analyses are applied, estimates of the fruit content of seedless raspberry jam should be based on conversion factors which account for the disproportionately low mineral content of the seedless pulp. Some factors are suggested.

Jam manufacturers have long been required to meet standards which specify minimum fruit contents for their products. The Jam and Similar Products Regulations 1981 (S.I. 1981 No. 1063), now compel manufacturers to label jams with their fruit content. The same regulations also define several categories of jam, based upon compositional differences. Conventionally, the fruit content of jam is estimated from the minerals present. The results of determinations of elements, such as potassium, nitrogen and phosphorus, are multiplied by various factors intended to relate the composition of the jam to the average composition of the fruit used to make it. This approach suffers the well-recognised defect that the composition of fruit is subject to very large natural variations. An additional difficulty arises when the fruit content of seedless raspberry jam is estimated from inorganic analyses. The available conversion factors relate to whole raspberries, whereas the jam is made with fruit from which the seeds have been removed by sieving. The effects of seed removal on the inorganic composition of the fruit are not known. Accordingly, a study was undertaken to provide this information.

Materials

Samples of both frozen fruit and sulphited pulps were analysed (Table I). Sulphited pulps comprised pulped raspberries preserved with a solution of sulphur dioxide in water, which is normal commercial practice in preference to

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TABLE I

RASPBERRIES ANALYSED

Sample number	Description (cultivar and harvest date, or country of origin)		Supplier (grower or jam manufacturer)
	Frozen fruit		
1	Glen Prosen	1981	Efford Experimental
2	Glen Moy	1981	Horticultural
3	Malling Jewel	1981	Station
4	England		manufacturer F
5	Glen Clova	1981	
6	Glen Clova	1982	Scottish Crop
7	Glen Prosen	1982	Research
8	Malling Jewel	1982	Institute
9	Glen Moy	1982	
	Sulphited pulp		
10	Poland		manufacturer A
11	Poland		manufacturer B
12	Poland		manufacturer C
13	Scotland		manufacturer D
14	Scotland		manufacturer E
15	Scotland		manufacturer B
16	Scotland		manufacturer C
17	Ireland		manufacturer B

the use of sulphite or bisulphite salts. The frozen fruit was taken from stocks, held at the Leatherhead Food Research Association for research on jam quality. For all but one of these samples the cultivar, harvest date and grower were known. For sample 4, harvested in 1981, the cultivar was unknown but was either Glen Clova or Malling Jewel. Sulphited pulps were obtained from five UK jam manufacturers and represented their normal commercial stocks. Only the countries of origin of these were known.

Methods

SAMPLE PREPARATION

Frozen fruit was placed in closed containers to prevent any condensation of moisture from the atmosphere. These containers were then immersed in hot water to assist thawing. Any juice lost during thawing was incorporated at the subsequent homogenisation stage. To prepare homogeneous samples, 200–300 g of thawed fruit was pulped at room temperature in an Atomix mixer. A portion of pulp, including seeds, was put aside for analysis and the remaining pulped material was sieved quantitatively to remove the seeds, which were then washed with warm distilled water to loosen any adhering tissue. The washings were added to seedless pulp and their combined weights recorded. The seeds were allowed to dry exposed to the air overnight at ambient temperatures and then weighed.

Sulphited pulps were well mixed, sampled to provide material containing both seeds and fruit flesh, and the remainder then sieved as described above.

ANALYSES

Duplicate weighed portions of all the air-dried seeds were further dried in an oven at 105°C for 16 h, cooled in a desiccator, and re-weighed.

Ash contents of fruit and sieved pulps were determined on duplicate samples which were dried and charred before ashing at 525°C. The ashes were subsequently dissolved in 10 ml of nitric acid (AR grade, diluted 1 : 1 with water) and, after suitable dilution of these solutions, potassium was determined by flame emission spectrophotometry.

The acid solutions of ash were also used for the determination of total phosphorus by the method of Fogg and Wilkinson[1]. Total nitrogen was determined with an automated Kjeldahl procedure (KjelFoss Model 16210, Foss Electric, York).

Certain samples of seeds were also analysed as above, in order to check the validity of results for seedless pulps.

TREATMENT OF RESULTS

All the results for the mixtures of seedless pulp with washings were corrected for the water added as washings. The tabulated results thus represent the analysis of undiluted pulp.

