R_f VALUES OF SOME NON-PERMITTED SYNTHETIC WATER-SOLUBLE COLOURING MATTERS

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Procedures are described and quoted for determination of the R_f values of certain colouring matters which are not permitted by the Colouring Matters in Food Regulations, 1957, for use in foodstuffs, and the results are tabulated.

Many food analysts have found the table of R_f values of permitted water-soluble synthetic colouring matters, published by the A.P.A. [1], to be most helpful, particularly for selecting the best solvent to employ for chromatographic separations. For non-permitted colours no such similar table has been available based specifically on the solvents recommended by the A.P.A. (Table I). Table II gives the R_f values of the water-soluble colours in the "Solmedia"* non-permitted set. These include some which are included in the E.E.C. [2] permitted list (but not permitted in the U.K.). The table also quotes the R_f values of certain other E.E.C. colours which do not appear in the set.

The technique employed for A.P.A. solvents Nos. 1 to 6 is essentially that described by Minor[3] and Pearson[4]. Briefly the procedure is as follows.

Draw a line about 2 cm from the bottom of a sheet of No. 1 Whatman chromatography paper measuring approximately 24 x 13 cm. Apply spots of the concentrated aqueous solutions of the dyes on the line using capillary tubes. After drying, roll the paper into a cylinder and fasten it near the top and the middle with an office-type stapling machine so that the vertical edges do not quite touch. Place the paper centrally in a litre beaker containing solvent to a depth of not more than 1 cm. Cover the beaker immediately with a clock glass and after the solvent has travelled about 10 cm, remove the paper and mark the solvent front. Calculate the R_f values after drying. Using the A.P.A. solvent No. 7, run chromatograms overnight by the descending technique on large sheets (57 x 46 cm) of Whatman

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* Obtainable from Solmedia Ltd., 31 Orford Road, London, E. 17.

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No. 1 paper in a Shandon Chromatography Cabinet.

The R_f values given in Table II represent in most instances the position of the bisector of the spots (the mean of the leading and trailing edges). For "funnel-shaped" spots the value quoted is usually that corresponding to the region of maximum intensity. With the longer "streaks" (usually with parallel sides) the values of the trailing and leading edges (e.g.0.0 – 0.86) are given. Where appropriate, the R_f values for subsidiary spots or streaks (although presumably due to impurities) are stated also in brackets.

It will be appreciated that R_f values are affected by certain factors and there may be day to day variations even if the same technique is employed. Tables of R_f values therefore are mainly of assistance for selecting the best solvent for differentiating between the most probable colours present.

Some difficulty was experienced in trying to establish the exact identity of some of the colours against the Colour Index and in one or two instances the authors have taken the liberty of amending the name on the label attached to the tube (see Notes at end of Table II).

TABLE I

COMPOSITION OF A.P.A.[1] SOLVENTS.

No. 1. 1 ml 0.88 ammonia + 99 ml water.

No. 2. 2.5 per cent w/v aqueous sodium chloride.

No. 3. 2 per cent w/v sodium chloride in 50 per cent ethanol.

No. 4. iso-Butanol (1 vol.), ethanol (2 vol.), water (1 vol.).

No. 5. n-Butanol (20 vol.), water (12 vol.), glacial acetic acid (5 vol.).

No. 6. iso-Butanol (3 vol.), ethanol (2 vol.), water (2 vol.). Then to 99 ml add 1 ml 0.88 ammonia.

No. 7. 80 g phenol + 20 g water.

TABLE II

R_f VALUES OF SOME "NON-PERMITTED" FOOD COLOURING MATTERS

NON-PERMITTED COLOUR	R_f VALUES						
	A.P.A. SOLVENT NUMBER						
	1	2	3	4	5	6	7
REDS							
Acid Fuchsin	0.98	0.86	0.83	0.27	0.16 (0.06)	0.18	0.72
Benzil Bordeau	0.36	0.09	0.32	0.24	0.29	0.45	0.61
Bordeau B	0.23	0.05	0.30	0.31	0.27	0.37	0.57
Brilliant Croceine	0.28	0.10	0.54	0.52	0.36	0.56	0.83
Congo Red	0.00	0.00	0.03	0.18	0.03 (0.00)	0.11	0.15
Croceine Scarlet 5 R	0.16	0.02	0.26	0.47	0.38	0.50	0.73
Eosin	0.23	0.10	0.62	0.47	0.89	0.52	0.97
Eosin Scarlet	0.30	0.11 (0.33)	0.50	0.46 (0.94)	0.83 (0.00-1.00)	0.53 (1.00)	0.95
Fast Red A	0.13	0.00	0.00-0.76	0.53	0.55	0.73	0.97
Magenta	0.00-0.62	0.08	0.93	0.89	0.85	0.86	0.94
Palatine Red	0.33	0.10	0.38	0.37	0.22	0.42	0.54
Phloxine	0.52	0.21	0.86	0.68	0.00-1.00	0.67	0.96

