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A Note on the Differentiation of Soft Brown Sugars

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The sugar syrup coating and the crystal centre were isolated from soft brown sugars. By comparing the magnesium and potassium contents of each component, it was possible to differentiate between genuine muscovado sugar on the one hand and refined sugar with added molasses coating on the other.

Soft brown sugars have traditionally been produced by the crystallisation of raw cane sugar. In recent years, products have appeared on the market which are composed of refined or partly refined crystal with a molasses coating.

The purpose of this work was to determine whether the coated product could be distinguished analytically from the traditional muscovado product.

In both types of product there is a layer of unrefined syrup coating the crystal, but it might be expected that in the traditional product, the crystal and the coating would be of nearly similar composition, whereas in the newer product the composition of the crystal and the coating would be significantly different.

No specific method is available for stripping the coating from the sugar crystal. However, by careful choice of solvents, a sugar residue can be obtained which represents the base crystal and a solution can be obtained that represents the syrup coating.

In this experiment, eleven brown sugars were examined, some of which were known to be genuine muscovado sugars and others which were known to be refined or partly refined crystal with a molasses coating.

Choice of Solvents

Preliminary experiments were carried out in order to determine the solubility characteristics of ordinary white granulated sugar in mixtures of IMS/Water. The extraction method used was that described below under "Solvent Extraction".

Each solvent was prepared by pipetting the appropriate quantity of water into a volumetric flask, diluting with IMS and making up to volume with IMS at 20°C.

The approximate percentages of sugar dissolved by the solvent mixtures used are given in Table I.

From these results, it was considered that when examining soft brown sugar, the sugar dissolved from Solvent A would represent the syrup coating while the residue remaining undissolved from solvent D would represent the base crystal.

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(A more detailed justification is given later.)

The soft brown sugars were therefore analysed using only solvents A and D.

Apparatus and Reagents

Medicine flat bottles, 120 ml capacity, with screw cap and rubber liner.

End-over-end shaker.

Moisture tins—75 mm in diameter and 20 mm deep containing 10 g of acid washed sand.

IMS 94 per cent.

Solvent A: see Table I.

Solvent D: see Table I.

TABLE I
SOLUBILITY OF WHITE SUGAR IN AQUEOUS ALCOHOL

Solvent	IMS	Water	Approximate percentage of sugar dissolved
A	97·5	2·5	10
B	95·0	5·0	15
C	90·0	10·0	35
D	85·0	15·0	70

Procedure

1. SOLVENT EXTRACTION

Into each of two 120 ml medicine flat bottles weigh 10·0 g of sugar sample. To one, add by pipette, 100 ml of solvent A, and to the other, 100 ml of solvent D. Adjust the temperature to approximately 18°C, and mix in an end-over-end shaker for 60 ± 5 min. Filter the contents of each through No. 1 filter paper and retain the sugar solutions. Wash the sugar residues from solvent D with 2 × 20 ml portions of IMS and reject the washings. Dry the residue overnight at 44°C and retain in a desiccator. Dry 5·0 ml of each of the retained sugar solutions on sand in weighed moisture tins at 105°C for 2 h. Weigh the residue (W).

Calculation of dissolved sugar solids

Let w = wt of dry solids in 5·0 ml of final solution. Let the density of sugar = 1·6 g/ml (an approximation for coated sugars). Volume occupied by wg of sugar = $w/1{\cdot}6 = 0{\cdot}625\,w$ ml. Then 5 ml of final solution contain $(5–0{\cdot}625\,w)$ ml of solvent. The factor to allow for volume change is therefore:

$$\frac{5}{5–0{\cdot}625\,w} = \frac{1}{1–0{\cdot}13w}$$

∴ Dissolved sugar solids (as a proportion of the original sugar) per cent w/w

$$= w \times \frac{100}{5} \times \frac{100}{10} \times \frac{1}{1–0{\cdot}13\,w}$$

$$= \frac{200 \times w}{1–0{\cdot}13\,w}$$

2. DETERMINATION OF POTASSIUM AND MAGNESIUM CONTENT

Determine the potassium content by flame photometry and the magnesium content by atomic absorption spectrophotometry of the extract obtained by solvent A, and calculate the results as mg/kg of the dissolved sugar solids.

Similarly, determine the potassium and magnesium contents of the dry residues retained after extraction with solvent D.

Results of Analysis

Eleven soft brown sugars were analysed. Five of these sugars were commercial brands known to be genuine Muscovados, having been supplied as authentic by a major importer. Four of the sugars were commercial brands labelled as being mixtures of sugar with molasses. Two of the sugars were commercial brands with no indication of their nature.

Table II lists type of sugar and the contents of magnesium and potassium in the syrup coating solids and the crystal centre of each sugar.

Discussion

1. RATIONALE OF METHOD

The average solubility of the 11 sugars in solvent A is 9·2 per cent. w/w (range 7·4–11·6 per cent.). On a model cube, this would mean that solvent A removes 1·6 per cent. of the total thickness from all surfaces of the cube. If a sugar grain is 500 microns in size, the thickness of coating removed by solvent A is 8 microns.

It is considered therefore, that the sugars dissolved in solvent A represent the syrup coating surrounding the sugar grain.

The average sugar residue obtained from solvent D is 30·3 per cent. (range 25·1–32·8) indicating that the solvent has dissolved approximately 70 per cent. of the product, i.e. solvent D has removed all coating from the sugar grain, as well as a significant part of the grain itself.

It is considered therefore, that the sugar residue from solvent D represents the centre of the base sugar grain.

Ratio M_A/M_D and K_A/K_D represent the proportions of mineral in the syrup coating relative to that in the crystal centre.

2. LIGHT BROWN SUGARS

Table II shows that the genuine light brown muscovados have M_A/M_D and K_A/K_D ratios below 10 while the compound sugars 3 and 4 have these ratios significantly greater than 20.

On that basis, sample 5 is shown to be a compound light brown sugar.

3. DARK BROWN SUGAR

Table II shows that the genuine dark brown muscovados have M_A/M_D and K_A/K_D ratios at 5 or below.