Sulphited pulps typically comprise 97 per cent. of fruit owing to the addition of significant amounts of sulphur dioxide solution as preservative[2]. The figure of 97 per cent. was used to correct the analytical results for these pulps to 100 per cent. fruit equivalent. It is possible that sulphited pulps are diluted with water in excess of that required to add preservative. However, as shown later, the results for sulphited pulps are not significantly different from those for frozen fruit. This observation suggests that excessive dilution has not taken place and that the figures given for sulphited pulps and those for frozen fruit are thus directly comparable.

The proportion of the total minerals in the fruit located in the seedless pulp (Table IV) was calculated from the following equation:

$$\text{Proportion of specified mineral in the seedless pulp, expressed as a percentage of the total mineral present in the fruit} = (100 - a)\frac{b}{c}$$

where a is the weight of air-dry seeds (g/100 g of fruit); b is the concentration of specified mineral in the seedless pulp (mg/100 g of pulp); and c is the concentration of specified mineral in the whole fruit (mg/100 g of fruit).

All analytical results were subjected to Filliben's test[3] for normality of distribution and, where appropriate, Dixon's test[4] for outliers. In order to compare the two methods of fruit preservation, F-tests and then unpaired, two-tailed t-tests were applied to the results. The sieved pulps were compared with the whole fruits by means of paired, two-tailed t-tests.

Results

The yield of seeds is summarised in Table II. The mean analytical results for whole fruit and the corresponding seedless pulps are shown in Table III. Table

TABLE II

YIELD OF SEEDS

Fruit type	Number of samples	Mean yield of seeds ± s.d. *per cent. m/m of whole fruit*		Mean weight of air-dried seeds associated with 100 g of seedless pulp ± s.d. *g*
		Air dried	Oven dried	
Frozen	9	5·06 ± 1·28	4·16 ± 1·08	5·33 ± 1·46
Sulphited	8	3·91 ± 1·73	3·28 ± 1·38	4·09 ± 1·90
All	17	4·52 ± 1·57	3·74 ± 1·27	4·74 ± 1·75

TABLE III

MEAN MINERAL CONTENTS OF RASPBERRIES AND SEEDLESS PULPS

Fruit type		No. of samples	Mean mineral content ± s.d. *mg/100 g*			
			Ash	Potassium	Nitrogen	Phosphorus
Whole fruit	frozen	9	459 ± 94	176 ± 37	178 ± 32	22·1 ± 3·5
	sulphited	8	415 ± 110	153 ± 42	165 ± 41	19·3 ± 3·5
	all	17	438 ± 101	165 ± 40	172 ± 36	20·8 ± 3·7
Seedless pulps	frozen	9	246 ± 61	142 ± 23	122 ± 27	13·7 ± 2·5
	sulphited	8	281 ± 99	117 ± 31	136 ± 36	13·3 ± 2·3
	all	17	262 ± 80	131 ± 29	129 ± 31	13·5 ± 2·3

TABLE IV

PROPORTION OF TOTAL MINERALS IN RASPBERRIES PRESENT IN SEEDLESS PULP

Fruit type	Number of samples	Minerals in seedless pulp expressed as mean percentage of total in whole fruit *m/m*			
		Ash	Potassium	Nitrogen	Phosphorus
Frozen	9	51·8 ± 13·7	77·7 ± 9·0	64·9 ± 7·9	59·0 ± 7·1
Sulphited	8	65·2 ± 14·5	74·4 ± 8·6	80·2 ± 12·5	66·7 ± 8·4
All	17	58·1 ± 15·3	76·2 ± 9·5	72·1 ± 12·7	62·7 ± 8·5

IV summarises the amount of each mineral found in seedless pulps expressed as a percentage of the quantity in the whole fruit.

Discussion

COMPARISON WITH PREVIOUSLY PUBLISHED ANALYSES

The published data of numerous workers, as compiled by Goodall[5], was used to calculate the average compositional figures shown in Table V. Comparison of Table V with Table III reveals an almost exact agreement between the mean results found here and those in the literature on the composition of whole raspberries. The sole exception is total nitrogen, but it should be noted that the

TABLE V

SUMMARY OF COMPOSITIONAL DATA FOR RASPBERRIES, COMPILED FROM THE SURVEY OF GOODALL[5]

	Ash	Potassium	Nitrogen	Phosphorus
No. of studies	5	6	3	6
Total No. of samples	207	184	46	231
Mean value (*mg/100 g*)	448	167	117	19·9
Omitting black varieties:				
No. of samples	162	156	no data	186
mean value (*mg/100 g*)	432	162		20

values for nitrogen concentration tabulated by Goodall all relate to black-fleshed varieties and are thus not likely to be representative of the more commonly-used red cultivars.