NON-PERMITTED COLOUR	R_f VALUES						
	A.P.A. SOLVENT NUMBER						
	1	2	3	4	5	6	7
REDS (continued)							
Ponceau 2 G	0.00-0.74	0.14	0.22	0.28	0.26	0.30	0.41
Ponceau 6 R	0.52	0.13	0.67	0.47	0.34	0.52	0.75
Ponceau Cryst 6 R	0.47	0.13	0.64	0.44	0.28	0.54	0.72
Rhodamine B	0.48 (0.00-0.75)	0.00-0.68	0.93	0.84	0.84	0.84	0.96
Rhodamine G	0.25	0.26	0.86	0.89	0.84	0.87	0.93
Rhodamine 6 G	0.16	0.26	0.92	0.85	0.82	0.85	0.97
Rose Bengal	0.39	0.33 (0.06)	0.95 (0.50)	0.93 (0.47)	0.84	0.88 (0.50)	0.96
Scarlet GN	0.91	0.31	0.73	0.52	0.40	0.51	0.78
Xylene Red B	0.77	0.41	0.83	0.67	0.43	0.72	0.94
ORANGES							
Acridine Orange 2 G	0.00	0.00	0.62	0.72	0.75	0.85	0.96
Orange G G N	0.80	0.28	0.68	0.39	0.36	0.46	0.97 (0.00-1.00)
Orange I	0.51	0.09	0.78	0.73	0.59	0.57	0.93
Orange II	0.33	0.08	0.78	0.74	0.61	0.74	0.96

NON-PERMITTED COLOUR	R_f VALUES						
	A.P.A. SOLVENT NUMBER						
	1	2	3	4	5	6	7
YELLOWS							
Acid Yellow G	0.53 (0.84)	0.25 (0.48)	0.76	0.69 (0.41)	0.43 (0.23)	0.67 (0.42)	0.89 (0.11)
Aniline Yellow (a)	0.18	0.10	0.89	0.84	0.89	0.80	0.23-1.00
Auramine	0.17	0.23	0.95	0.92	0.85	0.93	0.98
Azo-flavone (b)	0.07 (0.24)	0.05	0.79 (0.55-1.00)	0.83	0.63	0.73 (1.00)	0.92
Chrysoidine	0.07	0.06	0.76	0.88	0.78	0.91 (0.00-1.00)	0.95
Chrysoine	0.59	0.17	0.43	0.33	0.14	0.22 (0.00-0.29)	0.76 (0.15)
Chrysoine S	0.71	0.21	0.75	0.59	0.56	0.43	0.77
Chrysoine SGX	0.73	0.19	0.39	0.32	0.16	0.23 (0.00-0.29)	0.80
Fast Yellow	0.86	0.50	0.74	0.39	0.24	0.42	0.24
Fluorescein (c)	0.77	0.45	0.60 (0.85)	0.39	0.86	0.42	0.95
Metanil Yellow	0.23	0.10	0.86	0.80	0.63	0.75	0.92
Quinolin Yellow	0.00-0.76	0.13	0.58	0.50	0.35	0.50 (0.22)	0.97
Yellow 27. 175 N	0.73	0.33	0.53	0.25	0.09	0.25	0.06