The compound dark brown sugar, sample 9 has these ratios significantly greater than 20 but the compound sugar 10 has ratios between 5 and 20. Examination of the magnesium and potassium contents M_D and K_D shows that

TABLE II
MINERAL CONTENT OF SYRUP COATING SOLIDS AND CRYSTAL CENTRE OF SOFT BROWN SUGAR

Sample	Solids extracted by solvent A *per cent.*	Dry residue after extraction with solvent D *per cent.*	Mg in solids extracted by solvent A (M_A) *mg/kg.*	K in solids extracted by solvent A (K_A) *mg/kg.*	Mg in residue after solvent D extraction (M_D) *mg/kg.*	K in residue after solvent D extraction (K_D) *mg/kg.*	Ratio M_A/M_D	Ratio K_A/K_D
Light brown sugar								
1. Genuine muscovado	9·7	29·8	800	11 640	190	1590	4·2	7·3
2. Genuine muscovado	8·5	32·3	480	5110	110	590	4·4	8·7
3. Soft brown sugar (beet sugar + molasses)	7·4	32·3	460	4870	10	70	46·0	69·6
4. Soft brown sugar (cane sugar + molasses)	7·9	32·8	520	7720	20	210	26·0	36·8
5. Light brown soft sugar	9·6	31·2	500	8580	15	100	33·3	85·8
Dark brown sugar								
6. Genuine muscovado	8·9	32·0	870	11 630	1280	14 120	0·7	0·8
7. Genuine muscovado	11·6	25·1	1280	6500	510	4570	2·5	1·4
8. Genuine muscovado	11·1	26·8	1500	15 210	380	3070	3·9	5·0
9. Soft brown sugar (beet sugar + molasses)	8·2	32·2	310	5560	8	170	38·9	32·7
10. Soft brown sugar (cane sugar + molasses)	8·9	29·4	1040	28 270	210	1680	5·0	16·8
11. Dark brown soft sugar	9·7	29·2	1000	13 430	40	320	25·0	42·0

the base crystal in this sample is not as refined as in the other compound products. If this sample had not been known to be a mixture of sugar and molasses, care would have been needed in interpreting the mineral ratios. Nevertheless, K_A/K_D could indicate that it was likely to be a compound product.

Sample 11 is shown to be a mixture of sugar and molasses.

Conclusions

By use of the ratios of magnesium and potassium between the syrup coating and the base crystal, it has been possible to show the difference between genuine muscovado sugars and compound articles which have been prepared from refined sugar crystal with a molasses coating.

Intermediate compound products prepared from relatively unrefined crystal with a molasses coating might be more difficult to categorise. However, consideration of the minerals actually present in the base crystal can provide an assessment additional to that gained from the mineral ratios.

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Determination of Synthetic Colouring Matter in Foodstuffs—Collaborative Trial.

Part I: Initial Trial

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A collaborative trial by twelve U.K. Public Analyst and Government laboratories, and one Danish Government laboratory to assess the suitability of a method of analysis for the determination of synthetic colouring materials (dyes) in foodstuffs has been carried out. Six samples of soft drinks and two samples of sponge cake spiked with a range of dyes were used. From the statistical analysis of the trial data, it was found that the method produced variable results with respect to repeatability and reproducibility, notably for samples known to contain several different dyes and those samples containing dyes known to be unstable during extraction. There were also several instances of dye mis-identification.

In 1979, the Food Additives and Contaminants Committee (FACC), in their Interim Report on the Review of the Colouring Matter in Foods Regulations (1973), stated that to complete the review it would "next consider the levels of use of synthetic colouring materials in individual foodstuffs with a view to deciding the specific recommendations necessary for proposed stricter controls on their use"[1]. In the Final Report of the Food Advisory Committee (successor Committee to the FACC) Review of the 1973 Regulations[2], detailed recommendations were made regarding stricter controls on the classification and use of colouring materials in food, including maximum permitted levels where appropriate. These recommendations were partly based on the results of a survey conducted on behalf of the Working Party on Colours of the Interdepartmental Steering Group on Food Surveillance[3].

Development of Survey Method

The Food Science Laboratory, in conjunction with the Laboratory of the Government Chemist (LGC), analysed a wide range of foodstuffs using a procedure developed initially at LGC[4], in order to provide the Working Party with data on colouring matter usage. Poor results were obtained by the laboratories in their initial work which was carried out on jam and fruit gum samples, probably due to the inexperience of staff with the analytical procedure being used. The analytical procedure was refined and modified in an effort to improve its qualitative and quantitative aspects and to enable it to be applied to

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a wider range of food matrices. The results from this work again highlighted problems associated with inexperience of staff with the analyses, particularly with respect to dye mis-identification. Nevertheless, analysis of a wide range of commodities (cake crumbs, canned peas, ice creams, instant desserts, jam, sweets and smoked fish) resulted in a reasonable correlation between the levels of colours determined and manufacturers' estimates of colour content. Several discrepancies between MAFF and LGC were observed when the same samples were analysed, again indicating that the analysis is not routine.

Since the results from the last survey were reported the availability of improved HPLC instrumentation and methodologies has also made it possible further to refine the procedure. It is this refined procedure that has been collaboratively tested in the trial being reported.

The trial was organised by the Food Science Laboratory to test the robustness of and to validate a method for the determination of synthetic colouring materials in foodstuffs. Such a method could then be recommended for inclusion in Regulations, should there be such a need, as a reference method for enforcement purposes. Ideally such a method would be required to be readily accessible to any competent analytical laboratory, and not to be unduly time consuming nor require the use of unnecessarily costly or sophisticated materials and equipment.

Collaborative Trial Organisation, Samples, Method and Results

PARTICIPANTS

Thirteen analysts participated in the trial in the laboratories of 10 U.K. Public Analysts, the Laboratory of the Government Chemist, the Government Laboratory from the Isle of Man and the Laboratory of the National Food Institute of the Danish Government.

SAMPLE PREPARATION

The following samples were prepared in the Food Science Laboratory. Samples were spiked with synthetic colour standards supplied by Williams Ltd. of Hounslow, Middlesex:

Lemonade (purchased at retail) was spiked to contain the following colours:

1. Tartrazine at 250 mg/l.
2. Tartrazine at 250 mg/l and Green S at 50 mg/l.
3. Amaranth at 100 mg/l and Carmoisine at 120 mg/l.
4. Ponceau 4R at 20 mg/l, FD&C Red 40 at 10 mg/l and Erythrosine at 50 mg/l.
5. Green S at 10 mg/l, Brilliant Blue FCF at 10 mg/l and Sunset Yellow FCF at 50 mg/l.
6. Tartrazine at 350 mg/l and Green S at 30 mg/l.

Each lemonade sample was prepared in 2-litre batches and distributed to participants in polythene bottles.

