CHROM. 6175

SEPARATION AND IDENTIFICATION OF FOOD COLOURS

IV. EXTRACTION OF SYNTHETIC WATER-SOLUBLE FOOD COLOURS

R. A. GILHOOLEY, R. A. HOODLESS, K. G. PITMAN AND J. THOMSON
Department of Trade and Industry, Laboratory of the Government Chemist, London, SE1 9NQ (Great Britain)

(Received May 29th, 1972)

SUMMARY

A method of extraction of synthetic water-soluble food colours using polyamide columns for the purification of the dye extracts is described. An attempt has been made to produce a method for the quantitative determination of the colour content of foodstuffs. The method has been applied to jellies, jams, sweets, cakes, canned meats and sausages.

INTRODUCTION

The direct identification of the synthetic food colours is generally impossible. The colour must be extracted from the foodstuff and purified, as co-extractives interfere with the identification of the colour by thin-layer chromatography and spectrophotometry. The extract must also be concentrated because many food colours are used at very low concentrations.

The wool dyeing technique[1] that is commonly used has several disadvantages. A number of dyes are taken up slowly from the hot acidic solution and the adsorbed dyes often undergo changes during the stripping from the wool with ammonia solution. This technique, although useful qualitatively, does not lend itself to the quantitative determination of the colour in a foodstuff. Various other procedures have been suggested for the extraction and purification of food colours, including solvent extraction[2], column chromatography[3], use of liquid ion exchangers[4] and extraction with quinoline[5] and quaternary ammonium compounds[6]. Of these, a method described by LEHMANN *et al.*[7] using polyamide powder for the purification of the food colours appeared to be the most promising, as the organic colours can be quantitatively absorbed and desorbed from the polyamide powder.

Modification of the LEHMANN *et al.*[7] extraction and clean-up technique enabled the extract to be applied to the thin-layer chromatographic identification scheme[8] and made the quantitative determination of the food colour possible.

MATERIALS AND METHODS

Apparatus

The chromatographic columns used were: (a) 300 mm long, 22 mm I.D., fitted with a ground-glass stopcock; (b) 250 mm long, 15 mm I.D., fitted with a ground-glass stopcock; (c) 200 mm long, 10 mm I.D. with an exit tube 40 mm long and 3 mm I.D. A Soxhlet apparatus was used for extraction.

Reagents

All reagents should be of analytical-reagent grade quality. The following reagents were used:

Acetone.

Acetone–ammonia solution: mix 40 ml of acetone, 9 ml of water and 1 ml of ammonia solution (sp. gr. = 0.88). This should be freshly prepared.

Celite 545.

Chloroform.

Ethanol, absolute.

Formic acid, 90%.

Hydrochloric acid, 0.5 *N* and 0.1 *N*.

Methanol.

Methanol–ammonia solution: mix 90 ml of methanol, 5 ml of water and 5 ml of ammonia solution (sp. gr. = 0.88).

Petroleum spirit, boiling range 40–60°.

Polyamide powder for column chromatography: MN CC6 from Macherey, Nagel and Co.

Polyamide staple fibre: Nylon 66, 3.3 g per 10,000 m of fibre.

Polyoxyethylene sorbitan mono-oleate solution: mix 1 ml of polyoxyethylene bitan mono-oleate with 99 ml of water.

Sand: acid-washed.

Tetramethylammonium hydroxide solution, 25% w/w aqueous.

Procedures

Procedure for samples completely soluble in water (jellies, jams, sweets). Weigh about 5 g of the sample into a beaker, add 50 ml of water and warm the beaker on a water-bath until a solution is obtained. Acidify the mixture with acetic acid. Place a plug of polyamide staple fibre in the end of a chromatography tube (15 × 250 mm) and add a suspension of polyamide powder in water to the tube to give a column approximately 20 mm high. Rinse the walls of the tube with a small volume of acetone to aid the settling of the polyamide and then place sand on top of the polyamide to form a layer about 6 mm deep.

Pour the hot solution through the column and wash the column with six 10 ml portions of hot water and three times with 5 ml volumes of acetone. (Light air pressure may be used, if necessary.) Elute the colours with the minimum volume of acetone–ammonia solution, rejecting the eluate until the colours are eluted. Remove the ammonia by blowing a current of air over the surface of the liquid and then reduce the volume by about half on a steam-bath. Add an equal volume of water and adjust the pH to 5–6 with hydrochloric acid.