NON-PERMITTED COLOUR	R_f VALUES						
	A.P.A. SOLVENT NUMBER						
	1	2	3	4	5	6	7
GREENS							
Brilliant Crystal Green	0.08	0.21	0.96	0.87	0.27-1.00	0.91 (0.38-1.00)	0.92
Fast Green F C F	0.91	0.85	0.89	0.51 (0.00-0.91)	0.18	0.35 (0.00-0.66)	0.94 (0.27-1.00)
Guinea Green B	0.75	0.45 (0.00-0.89)	0.93	0.73	0.39	0.65	0.95
Light Green S F	0.85	0.87 (0.00-1.00)	0.85	0.54 (0.78)	0.19	0.47 (0.00-1.00)	0.96 (0.37-1.00)
Malachite Green	0.14 (0.00-0.84)	0.29	0.97	0.92	0.81	0.90	0.96
Naphthol Green B	0.90	0.62	0.67	0.33	0.09	0.25	0.06 (0.98)
Night Green 2 B	0.85	0.75	0.94	0.74	0.33	0.69	0.94
Patent Green OH	0.00-0.80	0.24	0.96	0.76	0.33	0.72	0.97
BLUES							
Alkali Blue	0.00-0.94	0.00-0.86	0.00-1.00	0.00-1.00	0.00-1.00	0.55 (0.32-1.00)	0.94
Brilliant Blue SCF (d)	0.89	0.74	0.86	0.53	0.27	0.48	0.94
Chlorozol Sky Blue FF	0.07	0.00	0.00	0.08	0.00	0.05	0.00 (0.21)
Coomasie Navy Blue	0.19	0.15	0.00-1.00	0.65	0.34	0.72	0.49-1.00

NON-PERMITTED COLOUR	R_f VALUES						
	A. P. A. SOLVENT NUMBER						
	1	2	3	4	5	6	7
BLUES (continued)							
Direct Blue 2 B	0.04 (0.00-0.27)	0.00	0.00	0.06	0.00	0.02	0.00
Induline Water Soluble	0.00-1.00	0.00-1.00	0.00-1.00	0.00-0.50	0.06	0.18	0.00-1.00
Naphthol Blue Black	0.20	0.04	0.15	0.40	0.14	0.35	0.19-0.97
Patent Blue	0.88	0.00-0.80	0.96	0.82	0.49	0.71	0.93
Patent Blue A	0.75	0.00-0.82	0.97	0.91	0.59	0.58	0.94
Patent Blue V	0.77	0.72	0.94	0:75	0.56	0.56	0.94
Turquoise Blue G 8	0.17	0.00-0.65	0.94	0.94	0.89	0.85	0.94
Water Blue	0.43-1.00	0.00-1.00	0.67 (0.00-1.00)	0.44 (0.00-0.51)	0.27, 0.16 (0.05)	0.22	0.00-0.98
BROWNS							
Baking Brown AW	0.00-0.98	0.00-0.69	0.28	0.18	0.07	0.18	0.14 (0.00-0.73)
Bismark Brown	0.00	0.00	0.00-1.00	0.00-1.00	0.00-1.00	0.76 (0.00-0.95)	0.95
Light Brown	0.22 (0.60)	0.15	0.37 (0.00-1.00)	0.63, 0.36 (0.00-1.00)	0.47 (0.24)	0.19, 0.56 (0.73)	0.96 (0.00-1.00)
Thiazine Brown R	0.00-0.85	0.00	0.06	0.20	0.15	0.13	0.96 (0.04)

NON-PERMITTED COLOUR	R_f VALUES						
	A. P. A. SOLVENT NUMBER						
	1	2	3	4	5	6	7
VIOLETS							
Acid Violet 4 BN	0.75	0.07	0.15 (0.00)	0.20 (0.00)	0.07 (0.64, 0.25, 0.00)	0.00 (0.13, 0.72)	0.97
Methyl Violet	0.12	0.10	0.95	0.90	0.85	0.90 (0.00-1.00)	0.96
Methyl Violet 5 B	0.13	0.10	0.95	0.91	0.88	0.89	0.96
Violet 5 BN	0.80	0.00-0.95	0.86	0.73	0.44	0.63 (0.00-1.00)	0.96
Wood Violet 5 BN (e)	0.81	0.00-0.82	0.96	0.77	0.52	0.72	0.96
BLACKS							
Acid Black B	0.21	0.04	0.17	0.36	0.23	0.31	0.96 (0.00-1.00)
Black 5410	0.22	0.02	0.08	0.10	0.00 (0.10)	0.00 (0.00-0.33)	0.09
Black 7984	0.00-0.68	0.05	0.06	0.12	0.07	0.10	0.04
Crispin Black G	0.10	0.04	0.00	0.09	0.00	0.00	0.03
Direct Black	0.22	0.00	0.00	0.11	0.00 (0.13)	0.08 (0.30)	0.09 (0.00-1.00)
Nigrosine	0.00-1.00	0.00-0.96	0.00-1.00	0.00-1.00	0.00-1.00	0.00 (0.00-1.00)	0.96 (0.00-1.00)

Notes.

(a) Marked "Analine Yellow".
(b) "- flavine" ?
(c) Marked "Fluoresine".
(d) Probably "FCF".
(e) "Wool Violet" ?