From packet cake mixes (purchased at retail outlet), two samples were prepared containing different ratios of colourings. For each sample, two packets were combined, spiked to contain the following colours (calculated on a final

TABLE IV

QUANTITATIVE DETERMINATION OF SYNTHETIC COLOURING MATERIAL: LEMONADE SAMPLE 1

	Sample code numbers	2,5
	Added colour	Tartrazine *mg/l*
Laboratory	Added level	250
1		291, 256
2		277[a], 56[a]
3		273, 263
4		349, 325
5		325, 340
6		203, 214
7		300, 291
8		300, 265
9		361, 362
10		293, 282
11		280, 260
12		297, 276
13		290, 272
Mean ($\bar{x}$)		290
Repeatability (r)		40.2
Reproducibility (R)		115.2

For key to superscripts, see Table XIII.

weight basis, assuming no colour degradation in cooking), and cooked to the manufacturer's instructions:

1. Tartrazine at 86 mg/kg and Ponceau 4R at 43 mg/kg.
2. Tartrazine at 69 mg/kg and Ponceau 4R at 72 mg/kg.

The browned outer layers of the cakes after cooking were removed prior to crumbling and homogenisation. Samples were homogenised and subjected to repeat analysis to verify homogeneity before the individual samples for participants were prepared. Samples were distributed to participants in sealed polythene bags.

Both the lemonade and cake samples were sent to each analyst coded as blind duplicates; i.e. each analyst received 12 samples of lemonade and 4 samples of cake crumb and was asked to analyse each once only. An additional aliquot of lemonade sample No. 1, diluted to contain 75 mg of Tartrazine per litre, was sent to each participant in the form of a pre-trial sample to enable participants to familiarise themselves with the method under trial conditions.

Each participant also received a complete set of synthetic colour standards covering the range of added colours as well as other permitted and non-permitted colours. These standards originated from the same batches as those used to prepare the samples.

TABLE V

QUANTITATIVE DETERMINATION OF SYNTHETIC COLOURING MATERIAL: LEMONADE SAMPLE 2

	Sample code numbers	3,10	3,10
	Added colour	Tartrazine *mg/l*	Green S *mg/l*
Laboratory	Added level	250	50
1		293, 301	41·6, 41·2
2		337, 265	N.d[a], 38·5[b]
3		278, 284	42, 49
4		334, 343	1·7[b], N.d.
5		315, 350	40[a], 64[a]
6		228, 178	46·8, 49·0
7		287, 268	43·9, 40·3
8		273, 276	N.d.[c], N.d.[c]
9		343, 336	68[d], 59[d]
10		307, 303	49·2, 49·2
11		270, 260	44, 43
12		307, 288	50·3, 45·7
13		306, 293	49, 50
Mean ($\bar{x}$)		293	45·9
Repeatability (r)		55·9	6·7
Reproducibility (R)		109·7	10·3

N.d. = not detected.

For key to superscripts, see Table XIII.

Mis-identification: Laboratory 8—Green S reported as Brilliant Blue FCF at levels of 28 and 18·2 mg/l for samples 3/10 respectively.

TABLE VI

QUANTITATIVE DETERMINATION OF SYNTHETIC COLOURING MATERIAL: LEMONADE SAMPLE 3

	Sample code numbers	1,12	1,12
	Added colour	Amaranth *mg/l*	Carmoisine *mg/l*
Laboratory	Added level	100	120
1		50·4, 47·6	131, 132
2		73·0, 78·3	132, 136
3		78, 83	155, 152
4		5·6, 2·4	117, 109
5		40, 26	98, 90
6		48·1, 42·4	128, 125
7		29·6, 27·1	118, 101
8		20·2, 22·9	115, 107
9		88·3, 72·9	127, 121
10		N.d[a],170[b]	225[a],128[a]
11		60[a], 28[a]	290[d],310[d]
12		69·6, 53·8	131, 122
13		78, 79	129, 125
Mean ($\bar{x}$)		50·7	123
Repeatability (r)		17·1	15·4
Reproducibility (R)		76·8	45·5

For key to superscripts, see Table XIII.

TABLE VII

QUANTITATIVE DETERMINATION OF SYNTHETIC COLOURING MATERIAL: LEMONADE SAMPLE 4

	Sample code numbers	9,11	9,11	9,11
	Added colour	Ponceau 4R *mg/l*	FD & C Red 40 *mg/l*	Erythrosine *mg/l*
Laboratory	Added level	20	10	50
1		19·5, 18·7	10·9, 10·6	N.d., N.d.
2		18·7, 19·9	10·5, 10·3	2·8, 1·4
3		20, 20	10, 10	17[d], 17[d]
4		15·0, 12·9	8·5, 8·0	1·3, 0·9
5		5·1, 9·6	2·8, 4·9	1·3, 1·5
6		17·5, 19·6	8·1, 9·1	N.d., N.d.
7		16·0, 14·6	9·4, 9·1	N.d., N.d.
8		13·9, 12·1	7·1, 5·7	N.d., N.d.
9		23·5, 22·1	10·7[a], 15·1[a]	N.d., N.d.
10		34·1, 34·1	N.d., N.d.	N.d., N.d.
11		30, 29	N.d.[c], N.d.[c]	N.d., N.d.
12		N.d.[c], N.d.[c]	12·4, 13·5	N.d., N.d.
13		21, 20	10·4, 10·4	2·9, 1·8
Mean ($\bar{x}$)		19·5	9·1	1·74
Repeatability (r)		3·7	1·91	1·84
Reproducibility (R)		20·4	7·33	2·10

N.d. = not detected.

For key to superscripts see Table XIII.

Mis-identifications: Laboratory 11, FD & C Red 40 reported as Red 10B at levels of 7·1 and 6·6 mg/l for samples 9/11 respectively; Laboratory 12, Ponceau 4R reported as Red 2G at levels of 10·8 and 9·9 mg/l for samples 9/11 respectively.

METHOD

The method tested in the trial uses HPLC determination after extraction and isolation of the colouring matter from the food matrix.

(i) For water-soluble samples such as soft drinks, the colouring materials were extracted and isolated by direct adsorption on to a polyamide column.

(ii) For water-insoluble samples such as the cakes, the colouring materials were first extracted from the food matrix using a liquid ion-exchange resin followed by adsorption on to a polyamide column.

The eluate from the polyamide column was qualitatively examined using thin-layer chromatography (TLC) and the colours were quantitatively analysed using reverse-phase high performance liquid chromatography (HPLC).

Details of the method used are given as Appendix I to this Report.

RESULTS

The results for the determination of synthetic colouring materials in soft drinks and cake crumbs are given in Tables IV–XI and summarised in Table XII.