SCATTER OF RESULTS

Unfortunately, Goodall's survey does not present data in a way which allows standard deviations to be calculated. It is not possible, therefore, to compare the variation between samples found here with earlier work. However, the figures shown in Table III do confirm that large deviations from the mean composition are common.

The results from the present study were subjected to Filliben's test for normality of distribution. There was no evidence that the ash, potassium and nitrogen concentrations of the whole fruit, or those of the seedless pulps, did not follow a normal (Gaussian) distribution. Although the phosphorus content of the seedless pulps also failed to reveal any indication of a non-normal distribution, the phosphorus concentrations found for the whole fruits were significantly different from a normal distribution at the 5 per cent. confidence level. Application of Dixon's test showed the result for phosphorus in one sample to be an outlier, and this result was also identified as the cause of the deviation from normal distribution. However, repeated chemical analyses showed no analytical error. There were, therefore, no grounds upon which to reject the result, and it was retained.

The results for frozen and sulphited fruit were compared to check the possibility that the method of preservation influenced composition. *F*-tests and then unpaired, two-tailed *t*-tests were applied to the results for each element in frozen and sulphited fruit as well as to those for each element in the corresponding seedless pulps. No significant differences were found, and it was concluded that the results obtained from fruit subjected to the two methods of preservation were not significantly different.

It should be noted that the non-normal distribution of phosphorus in whole fruit might influence the validity of the above conclusion for phoshorus. However, the deviation from normality was small and *t*-tests are robust. Accordingly, a fair measure of confidence can be placed in the findings.

The results shown in Table III for whole fruit provide coefficients of variation for potassium, nitrogen and phosphorus concentrations of 24, 21 and 18 per cent. respectively. These coefficients suggest that, for those results which are

normally distributed, there is about a one in twenty chance of samples occurring naturally with mineral compositions which deviate from the mean values by more than ±40 per cent. of the mean.

From Tables II and III it can be calculated that the mean potassium and nitrogen concentrations in the seeds of the frozen fruit are about 815 and 1230 mg/100 g, respectively. The corresponding figures for seeds of the sulphited fruit are 1040 and 880 mg/100 g respectively. The differences between these are quite large for average figures, and it might therefore seem that changes may take place during storage. However, it should be remembered that the seed coat is quite impermeable: it is the seed's protection against the digestive systems of birds and animals. In addition, it is difficult to envisage much movement of the elements concerned in frozen fruit. Transfer in the sulphited pulp would be more likely, but it would then be necessary to explain how potassium and nitrogen appear to move in opposite directions across the seed coat (assuming the analysis of frozen fruit indicates the pre-storage state). Furthermore, the postulated increase of potassium in sulphited seeds would represent a movement up a concentration gradient. For these reasons, change on storage seems an unlikely explanation, and it is probably the different origins of the fruit tested which give rise to the differences in seed composition.

DISTRIBUTION OF MINERALS BETWEEN SEEDS AND FRUIT PULP

Preliminary comment is necessary concerning the method of calculating seed, and hence pulp, contents. The procedure used assumes that the weight of air-dried seeds represents the weight of seeds as they are to be found in the whole fruit. This is open to the criticism that the seeds may have lost some of their natural moisture during air-drying, and that their yield is correspondingly underestimated. It might also be argued that the seeds acquired excess moisture during their washing, and that this has been retained leading to over-estimates. The latter seems unlikely and, in view of the actual yields recorded, the former would not appear to be a significant source of error. In either case it is not apparent what measure other than air-dry weight could be taken as representative of the true weight of seeds in the fruit. The results cited are thus the best practical estimates available.

Even when due allowance is made for any small error which might be present, Table III still shows clearly that all the seedless pulps contained markedly lower concentrations of minerals than the corresponding whole fruit. Table IV underlines this observation and, when the mean yield of pulp (95·3 per cent.) is taken into account, it is apparent that the seedless pulps contained disproportionately lower mineral concentrations. This finding was confirmed by carrying out paired, two-tailed *t*-tests on results for whole fruit and for the corresponding seedless pulps. For each of the three elements determined there was a very highly significant difference ($P = 0{\cdot}001$) between the concentrations found in the fruit and those in the seedless pulps.

The practice of applying conversion factors for whole fruit to analyses of seedless material assumes that the minerals determined are equally concentrated in seeds and fruit flesh alike. The above results show this assumption to be

false. For example, the mean potassium concentration of the seedless pulps was 76 per cent. of that to be expected were this element distributed homogeneously between seeds and flesh. In contrast, the seeds contain, on average, five times the potassium expected on the same basis, although the individual results are widely scattered. Similar calculations show that the raspberry seeds also contained more than six times the nitrogen, about eight times the phosphorus and, indeed, 8·75 times the ash level to be expected if these components were of uniform concentration throughout the whole fruit. It follows that conversion factors for whole fruit are not appropriate for products made with sieved pulp. The errors which arise from the application of such unsuitable factors are disussed below.