The authors would like to thank Dr. L. Golberg, Director of the British Industrial Biological Research Association for the gift to the College of authentic samples of several of the E.E.C. colours examined including Black 7984, Chrysoine S (C.I. No. 14270), Fast Yellow (C.I. No. 13015), Patent Blue V (C.I. No. 42051), and Scarlet GN (C.I. No. 14815).

References.

1. "Separation and Identification of Food Colours Permitted by the Colouring Matter in Food Regulations, 1957", Association of Public Analysts, London, 1960, page 8.
2. Board of Trade J., 1962, 183, 1184.
3. Minor, R.G., Lab. Practice, 1962, 11, 130.
4. Pearson, D., "Chemical Analysis of Foods", 5th Edition, Churchill, London, 1962.

SOME R_f VALUES OF PERMITTED SYNTHETIC WATER-SOLUBLE COLOURING MATTERS

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The table of R_f values of permitted water-soluble synthetic colouring matters, published by the A.P.A. [1] is incomplete in certain respects. R_f values are now given relating particularly to the permitted chocolate browns, indigo carmine and to the use of the Solvent No.7.

Results of collaborative work by members of the Association of Public Analysts on the R_f values of food colourings permitted by the Colouring Matters in Food Regulations, 1957 have been published,[1] but figures were not recorded for the behaviour of the colours with the seventh solvent, which was a mixture of 80 g of phenol with 20 g of water. The table below lists results obtained in experiments with this solvent and also the R_f values for the permitted colours indigo carmine, chocolate brown FB and chocolate brown HT, under the influence of the other[2] six solvents. The techniques employed and the method of reporting R_f values are similar to those previously described by Pearson and Chaudhri.[3]

R_f VALUES OF "PERMITTED" FOOD COLOURING MATTERS

PERMITTED COLOUR	R_f VALUES						
	A. P. A. SOLVENT NUMBER[2]						
	1	2	3	4	5	6	7
REDS							
Ponceau MX							0.66
Ponceau 4 R							0.19
Carmoisine							0.82
Amaranth							0.12
Red 10 B							0.36
Erythrosine BS							0.95
Red 2 G							0.46-1.00
Red 6 B							0.48-0.98
Red FB							0.00-1.00
Ponceau SX							0.33-1.00
Ponceau 3 R							0.58
Fast Red E							0.84

PERMITTED COLOUR	R_f VALUES						
	A. P. A. SOLVENT NUMBER						
	1	2	3	4	5	6	7
ORANGES							
Orange G							0.72
Orange RN							0.95
Sunset Yellow FCF							0.46-1.00 (0.08)
YELLOWS							
Tartrazine							0.11
Naphthol Yellow S							0.19
Yellow 2G							0.95
Yellow RFS							0.51
Yellow RY							0.10 (0.00-1.00)

PERMITTED COLOUR	R_f VALUES						
	A. P. A. SOLVENT NUMBER						
	1	2	3	4	5	6	7
GREENS, BLUES AND VIOLETS							
Green S							0.97
Blue VRS							0.95
Indigo Carmine	0.84 (0.71, 0.39)						0.00 (0.24,0.98)
Violet BNP							0.98
BROWNS, BLACKS							
Brown FK							0.00-1.00
Chocolate Brown FB	0.00-1.00	0.00-0.85	0.00-1.00	0.00-0.44	0.00-0.39	0.00-0.42	0.00
Chocolate Brown HT	0.00-1.00	0.00-0.87	0.00-1.00	0.00-0.45	0.00-0.46	0.00-0.50	0.15 (0.00-1.00)
Black PN							0.16

References

1. "Separation and Identification of Food Colours Permitted by the Colouring Matter in Food Regulations, 1957". Association of Public Analysts, London, 1960.
2. Ibid, page 8.
3. Pearson, D. and Chaudhri, A.B., J. Ass. Publ. Analysts, 1964, 2, 22.

DETERMINATION OF RESIDUAL CHLORINE IN SWIMMING BATH WATERS

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In view of persistent discrepancies between the figures for residual chlorine obtained at the public analyst's laboratory and at the public baths in Kensington an investigation was made to ascertain the cause.

As the analyst's results, using the o-tolidine-arsenite method, were invariably lower than those found at the baths, by Palin's method,[1] there was an obvious possibility of loss of chlorine during the 2 to 4 hour interval taken in transporting samples to the laboratory.