TABLE VIII

QUANTITATIVE DETERMINATION OF SYNTHETIC COLOURING MATERIAL: LEMONADE SAMPLE 5

	Sample code numbers	7, 8	7, 8	7, 8
	Added colour	Green S *mg/l*	Brilliant Blue FCF *mg/l*	Sunset Yellow FCF *mg/l*
Laboratory	Added level	10	10	50
1		9·4, 10·0	12·3, 11·8	52·1, 52·0
2		8·8, 5·0	10·3, 10·7	N.d., 51·4[b]
3		N.d., N.d.	18[d], 20[d]	48, 47
4		N.d., N.d.	10·8, 10·4	45·4, 45·1
5		3·2, 5·4	6·2, 5·0	56, 42
6		9·9, 6·4	9·5, 6·4	41·4, 30·9
7		8·9, 7·0	13·7, 12·2	52·7, 51·2
8		N.d., N.d.	11·0[a]17·6[a]	54·7, 50·7
9		13·7, 11·9	10·5, 12·3	58·9, 67·7
10		18·4, 15·4	N.d., N.d.	56·8, 56·8
11		8·7, 8·4	11·0, 11·0	59, 55
12		1·8, 4·2	11·1, 11·9	50·6, 52·8
13		9·8, 8·6	11·1, 10·8	56, 53
Mean ($\bar{x}$)		8·8	10·5	51·5
Repeatability (r)		4·7	2·7	12·0
Reproducibility (R)		11·7	6·3	21·0

N.d. = not detected.
For key to superscripts, see Table XIII.

Statistical Analysis of the Results

The results reported were examined for evidence of individual systematic error ($P < 0{\cdot}05$) using Cochran's and Dixon's tests progressively, by procedures described by the British Standards Institution[5].

REPEATABILITY AND REPRODUCIBILITY

Calculations for repeatability (r) and reproducibility (R), as defined by the procedures given by the British Standards Institution[5], were carried out on those results remaining after removal of outliers for each sample calculation.

Comments on Results Obtained

REPEATABILITY AND REPRODUCIBILITY

Overall, the results were somewhat disappointing with respect to the calculated precision values. It is suggested that the method may be intrinsically repeatable within experienced laboratories but its value as a reproducible method is undoubtedly poor. The large values calculated for reproducibility, and to a lesser extent for repeatability, suggest that the method in its present form, is neither sufficiently accurate nor precise to be considered as a suitable reference method for the determination of such a range of synthetic colouring materials in foodstuffs as were employed in this trial.

TABLE IX

QUANTITATIVE DETERMINATION OF SYNTHETIC COLOURING MATERIAL: LEMONADE SAMPLE 6

	Added colour	Tartrazine *mg/l*	Green S *mg/l*
	Added level *mg/l*	350	30
Laboratory	Sample code numbers	6,4	6,4
1		407, 400	20·2, 23·5
2		384, 196	24·6[a], 11·2[a]
3		422, 410	31, 28
4		499, 451	3·0[d], 3·7[d]
5		470, 290	5·3[d], 1·4[d]
6		239, 290	18·5, 21·3
7		365, 366	30·9[a], 5·2[a]
8		387, 395	N.d.[c], N.d.[c]
9		451, 464	39·4, 39·4
10		389, 391	29·5, 29·5
11		400, 410	28, 29
12		472, 414	24·6, 26·6
13		373, 424	26[a], 17·2[a]
Mean ($\bar{x}$)		391	27·8
Repeatability (r)		156	4·3
Reproducibility (R)		201	18·3

N.d. = not detected.

For key to superscripts, see Table XIII.

Mis-identifications: Laboratory 8—Green S reported as Brilliant Blue FCF at levels of 14·6 and 16·9 mg/l for samples 6/4 respectively

RECOVERIES

These appear to be variable in some of the results obtained.

This would tend to suggest that the efficiency of the extraction procedures is not ideal. This is confirmed by the poor recovery figures observed in the analysis not only of the cake samples, where dye-protein binding is known to occur, but also of the soft drink samples where consistently low recoveries for certain dyes were reported. In particular, Green S (88–93 per cent. recovery range) and Amaranth (50 per cent. recovery from sample No. 3) are known to be sensitive to light and heat, especially during extraction; hence losses of these colours would be expected. However, in the case of Tartrazine, especially at the relatively high level of spiking in the soft drink samples, recovery figures of over 100 per cent. (111–117 per cent.) were frequently reported. This may suggest either that error occurred in the preparation of the Tartrazine stock standard solution used to spike the samples, or that a non linear relationship between dye concentration and HPLC detection occurs at very high Tartrazine concentrations (i.e. in the volume of the final extract). Moreover, it appears likely that the final dissolution of the highly concentrated extract in a small (1 ml) volume of solvent could give rise to significant errors. However, high recovery figures were not observed for Tartrazine in the cake samples. It is also suspected that the

TABLE X

QUANTITATIVE DETERMINATION OF SYNTHETIC COLOURING MATERIAL: CAKE CRUMB SAMPLE 1

	Sample code letters	A, D	A, D
	Added colour	Tartrazine *mg/kg*	Ponceau 4R *mg/kg*
Laboratory	Added level	86	43
1		75·7, 76·8	15·7, 15·8
2		89·6, 86·0	27·1, 27·6
3		85, 65	N.d.[c], N.d.[c]
4		83·8, 77·7	21·3, 20·4
5		27, 37	7·3, 9·2
6		38·8, 31·5	3·3, 1·8
7		180[d], 179[d]	24·9, 28·6
8		78·2, 86·7	15·3, 18·1
9		109, 121	15·4, 11·0
10		40·7, 12·5	19·3, 6·6
11		40, 79	20, 19
12		85·1, 44·7	20·5, 11·1
13		33, 36	19·1, 33
Mean ($\bar{x}$)		64·2	17·1
Repeatability (r)		39·9	12·8
Reproducibility (R)		82·3	23·2

N.d. = not detected.

For key to superscripts, see Table XIII.

Mis-identifications: Laboratory 3—Ponceau 4R reported as Red 2G at levels of 10 and 8 mg/kg for samples A/D respectively.

acidity of the soft drink samples may have had some effect on the relative stability and/or solubility of certain dyes such as Erythrosine, which is insoluble at low pH values, thus giving rise to low apparent recovery figures.

As the dyes used in the preparation of the samples are formed from a number of different chemical classes, it might be expected that the method studied would be more suitable for some dyes than others.

MIS-IDENTIFICATIONS

A number of instances of dye mis-identification were reported and these are listed below:

Laboratory 8 mis-identified Green S for Brilliant Blue FCF in lemonade samples 2 and 6,
Laboratory 11 mis-identified Red 10B for FD&C 40 in lemonade sample 4,
Laboratory 12 mis-identified Red 2G for Ponceau 4R in lemonade sample 4,
Laboratory 3 mis-identified Red 2G for Ponceau 4R in both cake-crumb samples, and
Laboratory 10 mis-identified Yellow 2G for Tartrazine in cake-crumb sample 2.