EFFECTS ON ESTIMATES OF FRUIT CONTENT

The objective of this section is to compare the different estimates of fruit content which may be derived from identical analytical data by the application of various factors. For this purpose, the factors representing whole raspberries will be taken as those which may be derived from Table III. The resulting equations for fruit content are:

$$\begin{aligned}\text{Fruit content, } &= (100/165) \times \text{concentration of potassium in mg/100 g} \\ &= (100/172) \times \text{concentration of nitrogen in mg/100 g} \\ &= (100/20{\cdot}8) \times \text{concentration of phosphorus in mg/100 g}\end{aligned}$$

The above factors for potassium and phosphorus are virtually identical with those which can be derived from historical data as represented in Goodall's survey. The disparity between nitrogen results here and those in the literature has already been explained, with the consequence that those shown in Table III are to be preferred.

Substitution in the above equations of the mean results for whole raspberries gives a result of 100 per cent. of fruit in each case. However, although the seedless pulps are still 100 per cent. of fruit, their mean apparent fruit contents are as follows:

Based on	Apparent fruit content (*per cent.*)
Potassium	79·4
Nitrogen	75·0
Phosphorus	64·9
Mean of three elements	73·1

These figures show that a genuine seedless raspberry jam, made with 35 g of average quality seedless pulp per 100 g of jam, would appear to contain only 25·6 g of fruit, based on determinations of potassium, nitrogen and phosphorus averaged together.

It should be emphasised that this is the result expected of an average raspberry pulp. In practice, these errors will be further compounded with those previously mentioned which arise from natural variation. The least favourable combination of circumstances for a manufacturer would arise when a batch of fruit naturally low in minerals also contained a particularly large proportion of those minerals in the seeds. It follows that particular caution should be exercised in estimating the fruit content of seedless raspberry jam, and it is desirable to use conversion factors which take into account the disproportionately large quantity of minerals discarded with the seeds. On the basis of results described above, suitable conversion factors could be those in the following equations:

$$\text{Estimated fruit content, (per cent.)} = (100/131) \times \text{mg of potassium/100 g of jam}$$
$$= (100/129) \times \text{mg of nitrogen/100 g of jam}$$
$$= (100/13{\cdot}5) \times \text{mg of phosphorus/100 g of jam.}$$

These factors represent the mean composition of seedless raspberry pulp, as described by the results in Table III. Such mean factors have the attraction that they are likely to give the most realistic estimates of fruit content. However, their application to a large number of samples would tend to lead to underestimates of the true fruit content of about half the jams tested.

Fortunately, the data in Table III permit the chance of such an underestimate to be calculated for any chosen factor. Table VI lists a range of factors, together with the corresponding chances of underestimating fruit content. Each factor shown in Table VI is intended to be used in an equation of the form:

$$\text{estimated fruit content (per cent.)} \leqslant (\text{factor}) \times (\text{mg of element/100 g jam}).$$

TABLE VI

CONVERSION FACTORS FOR ESTIMATING MINIMUM FRUIT CONTENT FROM THE ELEMENTAL COMPOSITION OF SEEDLESS RASPBERRY JAM

Probability of underestimating true fruit content	Conversion factor*		
	Potassium	Nitrogen	Phosphorus
0·5	0·763	0·775	7·41
0·1	1·09	1·14	9·65
0·05	1·25	1·34	10·6
0·01	1·8	2·07	13·5

* See text for equation to be used.

Tables III and VI show that potassium, nitrogen and phosphorus concentrations are poor indicators of fruit content. While factors based on the mean composition of fruit or seedless pulp are to be preferred because they are most likely to provide realistic results, there is a substantial risk that the estimate of fruit content in any particular case will be incorrect.

Conclusions

Potassium, nitrogen and phosphorus concentrations are poor indicators of the fruit content of raspberry jam. These elements, as well as the ash content, are also distributed unevenly between raspberry fruit flesh and seeds. The seedless flesh typically comprises 95 per cent. of the fruit, yet contains only about 59 per cent. of the ash, 76 per cent. of the potassium, 70 per cent. of the nitrogen and 63 per cent. of the phosphorus of the whole fruit. Thus, when seedless raspberry jam is analysed any factors used to convert results for mineral concentrations into estimates of fruit content should take into account the disproportionately low mineral content of the seedless pulp.