Pour the solution through a column of polyamide in a 10 × 200 mm chromatography tube prepared as above and wash the column five times with 5 ml portions of hot water. Elute the dyes with the minimum volume of acetone–ammonia solution. Remove the ammonia as before and evaporate the solution to near dryness on a steam-bath. Dissolve the residue in a few drops of 0.1 *N* hydrochloric acid and use this solution for thin-layer chromatography. (If erythrosine is suspected, dissolve the residue in water.)

Procedure for bakery products (cakes, cake powders, pastries). Weigh about 5 g of the chopped sample into a glass evaporating basin and place the basin in a drying oven at 100° for 30 min. Add sufficient petroleum spirit to cover the dried sample (about 30 ml) and stir the mixture. Allow the solid to settle and decant off the petroleum spirit. Repeat this procedure twice more and then allow the residual petroleum spirit to evaporate. Grind the sample gently so as not to form too fine a powder, add 4 g of Celite and mix.

Place a plug of polyamide staple fibre in the end of a chromatography tube (250 × 15 mm) and transfer the powdered sample to the tube. Pour 30 ml of acetone on to the top of the column and when the solvent has percolated the whole length of the column apply slight air pressure to aid uniform packing. Discard the eluate. Carefully pour 50 ml of a methanol–water–tetramethylammonium hydroxide (40:9:1) solution through the column. (Light air pressure may be used if necessary.) Adjust the pH of the eluate to approx. 6 by the addition of dilute hydrochloric acid. Add 5 ml of 1% polyoxyethylene sorbitan mono-oleate solution and reduce the volume by about one half on a steam-bath with the aid of a current of air blown over the surface of the liquid. Add an equal volume of water to the solution and allow it to cool.

Place a plug of polyamide staple fibre in a 10 × 200 mm chromatography tube and add a suspension of polyamide powder in water to the tube to give a column approx. 20 mm high. Rinse the walls of the tube with a small volume of acetone to aid the settling of the polyamide and then place sand on top of the polyamide to form a layer about 6 mm deep.

Pour the solution of extracted dye through the column and wash the column three times with 5 ml portions of acetone, five times with 5 ml portions of chloroform–absolute ethanol–water–formic acid (100:90:10:1), three times with 5 ml amounts of acetone and finally three times with 10 ml volumes of water. Elute the dyes with the minimum volume of acetone–ammonia solution, rejecting the eluate until the dyes are eluted. Remove the ammonia by blowing a current of air over the surface of the liquid and then reduce the volume by about half on a steam-bath. Add an equal volume of water and adjust the pH to approx. 6 with hydrochloric acid. Pour the solution through a column of polyamide in a 10 × 200 mm chromatography tube prepared as above and wash the column with the same volumes of solvents in the sequence as described above for the first polyamide column. Elute the dyes with the minimum volume of acetone–ammonia solution. Remove the ammonia by blowing a current of air over the surface of the liquid and then evaporate the solution to near dryness on a steam-bath. Dissolve the residue in a few drops of 0.1 *N* hydrochloric acid and use this solution for thin-layer chromatography. (If erythrosine is suspected, dissolve the residue in water.)

Procedure for meat products. Weigh about 25 g of sample on to a glass plate. Chop up the sample with a knife, add 5 g of acid-washed sand and grind the mixture

to a paste. Add 10 g of Celite and mix with a palette knife until a homogeneous mixture is obtained.

Transfer the mixture to a Soxhlet thimble and extract with chloroform for 2 h. After extraction, remove the sample from the thimble and place it in an evaporating basin to allow the residual chloroform to evaporate.

Place a plug of polyamide staple fibre in the end of a chromatography tube (22 × 300 mm) and add the powdered sample to the tube, tapping the column gently to aid packing. Pass methanol–ammonia solution through the column until all the dyes are eluted. (Light air pressure may be used if necessary.)

Add 5 ml of 1% polyoxyethylene sorbitan mono-oleate solution and evaporate the solution on a steam-bath with the aid of a current of air blown over the surface of the liquid until all the ammonia and methanol are removed. Add an equal volume of water and adjust the pH of the solution to 6 with hydrochloric acid.