There appears to be no consistent pattern among the mis-identifications. However, Laboratory 10 failed to detect Brilliant Blue FCF in Lemonade sample 5 but did report the presence of Green S at approximately twice the

TABLE XI

QUANTITATIVE DETERMINATION OF SYNTHETIC COLOURING MATERIAL: CAKE CRUMB SAMPLE 2

	Sample code letters	B, C	B, C
	Added colour	Tartrazine *mg/kg*	Ponceau 4R *mg/kg*
Laboratory	Added level	69	72
1		65·0, 65·1	25·3, 26·4
2		71·7, 66·3	38·1, 40·6
3		56, 65	N.d.[c], N.d.[c]
4		62·3, 69·0	32·6, 40·6
5		50, 37	26, 24
6		38·6, 28·8	10·2, 3·9
7		152[d], 165[d]	53·9, 54·4
8		62·6, 71·3	26·5, 31·4
9		51·5, 85·6	7·8, 20·4
10		N.d.[c], N.d.[c]	12·4, 9·0
11		72, 61	44, 46
12		88·7, 58·6	33·3[a], 10·6[a]
13		50, 35	32, 32
Mean ($\bar{x}$)		59·6	29·0
Repeatability (r)		32·6	10·7
Reproducibility (R)		44·0	42·0

N.d. = not detected.

For key to superscripts, see Table XIII.

Mis-identifications: Laboratory 3 reported Ponceau 4R as Red 2G at levels of 54·1 and 54·0 mg/kg for samples B/C respectively.

Laboratory 10 reported Tartrazine as Yellow 2G at levels of 25 and 22 mg/kg for samples B/C respectively.

mean level added/found. This suggests that this laboratory experienced difficulty in separating Green S and Brilliant Blue FCF by HPLC when both were present in the sample at similar levels, reporting them as a single combined peak.

ANALYSTS' COMMENTS

The participants also provided a number of comments about the method and trial; these are summarised in Appendix II.

Conclusions

The results obtained in the trial are too variable to enable the method to be recommended for legislative use at the present time.

Most of the comments received from the participating laboratories concerned aspects of the extraction as opposed to the HPLC procedures; further work is therefore required in the development of a better extraction method with respect to dye recoveries. The main reasons for low recoveries are probably:

1. dye-binding within the food matrix, e.g., as in baked commodities and
2. losses of dyes because of breakdown during extraction, e.g. as a result of the application of heat for concentration purposes.

TABLE XII

DETECTION AND DETERMINATION OF COLOURING MATTERS: SUMMARY OF RESULTS—LEMONADES AND CAKES

Sample	Dyes added	Mean added *mg/l*	Mean found *mg/l*	*r*	*R*	Recovery *per cent.*
Lemonades						
1	Tartrazine	250	290	40·2	15·2	116
2	Tartrazine	250	293	55·9	109·7	117
	Green S	50	45·9	6·7	10·3	92
3	Amaranth	100	50·7	17·1	76·8	50
	Carmoisine	120	123	15·4	45·5	103
4	Ponceau 4R	20	19·5	3·7	20·4	98
	FD & C Red 40	10	9·1	1·9	7·3	91
	Erythrosine	50	1·7	1·8	2·1	3·4
5	Green S	10	8·8	4·7	11·7	88
	Brilliant Blue FCF	10	10·5	2·7	6·3	105
	Sunset Yellow FCF	50	51·5	12·0	21·0	103
6	Tartrazine	350	391	156	201	111
	Green S	30	27·8	4·3	18·3	93
Cakes		*mg/kg*	*mg/kg*			
1	Tartrazine	86	64·2	39·9	82·3	75
	Ponceau 4R	43	17·1	12·8	23·2	40
2	Tartrazine	69	59·6	32·6	44·0	86
	Ponceau 4R	72	21·0	10·7	42·0	40

TABLE XIII

KEY TO TABLES IV–XI

a. Results rejected by Cochran's Test $P \leq 0{\cdot}05$. Values not used in the calculation of mean repeatability nor reproducibility.

b. Results reported as single values only. Values not used in the calculation of mean, repeatability nor reproducibility.

c. Results reported as mis-identified colouring material. Values not used in the calculation of mean, repeatability nor reproducibility.

d. Results rejected by Dixons' Test $P \leq 0{\cdot}05$. Values not used in the calculation of mean, repeatability nor reproducibility.

These problems are well known in this type of analysis. In addition, as the reproducibility figures reported are disappointing, it is suggested that improved techniques and equipment are required in order to provide the basis of a sound extraction methodology upon which a robust analytical method can be developed. Recently published material employing novel ion-pair extraction techniques appears to be promising and work on the development of a method along these lines is being undertaken at the MAFF Food Science Laboratory. It is anticipated that the use of a diode-array HPLC detector would facilitate better qualitative analysis particularly with regard to the problem of co-eluting dyes and subsidiary colours.

When the current work in the Food Science Laboratory is completed the method will be re-tested as the second part of this trial.

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APPENDIX I: DETERMINATION OF SYNTHETIC COLOURING MATERIALS IN SOFT DRINKS AND CAKES

1. SCOPE AND FIELD OF APPLICATION

The method extracts, separates, identifies and quantifies a range of synthetic colouring materials (see Annex A) when present in soft drinks and cakes.

2. DEFINITION

Synthetic colouring materials: any substance which is capable of being and is used for the purpose of colouring food, other than those substances occurring naturally.

3. PRINCIPLE

The colouring materials are extracted from the food matrix and isolated on a polyamide chromatography column. The column eluate is qualitatively exam-

ined on cellulose thin-layer chromatographic plates and the colouring materials quantitatively analysed using reverse-phase, ion-pair, high performance liquid chromatography (HPLC).

4. REAGENTS

Reagents should be of a recognised analytical grade, if available, except water and methanol which should be HPLC grade.

4.1 *Acetic acid, glacial*

4.2 *Acetone*

4.3 *Concentrated ammonia solution, (S.G. = 0·88)*

4.3.1 *Ammonia solution, 10 per cent. w/v*

4.4 *Acetone: ammonia solution:* Prepare by mixing 40 ml of acetone, and 10 ml of ammonia solution (4.3.1). Prepare fresh daily.