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Newer Techniques in Food Analysis—Immunoassays and their Application to Small Molecules

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Immunoassays can offer several advantages to the analyst, often in applications where conventional procedures suffer difficulties and drawbacks. The use of immunoassays for the determination of low molecular weight materials in food is a comparatively recent development. It appears that the use of enzymes as attached indicators or labels will become the most widely used of the many possibilities available, and some recent developments are described. Examples include the determination of total potato glycoalkaloids, and the analysis of ochratoxin A. The properties of the generated assays make it clear that the immunological technique will be used widely in future.

Since the description in 1959 by Berson and Yalow[1] of a radioimmunoassay for plasma insulin, this type of analytical technique has been used extensively in many fields of research. Immunoassays can be sensitive, specific, and applied to the rapid and routine analysis of large sample numbers. The combination of specificity and sensitivity means that assays can often be performed on complex biological matrices with minimal sample clean-up, giving consequent beneficial effects on assay performance, assay time and cost. These unique properties result from the nature and affinity of the antibody interaction with its specific antigen. Classical radioimmunoassays are based on the competition for a limited number of antibody binding sites by a trace amount of radiolabelled antigen and the antigen of the sample to be analysed. The amount of radiolabelled antigen that subsequently becomes antibody-bound is dependent on how much unknown antigen is present. Radioactivity can be determined in either the antibody-bound or antibody-free phases, and after reference to the behaviour of standard amounts of antigen the unknown antigen can be quantified.

In order to set up an immunoassay, the requirements are:

(i) a standard or reference preparation. This may present more problems with, for example, food proteins that have been subjected to processing than small molecules of defined chemical and physical properties.

(ii) a labelled antigen or antibody preparation. Radioisotopic labels have been used traditionally, though their use for food application may be limited as discussed later.

(iii) an appropriate antiserum preparation. The antiserum is the most important component of any immunoassay system and needs to be (a) of the correct specificity for the desired purpose, (b) able to contribute to desired assay sensitivity, and (c) available in sufficient amounts for continuity of work.

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(iv) a suitable phase separation system. There are many methods available to separate the antibody-bound from the antibody-free phase and the analyst should choose the most appropriate one for the circumstances.

Small molecules, those of molecular weight less than about 1000 daltons, present different problems to the immunoanalyst than those of larger size. Small molecules do not stimulate antibody formation, i.e. they are not immunogenic. In order to obtain antibodies, the small molecule must first be chemically coupled to a larger, immunogenic molecule. Thus many different antibodies are generated, some of which will recognise the coupled compound (known as the hapten). How the conjugation of hapten to carrier immunogen (almost always a protein) is carried out is crucially important in defining the specificity of the antiserum that is produced subsequently. As Landsteiner discovered[2], antibodies have greater specificity to those functional groups on the hapten distal to the point of conjugation, and little or none around the point of linkage. Synthesis of hapten-protein conjugates for use as immunogens requires careful consideration of the specificity required ultimately in the assay. This needs knowledge of related compounds and metabolites, and their likely levels in the samples to be analysed. A decision can then be made as to whether a high or broad specificity antiserum is required, and how to carry out the conjugation.

Having obtained appropriate antiserum, the immunoassay can be set up and validated for the desired application. It should be emphasised that assays for different applications must be re-validated.

Enzyme Immunoassays

Much of the earlier work on hapten immunoassays has employed radiolabelled tracer, usually tritiated though less often of the ^{125}I form. Application of radioimmunoassays in food analysis is exemplified by the work on anabolic residues in meat[3,4]. In general, however, the use of radiolabelled tracers is hindered by the commercial non-availability of suitable material of high specific activity and by the fact that their use may be unacceptable in a food context. Tritiated labels require expensive and time-consuming liquid scintillation counting, and the use of ^{125}I is severely limited by its lack of stability. Therefore, despite the advantages when working with tritiated analyte of having a tracer very similar in properties to the unlabelled analyte, there has been a lot of interest in the use of non-isotopic labels. At present, the most popular approach is to use enzyme labels, pioneered by two groups of workers[5,6]. Different types of enzyme immunoassay, based on different principles, have now been described and include competitive, immunometric and "sandwich" assays as well as the novel homogeneous, non-separation techniques[7]. For food-related applications, the use of enzyme-linked immunosorbent assays (ELISAs) carried out in the wells of plastic microtitration plates have been widely reported and have a number of advantages. Phase separation is quick and simple because one of the components of the assay is immobilised to the well surface. The assays can be carried out very cheaply—using simple pipettes, wash bottles to wash the plates and culminating in visual assessment of enzyme-generated optical densities—or a much higher level of automation (still comparatively cheap) can

be used by laboratories with high sample throughput. The assays employ stable materials and reagents, and are technically simple to carry out.