An inspection was accordingly made at the site and tests carried out on samples from the shallow and deep ends of the two baths involved by the analyst and by the baths superintendent simultaneously. Further portions of the same samples were then brought back to the analyst's laboratory and tested 3 hours, and 20 hours, later.

Considerable differences were found between the two different methods, and an additional determination of total residual chlorine by iodide and thiosulphate titration gave results even higher than those using Palin's method.

All determinations were carried out in duplicate, and results on samples from the shallow and deep ends of each bath have been averaged in the Table shown below.

	Residual Chlorine, p. p. m.					
	Small Bath			Large Bath		
	Free	Combined	Total	Free	Combined	Total
(1):						
At Baths						
(a) Palin's method	1.9	1.6	3.5	1.9	0.9	2.8
(b) o-Tolidine	1.4	0.2	1.6	1.4	0.3	1.7
(2):						
At Laboratory						
3 hours later.						
(b) o-Tolidine	1.3	0.2	1.5	1.1	0.3	1.4
(c) Thiosulphate			4.6			3.3
(3):						
At Laboratory						
20 hours later.						
(b) o-Tolidine	0.2	0.2	0.4			
(c) Thiosulphate			3.0			

To test the validity of the o-tolidine reagent, a fresh solution was prepared at the laboratory and both were standardised against a freshly prepared solution of free chlorine, itself standardised against thiosulphate. Both solutions of o-tolidine were found to be in agreement, within experimental error, and to give chlorine values for the standard solution of chlorine only slightly below its known chlorine content.

The standardisation of the o-tolidine solution applies, however, only to the determination of free chlorine. It is difficult to envisage any means of checking the determination of combined residual chlorine, because chlorine forms a number of different compounds in used water and the figure would depend upon the way in which the chlorine was combined, which might be peculiar to the particular water in question.

As regards Palin's method, the procedure carried out by

the baths superintendent was checked by the analyst and found to be in accordance with the instructions issued with the tablets. In this method, diethyl-para-phenylene diamine (DPD) in tablet form is employed to give a red colour with free chlorine, which is read against a comparator disc, and total residual chlorine is given by the further addition of potassium iodide tablets.

From the above Table it will be seen (1) that the o-tolidine method gave substantially lower results than Palin's method which having regard to the high chlorine dosage and agreement between duplicates could not have been due to experimental error; (2) that the differences arose mainly in the combined residual chlorine figures; and (3) that the loss of chlorine in transit to the analyst's laboratory was comparatively small and did not account for the discrepancies.

On referring to the literature one finds it is known that Palin's method gives higher results than the o-tolidine method, and that Palin considers that o-tolidine does not respond fully to combined chlorine compounds.[1] The question arises whether the more firmly combined forms of residual chlorine, which are included in Palin's results have any value as bactericides. Unless this can be established it will be safer to take the more easily dissociated compounds measured by the o-tolidine method. It has, indeed, been questioned by Windle Taylor whether chloramine itself is a disinfectant at all, its effect being ascribed to gradual dissociation into free chlorine.[2]

It should perhaps be pointed out that official recommendations for the purification of water for public swimming baths have in general been based upon data obtained by o-tolidine-arsenite methods; and that these methods are still recommended as a standard, in spite of various criticisms, by the Joint Committee composed of representatives of the Institute of Water Engineers, the R.I.C., the S.A.C. and the Society for Water Treatment and Examination.[3]

In these circumstances it was suggested that chemical tests for the adequacy of chlorination should be restricted to the determination of pH and free residual chlorine only; and that where a discrepancy arises between the figures for free chlorine obtained by different methods the o-tolidine figures should from considerations of safety be preferred.

References

1. Palin, A.T., Tablet Tests for Treatment Control in Swimming Baths, National Association of Bath Superintendents, Annual Conference Report, 1958.
2. Windle Taylor, E., 36th Annual Report of Metropolitan Water Board, London.
3. Approved Methods for the Examination of Water, Institute of Water Engineers, London 2nd Edition, 1960.

THE DETERMINATION OF SORBIC ACID IN DRIED PRUNES AND PRUNES IN SYRUP

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Methods are described for the determination of sorbic acid in prunes and prunes in syrup by U.V. spectrophotometry and colorimetrically using thiobarbituric acid after oxidation. Experiments with the former method give recovery of 92 to 105 per cent. after correction for irrelevant absorption, and with the colorimetric procedure, of 88 to 106 per cent.