Place a plug of polyamide staple fibre in the end of a chromatography tube (15 × 250 mm) and add a suspension of polyamide powder in water to the tube to give a column approx. 20 mm high. Rinse the walls of the tube with a small volume of acetone to aid the settling of the polyamide and then place sand on top of the polyamide to form a layer about 6 mm deep.

Pour the solution of dyes through the column and wash the column three times with 10 ml portions of water, twice with 5 ml volumes of acetone, twice with 5 ml portions of a chloroform–absolute ethanol–water–formic acid (100:90:10:1) mixture and twice with 5 ml portions of acetone. Elute the dyes with the minimum volume of acetone–ammonia solution, rejecting the eluate until the dyes are eluted. Remove the ammonia by blowing a current of air over the surface of the liquid and then reduce the volume by about half on a steam-bath. Add an equal volume of water and adjust the pH to approx. 6 with hydrochloric acid. Pour the solution through a column of polyamide in a 10 × 200 mm chromatography tube prepared as above and wash the column as previously described. Elute the dyes with the minimum volume of acetone–ammonia solution. Remove the ammonia by blowing a current of air over the surface of the liquid and then evaporate the solution to near dryness on a steam-bath. Dissolve the residue in a few drops of 0.1 *N* hydrochloric acid and use this solution for thin-layer chromatography. (If erythrosine is suspected, dissolve the residue in water.)

RESULTS AND DISCUSSION

The products tested were divided into three groups: (a) samples completely soluble in water (jellies, jams and sweets); (b) bakery products (cakes, cake powders and pastries); (c) meat products (canned meat and sausages).

Samples completely soluble in water

No major difficulties were experienced with this group. Good recoveries were obtained when the following food colours (Colour Index Number, 1971, in brackets) were incorporated in jellies: Acid Yellow (13015), Amaranth (16185), Brilliant Blue FCF (42090), Erythrosine (45430), Fast Green FCF (42053), Green S (44090), Ponceau SX (14700), Sunset Yellow FCF (15985) and Tartrazine (19140).

Equally good recoveries were also obtained when the following dyes were added

to home-made blackcurrent or apricot jam: Acid Yellow (13015), Amaranth (16185), Black PN (28440), Green S (44090), Ponceau SX (14700), Red 6B (18055), Sunset Yellow (15985) and Tartrazine (19140).

Difficulties were encountered with Indigo Carmine, which decomposes very easily in alkaline solutions.

In order that the recoveries should be as quantitative as possible, the solutions containing the colour were passed through columns of polyamide so that the dyes were adsorbed as a narrow band on the top of the column instead of adding the polyamide to the dye solution as suggested by LEHMANN *et al.*[7]. It was also shown that methanol impaired the adsorption of the dyes by the polyamide and so the excess of methanol was removed from solutions before passing them through the polyamide columns. Acetone–ammonia was used for eluting the dyes from the polyamide column in place of methanol–sodium hydroxide, as the ammonia and acetone can be removed on a water-bath and on addition of acid no salts are formed that interfere with the adsorption of the dyes by the polyamide and also with some of the thin-layer chromatographic systems used for the identification of the dyes.

Bakery products

The procedure described by LEHMANN *et al.*[7] for pastries and baked products gave low recoveries when applied to the sponge and madeira cakes available in Great Britain. It was thought that this was mainly due to incomplete elution of the dyes from the cake–Celite column although much larger volumes of methanol–ammonia solution were used than were specified in the original method.

Consequently, various changes in the eluting solvent were tried. A known volume of a solution of Sunset Yellow FCF was added to 20 g portions of sponge cake and each portion was dried, mixed with Celite and packed into a chromatography column. Then 50 ml of acetone was passed through each column and each column was treated with a different eluting solvent, as listed in Table I. It can be seen from Table

TABLE I

ELUTING SOLVENTS

Solvent mixture		*Amount of dye recovered (%)*
Components	*Proportions*	
Methanol–0.88 ammonia	95:5	50
	85:15	58
Methanol–0.88 ammonia–water	90:5:5	58
	85:10:5	53
	80:15:5	46
Methanol–diethylamine–water	80:1:19	62
Methanol–tetramethylammonium hydroxide (25% w/w aqueous solution)–water	80:0.5:19.5	72
	90:2:8	66
	80:2:18	80
Methanol–tetrabutylammonium hydroxide (40% w/w aqueous solution)–water	90:2:8	56
	80:2:18	54