For the application of ELISA to small molecules on microtitration plates the majority of procedures described in the literature to date are of the direct, competitive format. Antibodies are immobilised to the plastic surface of the microtitration plate wells, and hapten and enzyme-labelled hapten compete for the binding sites. Phase separation is a simple matter of emptying the well contents and washing. Bound enzyme is then detected by addition of substrate. The rate of development of optical density is proportional to the amount of hapten present initially. This form of ELISA procedure as applied to ochratoxin A[8], aflatoxin B_1[9], and limonin[10], for example, does have some disadvantages. Antibody adsorption and availability for the subsequent binding reactions is not complete, resulting in inefficient use of the antiserum. Enzyme-labelled analyte must be synthesised to give a product of certain specification for maximum assay performance. In addition, the mixing of enzyme label and food matrix may be a potential source of problems.

At the Food Research Institute there has been a preference to work on indirect assays, where hapten rather than antibody is immobilised to the well surface. Primary antibody and analyte hapten are added to each well and the antibody distributes itself between immobilised and free hapten according to how much free hapten is present initially. After phase separation, again by well emptying and washing, bound primary antibody is detected by addition of a species-specific, enzyme-labelled second antibody (i.e. one which is active against the primary antibodies). After an appropriate incubation period, excess material is removed and substrate added. Well optical densities are determined after a suitable time. Although the indirect assay has one more incubation step than the direct form, the benefits that accrue, such as the economy of primary antisera used, more than offset this disadvantage. Enzyme-labelled second antibodies are widely available commercially, active against different species and labelled with a variety of enzymes.

Application to Food

At the Food Research Institute, indirect ELISA procedures have been set up and validated for the measurement of total potato glycoalkaloids[11,12], the determination of ochratoxin A in barley[13,14], and for the food bittering agents quassin[15,16], and quinine and quinidine[17]. The first two of these applications illustrate clearly the advantages of the immunological approach over that of conventional methods and will be described more fully.

The total potato glycoalkaloid fraction consists of α-solanine and α-chaconine (which together constitute more than 95 per cent. of the total under normal conditions) with minor components such as demissine and the aglycones solanidine and demissidine. The glycoalkaloids, which occur naturally in the potato, are poisonous and may be teratogenic. They also exert a nauseous, bitter taste to the cooked product, and so there is considerable interest in monitoring total glycoalkaloid levels and ensuring that they remain below a specified maximum value. Unfortunately, trying to do this and trying to assess the role of these compounds in human disease has been hindered by the unsuitability of the

available methodology. To set up an ELISA procedure an antiserum of broad specificity was required, and it was generated by judicious selection of the method of immunogen synthesis to give a product recognising the appropriate compounds equally well[11]. A direct measure of total glycoalkaloid is thus possible. Assay sensitivity and specificity are such that mere homogenisation and dilution of tuber material is all that is required as sample clean-up. At least 50 times as many samples can be extracted and assayed per week by ELISA as by conventional procedures, and at a much lower level of technical and analytical expertise. Results for the different types of assay correlate extremely well[12], and so the ELISA procedure would appear to be the method of choice. In addition, the sensitivity of the immunoassay procedure means that it can be used for the first time to study the metabolism of the glycoalkaloids in humans by being able to detect and quantify metabolites in serum[18]. Better assessments of the role of potato glycoalkaloids in human health should be possible because of the improved analytical technology.

Ochratoxin A, a toxic secondary metabolite of certain moulds, is found as a contaminant of a number of agricultural commodities such as cereals and nuts. Analysis of this compound is rendered difficult because (a) its distribution in foodstuffs is often such that it is necessary to analyse several sub-samples in order to assess the overall contamination, and many of these will be negative, (b) the analyst needs to be interested in concentrations at the parts per billion level, and (c) conventional methods of analysis are not sufficiently specific to avoid lengthy sample clean-up procedures. The advantages of immunoassays apply to problems of this type and a suitable indirect ELISA procedure has been set up at the Food Research Institute[13,14]. The antiserum is specific, cross-reacting very poorly with hydroxylated derivatives, and other metabolites such as ochratoxin α and phenylalanine. The much less toxic ochratoxin B also interacts to an insignificant extent in the assay. The ELISA procedure has been validated for application to barley and pig kidney, both requiring only simple and quick clean-up procedures prior to assay. Apart from beneficial effects on total assay time and cost, the reduction in the number of steps improves assay performance in terms of, for example, recovery and reproducibility.