Following the discovery of the non-permitted preservative sorbic acid in samples of dried prunes and prunes in syrup submitted for analysis, the determination of sorbic acid in these products at levels up to 500 p.p.m. was investigated.

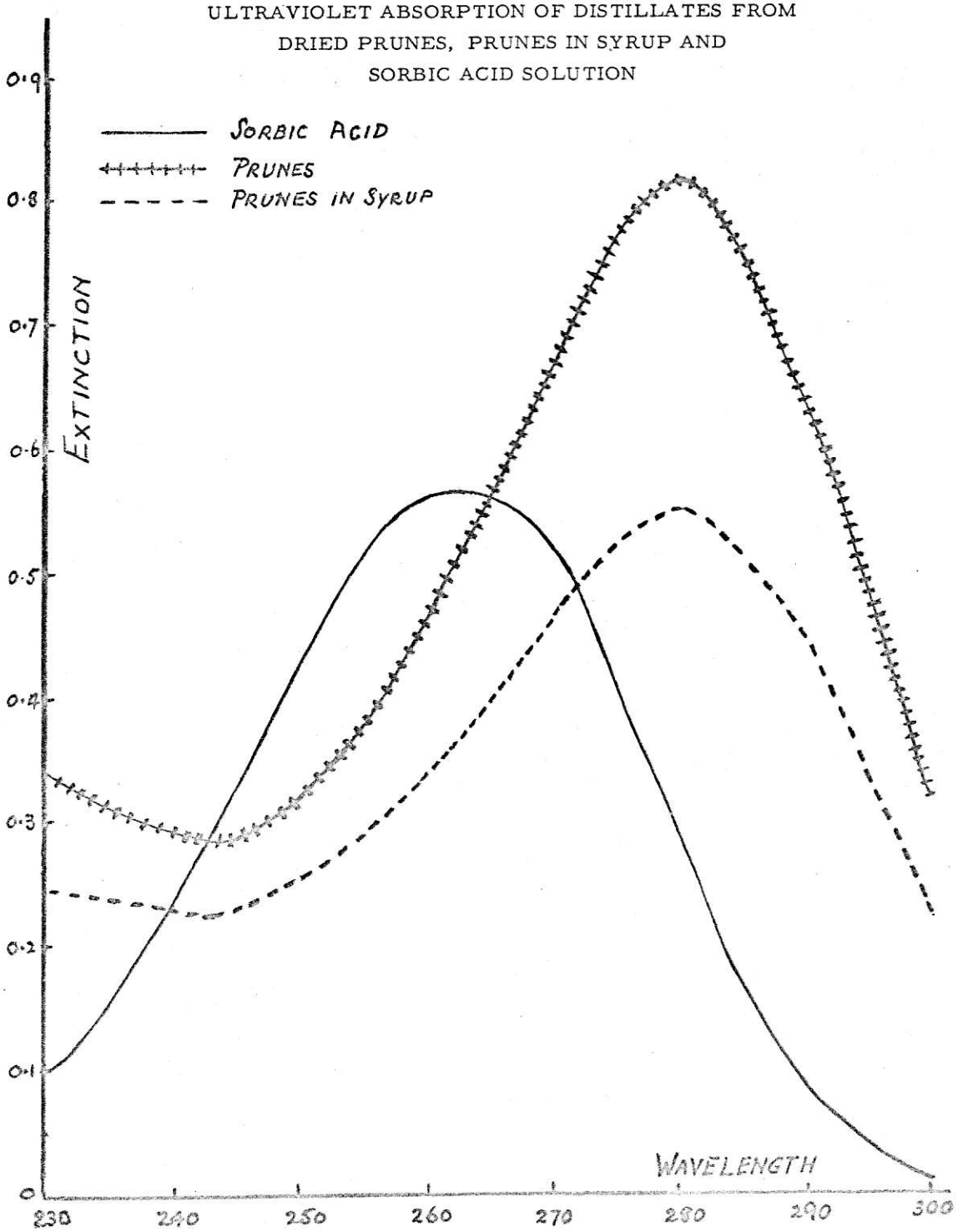
I. Ultra-violet absorption measurements using an S.P. 500 Spectrophotometer.

Pure sorbic acid has an absorption peak at 262 mμ in N/100 acid solution and at 254 mμ in N/100 alkali, and can be steam distilled.[1, 2]. Steam distillation of prunes from saturated brine acidified with phosphoric acid, followed by examination of the ultraviolet absorption spectrum of the distillate was investigated, but at first this proved to be unsatisfactory due to brown coloured and cloudy distillates. However, steam distillation from a solution containing sulphuric acid and magnesium sulphate produced clear, colourless distillates and this procedure was subsequently adopted.