4.5 *Butan-1-ol*

4.6 *Celite 545 filter aid*

4.7 *Chloroform*

4.8 *Diethyl-Ether*

4.9 *Glass wool*

4.10 *Hexane*

4.11 *Hydrochloric acid, concentrated*

4.11.1 *Hydrochloric acid, 0·1 Mol/l solution*

4.12 *Nitrogen, oxygen-free*

4.13 *Polyamide powder, grade NM-polyamide SC6/CC6 (CAMLAB LTD.)*

4.14 *Resin, Amberlite LA-2:* A 5 per cent. solution in butan-1-ol. Mix 50 ml of LA-2 resin with 900 ml of butan-1-ol and shake with 400 ml of water containing 19 ml of concentrated hydrochloric acid and allow to equilibrate in a 2 l separating funnel. Discard the lower aqueous layer.

4.15 *Sand, acid washed, 80–100 mesh*

4.16 *Sodium chloride solution, 1 per cent. w/v*

4.17 *Sodium chloride–ammonia solution:* Dissolve 10 g of sodium chloride (NaCl) in 500 ml of water, add 10 ml of ammonia (S.G. = 0·88) and dilute to 1 litre in a graduated flask.

4.18 *Trisodium citrate dihydrate*

4.19 *HPLC mobile phase:* Prepare by mixing methanol and 0·005 M potassium dihydrogen phosphate (KH_2PO_4) solution in various ratios (see (5.10.6) below) but containing tetra-*n*-butyl ammonium bromide (Fisons Ltd.) 0·005 M as counter-ion, overall. De-gas ultrasonically or by helium purge after removing any microparticulate matter by filtration through a 2 μm membrane filter, under vacuum.

4.20 *Standard colour solutions:* Dissolve 0·100 g of dye of known purity in 100 ml of water. Store in amber bottles or under subdued lighting. Dilute to give suitable strength working solution in the HPLC mobile phase.

4.21 *Thin-layer chromatographic developing solution:* Dissolve 2·0 g of *tri*sodiumcitrate dihydrate in 85 ml of water, add 15 ml of ammonia solution (S.G. = 0·88) and mix well.

5. APPARATUS

5.1 *Beakers, 250 ml glass*

5.2 *Buchner funnels:* Sintered glass, 200 ml capacity, porosity size 3 with disc diameter 65 mm.

5.3 *Chromatography columns:* Glass, approximately 280 mm in length and 18 mm in diameter, fitted with a glass sinter and tap.

5.4 *Evaporating basins, 100 ml silica or porcelain*

5.5 *Anti-bumping granules*

5.6 *Flasks, glass volumetric 100 ml and 1 l*

5.7 *Filters, disposable MILLEX-HV 0·45* μm *(Millipore)*

5.8 *Filter membranes 2* μm
For the removal of microparticulate matter from HPLC mobile phase.

5.9 *Funnels, glass wide-neck*

5.10 *HPLC system*

5.10.1 *Pump, capable of delivering solvent at 1 ml per min*

5.10.2 *Injection Valve, fitted with a 10 or 20* μl *loop*

5.10.3 *Column, 250 × 4·6 mm Spherisorb C8 5* μm *(or equivalent)*

5.10.4 *Guard column:* Packed with reverse-phase packing material.

5.10.5 *Detector, variable wavelength:* Operating in the visible wavelength range at:
430 nm for yellow dyes
480 nm for orange and brown dyes
520 nm for red dyes
600 nm for purple (black) dyes
640 nm for green and blue dyes

5.10.6 *Mobile phase composition:* Methanol: KH_2PO_4 soln. (0.005 M), adjusted between 50:50 and 60:40 so as to produce optimum resolution of peaks, depending on the age and condition of the column.

5.11 *Pipettes, glass:* 1, 50 and 100 ml.

5.12 *Separating funnel, 1 litre capacity*

5.13 *Sep-Paks, C 18 (Waters Associates)*

5.14 *Syringes, 10 ml glass with Luer-lock fitting*

5.15 *Thin-layer chromatography plates:* 20 cm × 20 cm glass coated with a 0·25 mm thick layer of microcrystalline cellulose. Activate at 110 degrees C for 30 min and cool in a desiccator just prior to use.

5.16 *TLC developing tank, glass with lid*

5.17 *Tubes, glass capillary for TLC:* Graduated to 5 μl.

5.18 *Water bath, boiling*

5.19 *pH Indicator paper, range around 5 to 8*

6. PROCEDURE

6.1 *Analysis of soft drinks—Extraction and concentration*

6.1.1 *Sample preparation*

Accurately pipette 50 or 100 ml of sample (depending on the expected amount of dyes present) into a 250 ml beaker and warm on a water bath to approximately 60°C. Ensure that the pH of the solution is between 6 and 7 by the addition of ammonia solution or hydrochloric acid solution (using indicator paper).

6.1.2 *Preparation of Polyamide column*

Slurry 10 g of Polyamide powder in approximately 100 ml of water and pour into a glass chromatography column via a wide-necked funnel. Allow the water to filter through, but at no time allow the column to become dry. Rinse the walls of the column with a few ml of acetone to aid settling of the polyamide. Add sand to the top of the polyamide bed to form a layer approximately 6 mm deep, followed by a small plug of glass wool. The column is now ready for use.

6.1.3 *Column chromatography*

Pour the warm sample solution (6.1.1) on to the prepared column (6.1.2) and allow the aqueous eluate to run to waste. Rinse the beaker with approximately 50 ml of warm water and pass this through the polyamide column, again allowing the aqueous eluate to run to waste. Wash the column with approximately 15 ml of acetone. Elute all synthetic colouring materials with the minimum volume of acetone: ammonia solution (4.4) and collect the eluate in an evaporating basin containing a few anti-bumping granules.

6.1.4 *Concentration*

Carefully reduce the volume of the column eluate (6.1.3) on a boiling water bath (in a fume cupboard) to approximately 3 ml, adding a few ml of acetone where necessary to azeotrope the water. Dry the basin contents finally by blowing over with nitrogen. *Never allow the residue to bake.* Dissolve the residue in 1 ml of water (a larger volume may be desirable if the extract is particularly concentrated). Retain for qualitative and quantitative analysis.

6.1.4.1 *Rapid concentration* (for certain colourings)

A rapid semi-quantitative concentration can be performed on a suitable sample aliquot the pH of which has been adjusted to between 6 and 7 with dilute ammonia or hydrochloric acid solution (using indicator paper), as follows:

Prime a C 18 Sep-Pak cartridge by pumping 10 ml of HPLC mobile phase (4.8) through it using a 10 ml glass syringe. Load the Sep-Pak with the sample aliquot by pumping through the cartridge with enough pressure on the syringe to allow a flow rate not exceeding 20 ml per min, rinse the cartridge and syringe with approximately 15–20 ml of water and elute the colouring materials from the Sep-Pak with the minimum amount of methanol. This has only been applied to a limited number of colouring compounds.