Future Trends

The immediate application of immunoassays to food problems will be the transfer of assays developed in the research laboratory to the routine analytical laboratory. It is envisaged that kits will be available shortly for use in laboratories with both high and low sample through-puts. In areas such as mycotoxin analysis, two forms of assay might be available—one a highly specific test, the other a broad specificity assay for groups such as the trichothecenes or the aflatoxins. This might be achieved by using "cocktails" of appropriate (monoclonal?) antibodies. The immunoassay methodology will improve with the availability of better antisera and with, if necessary, the use of end-points of a greater sensitivity of detection. Examples would be the use of fluorescent or chemiluminescent labels. The development of new analytical approaches utilising the antibody–antigen interaction, such as chromatography on immuno-

affinity strips[19], will continue. The advent in food analysis of the "dipstick" assay of high specificity and sensitivity can be foreseen.

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Crocetin Equivalent of Saffron Extracts. Comparison of Three Extraction Methods*

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Extraction of the colour of saffron may be performed with cold water, hot water, or a combination of hot water and maceration. The last method was found to be the most efficient.

Saffron is the most expensive of spices[1], and one criterion of the quality of any particular sample is the intensity of its colouring power[2]. The International Standard[2] requires that this be measured on a cold water extract. However, the principal pigment present, α-crocin, is reported to be only slightly soluble in cold water, but freely soluble in hot water[3–5]; hot water extraction therefore appears to be more suitable[6]. Even after hot water extraction, the "exhausted" material still often contains visible remaining pigment; complete extraction requires maceration of this material. The results obtained after extraction by the three methods were compared.

The small retail packets of saffron—seldom over 2 g and often only 0·125 g net, and its high retail price—equivalent to from £2 to £10 per gram in recent years, combine to necessitate micro-analysis; for reference the International Standard[7] requires a 2 g sample.

Materials

Twenty fresh samples of saffron were obtained from saffron crocuses (*Crocus sativus* L.) grown experimentally in pots. The stigmas, the spice of commerce, were plucked from the flowers, and air-dried at ambient temperature, at about 20°C, in the dark: drying was complete after about seven days.

For comparison, six retail samples of unknown age and of diverse geographic origin were obtained from retail sources. Four of these were of hay saffron[8] and two were of powdered saffron. The absorption spectra of the aqueous extracts of five of the six samples were very similar to one another and to those of the fresh saffron above. The characteristic pigments of saffron, and no others, were detected by thin-layer chromatography[2] in all except the sixth retail sample (hay saffron); this sample was therefore discarded.

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Procedures

Individual stigmas of hay saffron were tested; each constituted one-third of a flower's triad and weighed about 2 mg. They were weighed to an accuracy of ±0·1 mg and transferred to 20-ml ground-glass stoppered Pyrex test-tubes. The two retail samples of powdered saffron were tested in the same manner.

METHOD I: COLD WATER EXTRACTION

Add 5 ml of distilled water to the stigma in each test-tube, and allow the stoppered tube to stand for 24 h in the dark at ambient temperature with occasional shaking. Determine the absorbance of the supernatant solution against a water blank at 440 nm[2,9]. If the absorbance of the solution is above the range of the spectrophotometer, dilute the solution tenfold. Perform triplicate determinations.*

METHOD II: HOT WATER EXTRACTION

Add approximately 2 ml of distilled water to the stigma in each test tube, and then stopper with a sliver of plastic paper (e.g. Nalgene Polypaper) between the stopper and the tube, to prevent any pressure build-up. Place each tube in a boiling water bath for 20 min, with occasional shaking to ensure that the stigma is properly immersed in the distilled water. Cool to room temperature, make up to 5 ml in a standard volumetric flask, and determine the absorbance as in Method I. Perform triplicate determinations.

METHOD III: HOT WATER EXTRACTION WITH MACERATION

Follow the procedure of Method II, except that after cooling the aqueous extract, macerate the "exhausted" stigma (or powder) with a Teflon tissue grinder before diluting to standard volume. The solid residue now becomes virtually colourless. Perform triplicate determinations.