TABLE II

RECOVERIES OF DYES ADDED TO CAKES

Dye added	*Colour Index No. 1971*	*Recovery (%)*	
		Dye added after baking	*Dye added before baking*
Amaranth	16185	50–80	50–70
Brilliant Blue FCF	42090	89	69
Carmoisine	14720	88	75
Fast Red E	16045	73	—
Orange G	16230	89	—
Orange I	14600	80	50
Ponceau 4R	16255	88	80
Red 6B	18055	84	—
Sunset Yellow FCF	15935	80	79
Tartrazine	19140	87	61

I that the addition of a small amount of water and an increase in the basicity of the solvent enhanced the eluting power of the solvent. However, these changes also increased the co-extracted material. The addition of a small amount of a surfactant (polyoxyethylene sorbitan mono-oleate) dispersed these co-extractives and prevented them from clogging the polyamide column used for adsorbing the colours. The amount of surfactant must be kept to a minimum otherwise it will interfere with the adsorption of the dyes by the polyamide. Other co-extractives that interfered with the thin-layer chromatography of the dyes and the measurement of extinctions for recovery experiments were washed out of the polyamide column with a series of solvents before elution of the dyes with acetone–ammonia solution.

The recovery of various food colours in cakes was checked by adding a known amount of the food colour to weighed amounts of baked cake. The sample of cake was then treated as described above. The results are shown in Table II. Cakes were also prepared in which the food colour was incorporated before baking. A known amount of food colour was added to the flour used for making each cake and, after baking, a 5 mm layer was removed from the whole of the outside of the cake. The remainder of the cake was dried and mixed to a uniform powder and 10 g portions of this powder were taken for recovery experiments. The results obtained are shown in Table II. The procedure is not applicable to Chocolate Brown FB, Chocolate Brown HT or Indigo Carmine, as the two Chocolate Browns are not completely eluted from the polyamide columns and Indigo Carmine decomposes during the extraction.

Meat products

When the method of LEHMANN *et al.*[7] was applied to canned processed meats or sausages, it was found that some of the colour was removed by the treatment with acetone. In order to avoid recovering this colour from the acetone, the sample mixed with Celite is extracted with chloroform in a Soxhlet extraction apparatus. After evaporation of the residual chloroform, the sample is transferred to a chromatography column and the dyes are eluted with methanol–ammonia solution. The food colours are adsorbed on to a polyamide column and co-extracted material is washed out of

the column by a series of solvents before elution of the dyes with acetone–ammonia solution.

By using this procedure, recoveries of about 70% were obtained when Erythrosine (45430), Red 2G (18050), Red 6B (18055) were added to canned luncheon meat.

REFERENCES

1 *Separation and Identification of Food Colours Permitted by the Colouring Matters in Food Regulations, 1957*, The Association of Public Analysts, London, 1960.
2 *Official Methods of Analysis of the Association of Official Analytical Chemists*, A.O.A.C., Washington, 11th ed., 1970, p. 576.
3 Y. YANUKA, Y. SHALON, E. WEISSENBERG AND I. NIR-GROSFELD, *Analyst (London)*, 88 (1963) 872.
4 M. DOLINSKY AND C. STEIN, *J. Ass. Offic. Anal. Chem.*, 45 (1962) 767; C. GRAICHEN AND J. C. MOLITOR, *J. Ass. Offic. Anal. Chem.*, 46 (1963) 1022.
5 M. MOTTIER AND M. POTTERAT, *Anal. Chim. Acta*, 13 (1955) 46.
6 B. DREVON AND J. LAUR, *Ann. Fals. Fraudes*, 52 (1959) 155.
7 G. LEHMANN, P. COLLET, H. G. HAHN AND M. R. F. ASHWORTH, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 1182.
8 R. A. HOODLESS, K. G. PITMAN, T. E. STEWART, J. THOMSON AND J. E. ARNOLD, *J. Chromatogr.*, 54 (1971) 393.