6.2 *Analysis of cakes—Extraction and concentration*

6.2.1 *Sample preparation*

Weigh accurately between 5 and 10 g of sample (depending upon the expected amount of dyes present) and homogenise with 10 g of celite 545 (4.6) and 10 ml of 0·1 M hydrochloric acid (4.11.1). Quantitatively transfer to a sintered glass funnel and add 125 ml of chloroform, mix well and stand for 5 min. Apply a vacuum and discard the filtrate. Add 80 ml of LA-2 resin in butanol solution (4.14) to the dry homogenate, stir well, allow to stand for 10 minutes prior to filtering and retain the filtrate. Repeat the extraction with a further two 80 ml portions of resin in butanol solution, combine the filtrates and transfer to a 1 litre separating funnel. Wash the butanol layer with two separate portions of sodium chloride solution (4.16) and discard the aqueous washings. Add 240 ml of hexane, 100 ml of ammonia solution (4.3.1) and 50 ml of sodium chloride–ammonia solution (4.17). Shake vigorously and allow the layers to separate. Collect the aqueous layer and repeat the aqueous extraction a further two more times. Combine the aqueous extracts in a clean separating funnel, wash with 50 ml of diethyl ether, and discard the ether. Warm the extract on a steam bath for 30 min to remove the ammonia and residual solvent, cool and adjust the pH to 6–7 using glacial acetic acid. Carry out Sections 6.1.2 and 6.1.3.

6.3 *Determination*

6.3.1 *Thin-layer chromatography—Qualitative determination*

Allow the developing solvent to equilibrate in the TLC tank. Spot 5 µl of the sample extract obtained from 6.1.4 or 6.1.4.1 as appropriate in duplicate, on to an activated TLC plate (5.15) along with 5 µl spots of standard dye solutions (4.20) thought to be present in the extract. Allow the spots to dry and over-spot one of the sample spots as well as a duplicate set of standard spots, with 5 µl of Orange G standard (250 ppm). Allow the spots to dry thoroughly and develop the plate in the tank until the solvent front has risen at least 15 cm. Dry the plates in a fume cupboard and measure the retention distances.

6.3.1.1 *Calculation*

Calculate the R_f and R_x values of the sample extract components and the standard dye solutions;
Where

$$R_f = \frac{\text{Distance travelled by sample or standard spot (mm)}}{\text{Distance travelled by solvent front (mm)}}$$

and

$$R_x = \frac{\text{Distance travelled by sample or standard spot (mm)}}{\text{Distance travelled by Orange G spot (mm)}}$$

Attempt to identify any synthetic colouring materials present in the sample extracts by comparison with the R_f and R_x values of the standard dye solutions. See Annex B.

6.3.2 *Quantitative determination*

High Performance Liquid Chromatography

Inject a suitable amount of sample extract (10–20 µl) on to the column (5.10.3) and develop the chromatogram using the optimum mobile phase conditions (see note 5.10.6). Monitor the peak elution by selecting the

appropriate spectroscopic absorption wavelength (5.10.5). Identify the colouring materials by relative retention times and confirm identification if possible, by obtaining UV-visible spectra by collection of eluting peak fractions or by stopped-flow scanning, using the mobile phase as the reference solvent.

6.3.2.1 *Calculation*

Calculate the concentration of colouring materials present in the extract by comparison of peak areas with standard dye solution of known purity and concentration. The amount of colouring material present in the sample can be calculated by applying the appropriate dilution factor.

Results should be expressed as milligrams of colouring material per litre or kg of sample.

Annex A

Synthetic colouring materials currently permitted in the U.K. are as follows:

Common name	*EEC No.*
Amaranth	E 123
Black PN	E 151
Brilliant Blue FCF	133
Brown FK	154
Carmoisine	E 122
Chocolate Brown HT	155
Erythrosine BS	E 127
Green S	E 142
Indigo Carmine	E 132
Patent Blue V	E 131
Ponceau 4R	E 124
Quinoline Yellow	E 104
Red 2G	128
Sunset Yellow FCF	E 110
Tartrazine	E 102

Non-permitted synthetic colouring materials include:

Allura Red AC
Fast Green FCF
Orange G
Orange GGN
Orange RN
Ponceau SX
Ponceau 6R
Red 10B
Yellow 2G

Annex B

Tables I–III give guide to the R_f and R_x values (6.3.1) of some of the more commonly used synthetic colouring materials.

The analyses were carried out on activated microcrystalline cellulose TLC plates (5.15), developing with ammonia : trisodium citrate solution (4.21), and using Orange G as a standard.

TABLE I

R_F AND R_X VALUES OF SOME COMMON SYNTHETIC COLOURS

Synthetic colour	R_f	R_x
Brilliant Blue FCF	0·90	1·22
Patent Blue V	0·94	1·28
Indigo Carmine	0·20	0·26
Green S	0·94	1·28
Orange G	0·73	1·00

TABLE II

R_F AND R_X VALUES OF SOME COMMON SYNTHETIC COLOURS

Synthetic colour	R_f	R_x
Chocolate Brown HT	0·00	0·00
Red 2G	0·54	0·74
Carmoisine	0·21	0·28
Amaranth	0·42	0·57
Brown FK	0·11	0·15
Orange G	0·73	1·00

TABLE III

R_F AND R_X VALUES OF SOME COMMON SYNTHETIC COLOURS

Synthetic colour	R_f	R_x
Erythrosine BS	0·00	0·00
Tartrazine	0·78	1·05
Sunset Yellow FCF	0·53	0·71
Orange G	0·74	1·00

APPENDIX II: COMMENTS FROM PARTICIPANTS IN THE TRIAL

Several comments were received from participants concerning the method being tested. These are summarised below:

1. Some laboratories reported visibly inefficient colour extraction from the cake samples when using the resin in butanol solution. This illustrates the need for the development of the extraction procedures with respect to "dye-bound" samples; the problem had been identified in earlier work.

2. One laboratory reported that relatively higher levels of colour were found, with improved repeatability values, when the soft drink samples were analysed for a second time by direct HPLC injection. Although this would be expected, the repeated direct injection of samples such as soft drinks will reduce the useful lifetime of a column. However, if it were to be found that such procedures far outweighed the risks of poor dye recoveries produced as a result of extraction and purification, then this type of procedure could be incorporated into a revised method for the analyses of certain samples, providing that some type of quality assurance parameter was also incorporated into the method such as regular checks on HPLC column performance, i.e., resolution and plate-count.