Units

The International Standard[2] expresses the colouring power of saffron as the specific extinction $E^{1\ \text{per cent.}}_{1\ \text{cm}}$ at 440 nm. The results below are expressed as crocetin, the digentiobiose ester of which is α-crocin[10]. The relationship between these units is given by equation (1):

$$E^{1\ \text{per cent.}}_{1\ \text{cm}}\ (440\ \text{nm}) = 40{\cdot}25 \times \text{crocetin (per cent.)} \qquad (1)$$

In fact, crocetin is not really water-soluble[12], but the extinction of α-crocin is not known. In order to obtain an approximation to the concentration of α-crocin, it is noted that the molar extinctions of crocetin and those of its dimethyl and diethyl esters are fairly close[11,13]:

crocetin	132 200
crocetin dimethyl ester	141 700
crocetin diethyl ester	147 800
(cf. β-carotene	134 500)

* This method is essentially equivalent to the ISO method except that triplicate determinations are performed, each with only 2 mg of sample.

Then, assuming that the molar extinctions of α-crocin (M.W. = 977) and of crocetin (M.W. = 328·38) are the same,

$$\text{Percentage of } \alpha\text{-crocin} = \frac{977{\cdot}00}{328{\cdot}38} \times \frac{1}{40{\cdot}25} \times E_{1\,\text{cm}}^{1\%} \text{ (440 nm)} \quad (2)$$

$$= 2{\cdot}975 \times \text{crocetin (per cent.)} \quad (3)$$

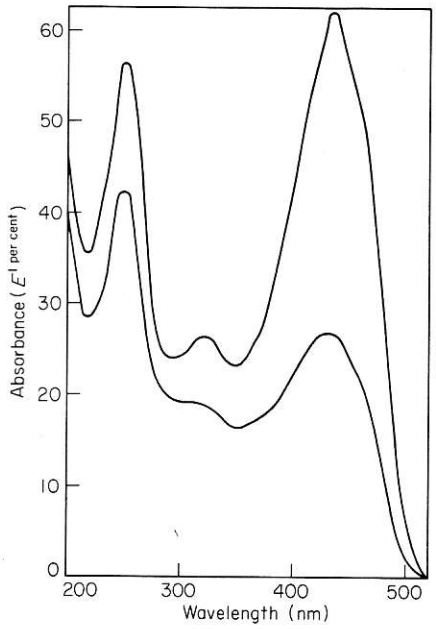

Fig. 1. Typical absorption spectra of aqueous saffron extracts.

Results and Discussion

Typical spectra of aqueous saffron solutions are shown in Figure 1. These were obtained from individual stigmas (each one-third of a flower's triad) from the same sample of fresh saffron, following extraction performed by Method II. The absorbance was calculated as for solutions of a hypothetical 1 per cent. concentration of saffron. The minor peak at 325 nm, and the ultraviolet peak at 255 nm are referred to in the Draft International Standard[14]; the absorbance measured at this latter wavelength is also reported below.

The mean results obtained from the comparison of the extraction methods are shown in Table I; identical lower-case letters indicate, for each group of samples, means which did not differ at the $P = 0{\cdot}05$ statistical significance level, by Duncan's multiple range test[15].

The precision of the test procedures may be gauged from the pooled standard deviations[16] obtained on the powdered saffron samples (see Table II); the greater homogeneity of these samples as compared with hay saffron makes them especially useful for this purpose.

TABLE I

COMPARISON OF PROCEDURES FOR AQUEOUS EXTRACTION OF SAFFRON

Samples	Extraction method	Carotenoid content (*as per cent. crocetin*)	$E^{1\ per\ cent.}_{1\ cm}$ (*255 nm*)
Fresh saffron	Cold water	1·6b	60b
	Hot water	2·4a	51c
	Hot water + maceration	2·6a	69a
Purchased saffron	Cold water	1·3a	55a
	Hot water	1·5a	41a
	Hot water + maceration	1·8a	46a

Note

In each group of three means, identical lower case letters indicate values which do not differ significantly.

TABLE II

PRECISION OF TEST PROCEDURES WITH POWDERED SAFFRON: COMPARISON OF POOLED STANDARD DEVIATIONS OF REPLICATE DETERMINATIONS

Extraction method	Carotenoid content (*as per cent. crocetin*)	$E^{1\ per\ cent.}_{1\ cm}$ (*255 nm*)
Cold water	0·13	3·0
Hot water	0·28	3·5
Hot water + maceration	0·14	3·9

Conclusions

Hot water extraction with maceration (Method III) gave the highest values of colouring power or carotenoid content; the difference between this method and the similar one without maceration (Method II) was not statistically significant. For fresh saffron, but not for saffron purchased by retail, cold water extraction (Method I) gave significantly lower values.

Method III gave significantly higher values of the 255 nm peak than the two other methods, and Method I gave significantly lower values, for fresh saffron only. The implications of this finding are unclear.

In general, Method III is to be preferred to Method II because of the higher precision (lower pooled standard deviation) obtained in determination of the colouring power.

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