Examination of the ultraviolet absorption spectrum of the

Figure 1

ULTRAVIOLET ABSORPTION OF DISTILLATES FROM DRIED PRUNES, PRUNES IN SYRUP AND SORBIC ACID SOLUTION



distillate showed irrelevant absorption leading to over-estimation of sorbic acid. It was thought that this might be due to hydroxymethyl furfural and other natural products derived from prunes.

Attempts to separate the preservative from interfering substances proved to be both time consuming and only partially successful. A mathematical correction for the irrelevant absorption present was devised.

Correction for irrelevant absorption

Distillation of sorbic acid-free prunes yielded a distillate having an absorption spectrum showing a minimum at 243 mμ and a maximum at 280 mμ (Figure 1). The height of the peak, however, varies considerably. It is possible to make a mathematical correction depending on the absorption at wavelength 280 mμ. The absorption curves obtained from six samples of sorbic acid-free prunes had a mean E_{280}/E_{262} ratio of 1.75, and using the values for pure sorbic acid measured under the conditions used in the determination, $E_{280}/E_{262} = 0.517$ for the acid. It can then be deduced that

$$E_{262}^{\text{Sorbic Acid}} = 1.418\ E_{262}^{\text{Sample}} - 0.811\ E_{280}^{\text{Sample}}$$

II Colorimetric Determination

Under specific conditions, sorbic acid can be oxidised by acidic potassium dichromate solution to give malonaldehyde and this, when heated with thio-barbituric acid solution, forms a deep red colour having maximum absorption at 530 mμ.[4] The products of distillation from sorbic acid-free prunes were shown to give negligible colour when subjected to this reaction and successful recoveries of sorbic acid from prunes over the range 0 to 500 p.p.m. were obtained.