3. When carrying out the polyamide column clean-up, some participants commented that it was necessary to "observe carefully" the elution in order to assess qualitatively the eluate for the presence of any colours. It is difficult to include such a procedure in the method protocol because of its subjective nature; such observations are better left to the discretion of the analyst.

4. Some participants reported observable inconsistent losses of blue/green colour and the formation of a brown residue during the concentration and drying stages of the column eluant. Two participants reported the presence of a secondary orange colour associated with the elution and concentration of amaranth, and observed the formation of a yellow colour of unknown origin.

   As noted previously certain dyes are known to be more susceptible to breakdown, quite often in association with secondary colour formation. This causes some difficulty but with careful modification and development of the method, the analyst should be able to exert more control over certain factors affecting dye stability (such as heat, air and light) during the extraction thus minimising their effects and giving improved recoveries. One participant reported using a rotary-evaporator, set at 80°C and incorporating an on-line vacuum, to speed-up the concentration stage without resorting to drying of the eluate, to concentrate the column eluate; improved recoveries were obtained. Furthermore, dissolving the extract in larger final volumes (10 or 25 ml in water instead of mobile phase) facilitated complete dissolution of the extract thus avoiding the formation of "syrupy" concentrates of very high dye concentration. The inclusion of such modification procedures will be necessary in order to make the method more rugged. Thus a move towards the use of less severe concentration techniques generally will be beneficial.

5. Relatively few comments were received with respect to the HPLC analysis. Several participants observed long elution times for Erythrosine when operating within the criteria of the given methodology. One participant had to use a mobile phase containing 70 per cent. methanol in order to achieve acceptable retention times.

6. One participant noted particularly that subsidiary colours associated with Brilliant Blue FCF interfered with the HPLC analysis. Since one of the main criteria of the method was to keep the HPLC methodology as simple as possible, it was restricted to isocratic elution. Gradient elution, though much the more powerful technique, is not available in many laboratories and, in addition, some older instruments do not yield reproducible gradients. However, considering the problems mentioned above and the several instances of dye misidentification reported (see text of Report), gradient elution may be usefully employed in improving the quantitative and qualitative aspects of the end-determination, especially since more relatively low-cost high performance gradient systems are now available. Full characterisation of the dyes as well as their subsidiary colours and breakdown products is required. This highlights the need for confirmatory procedures (i.e. stop-flow scanning or diode-array detection) especially for the analysis

of dyes at very low levels or where it has been suspected that non-permitted dyes have been detected.

Aspects of the participants' comments are being incorporated in the method being prepared for the next phase of the colours collaborative trial.

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Book Reviews

CEREALS AND CEREAL PRODUCTS: THIRD SUPPLEMENT TO MCCANCE AND WIDDOWSON'S "THE COMPOSITION OF FOODS." By B. HOLLAND, I. D. UNWIN and D. H. BUSS. London: The Royal Society of Chemistry and the Ministry of Agriculture, Fisheries and Food, 1988. Softback. 140 pp. plus index. Price £18.95.

This, the third supplement to the fourth edition of "McCance and Widdowson" published in 1978, is the first to make use of the computerised U.K. National Nutrient Databank. The Databank is a joint venture of the Ministry of Agriculture, Fisheries and Food and the Royal Society of Chemistry (who are the joint publishers of this volume) and, as a developing project, will be progressively updated by the merging, after full evaluation, of new or revised information on each food group with data already published.

The title "Supplement" is, in fact, somewhat of a misnomer in that, rather than adding to the corresponding section in the main edition, it completely replaces it. The section has been considerably expanded, containing 360 entries as against 122 in the main volume.

Changes in analytical methodology and manufacturing practices will obviously mean that new data are needed, and as an example of this, the present supplement includes a considerable amount of new information on dietary fibre, determined by two methods, based on the work of Southgate and Englyst respectively. A breakdown of the fibre fractions into cellulose, non-cellulosic polysaccharides, lignin and resistant starch is also given. Contents of individual sugars are listed, and figures are now provided for manganese, selenium and iodine.

Public Analysts will obviously need this well-produced volume on their shelves, as will all concerned with the composition and nutritional value of food. Further supplements are promised, with a complete new edition in about three years' time. It is the stated intention, however, that updated information for the whole range of foods will be made available for use with computer software, and later probably by means of on-line search systems.

M. C. FINNIEAR

BENZOPYRENES. By MARTIN R. OSBORNE and NEIL T. CROSBY. Cambridge 1987. 321 pp. plus index. Price: £45.

'Benzopyrenes' is the first in a series of Cambridge Monographs on Cancer Research and the subject is appropriate in that benzo(a)pyrene was the first chemical carcinogen to be isolated. In compiling this review of 50 years of research the authors faced no less daunting a task than the pioneers who,

starting with two tons of coal pitch, eventually ended up with a small quantity of the two isomers of perylene identified as benzo(a)pyrene and benzo(e)pyrene. The literature on benzo(a)pyrene is vast and according to the authors it is expanding exponentially, with no less than 800 papers published in 1980. The danger in reviewing such a subject may therefore be that shortly after publication, advances in analytical technique render much of the information of little more than historical interest.

The book is basically divided into two sections. The first section covers the chemistry and biology of the benzopyrenes and the second deals with analysis and occurrence. The ten opening chapters are devoted to the carcinogenic isomer benzo(a)pyrene while benzo(e)pyrene, which is non-carcinogenic, merits a single chapter. Each chapter is thorough and well referenced and I particularly enjoyed the opening chapter dealing with the history of benzo(a) pyrene. Chapters 6–9 are not light reading and some knowledge of biochemistry would be useful. These chapters deal with metabolism, diol-epoxides, binding to proteins and nucleic acids and effect on cells *in vitro*. The concluding chapter on benzo(a)pyrene deals with carcinogenesis and this is interesting and informative, though it concludes that for environmental pollutants we do not yet know the extent to which benzo(a)pyrene contributes to total carcinogenity in man.

The second section of the book on analytical methods for benzopyrenes covers definition of the problem and choice of analytical method, reference materials and standards, sampling, extraction, clean-up and concentration, separation and identification, occurrence in the environment and suggestions for further reading. The analyst already involved in polyaromatic hydrocarbon (PAH) analysis will identify with the thoughts and advice of the authors and may glean some useful information. I found the sections on solvent extraction, partition coefficients and levels of benzo(a)pyrene in foodstuffs of particular interest.

This is not a book which will appeal to everyone in the public analyst field but for those involved in the analysis of PAH in environmental and food samples it provides many hundreds of useful references and a great deal of important background information on the significance of the chemical carcinogen benzo(a)pyrene.

W. SWANSON