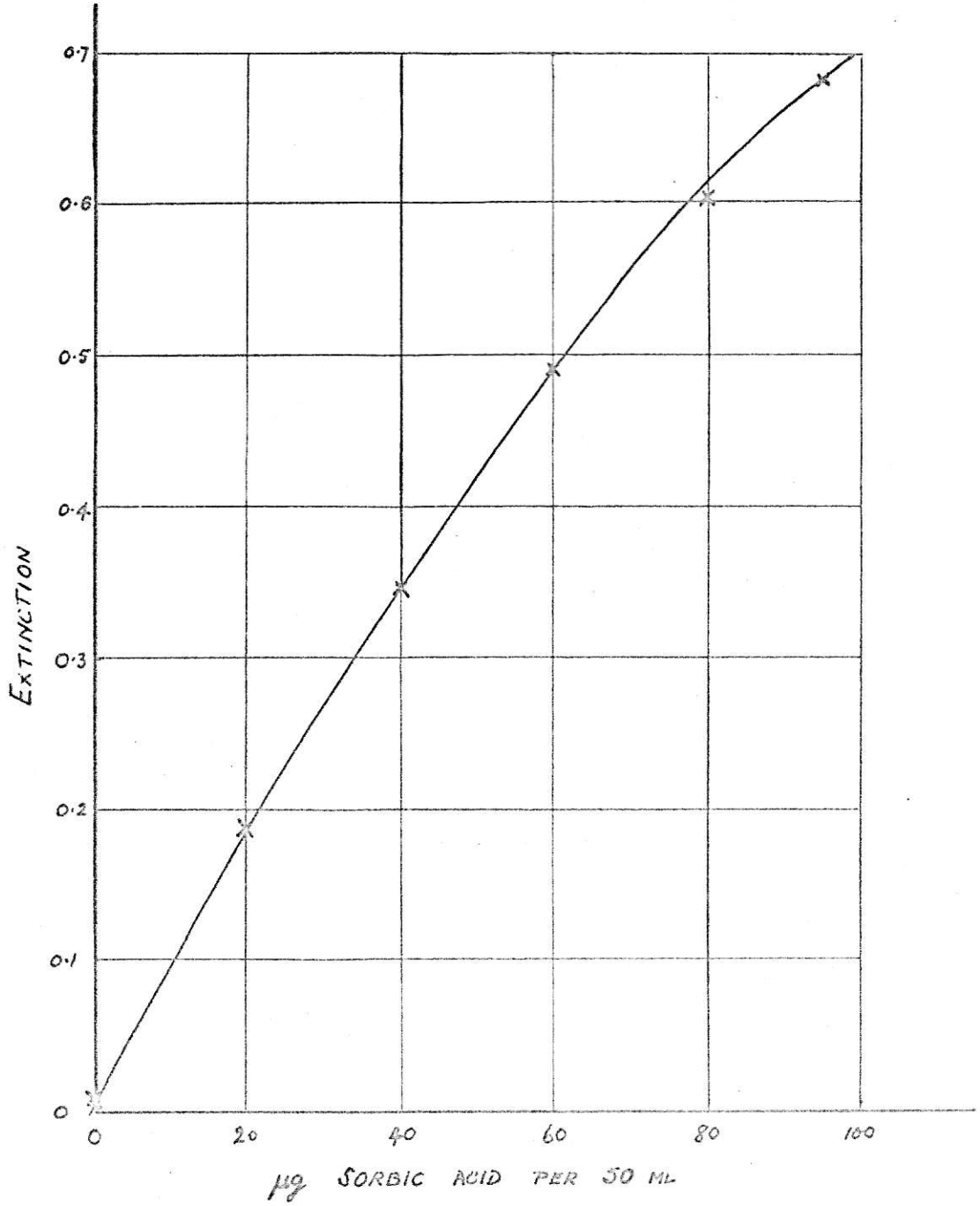
METHODS

Reagents

1. N sulphuric acid
2. N sodium hydroxide
3. 0.01 N sulphuric acid
4. Magnesium sulphate (B.P. grade Epsom Salts)
5. Equal volumes of 0.3 N sulphuric acid and 0.01 N potassium dichromate solution mixed just before use.
6. Freshly prepared 0.5 % w/v thio-barbituric acid in water.

Figure 2

$E_{1\ cm}$ FOR SOLUTIONS OF SORBIC ACID AT 530 mμ
AGAINST A WATER BLANK



(A) Steam Distillation

Transfer 35 grams of prunes (or prunes in syrup) to a 1 litre, Quickfit and Quartz double necked flask (Catalogue No. FRIL/3S/1A) together with 100 g magnesium sulphate and 100 ml N sulphuric acid and steam distil rapidly into 10 ml N sodium hydroxide, collecting approximately 450 ml in 30 minutes. Do not attempt to apply any heat directly to the flask containing the sample during distillation, otherwise a coloured distillate may result. Transfer the distillate to a 500 ml volumetric flask, add 15 ml N sulphuric acid and make up to volume.

(B) Ultraviolet Absorption Measurement

Make a suitable dilution of the distillate with 0.01 N sulphuric acid. Using water as a reference, measure the extinctions at 262 mμ and 280 mμ and determine the corrected value for E_{262} from the formula

$$E_{262}^{\text{Sorbic acid}} = 1.418\ E_{262} - 0.811\ E_{280}$$

Various figures have been reported for the extinction coefficient of sorbic acid.[1, 2, 5, 6] However, when measurements are taken in the pH range 2 to 4, sorbic acid shows a broad peak from 261 mμ to 264 mμ with little variation of the coefficient. By making up the distillate with 0.01 N sulphuric acid, the required conditions are achieved and the value of $E_{1\text{ cm}}^{1\%}$ may be taken as 2260.

(C) Colorimetric Determination

Pipette 2 ml of distillate into a test-tube (15 x 1.3 cm) and add an equal volume of acidified dichromate solution (5). Oxidise by heating at 100° C for 5 min. Cool, add 2 ml thio-barbituric acid solution (6) and heat in a boiling water-bath for 10 min. Cool rapidly, dilute with water to 50 ml and measure the extinction of the red colour at 530 mμ with reference to water. Calculate the sorbic acid content from a calibration curve covering the range 0 to 100 micrograms of sorbic acid per 50 ml of the final solution (Figure 2).

Results

Table I gives results obtained in recovery experiments in which known amounts of sorbic acid were added to samples of

prunes and prunes in syrup.

Table II compares the analyses of various samples using the two methods described above. It is apparent from these results that the spectrophotometric method is unreliable when only small concentrations of sorbic acid are present.

TABLE I

RECOVERY OF SORBIC ACID
ADDED TO PRUNES AND PRUNES IN SYRUP

Sample	Sorbic Acid added	Sorbic acid recovered					
		U.V. Method				Colorimetric Method	
		Uncorrected		Corrected			
	p.p.m.	p.p.m.	%	p.p.m.	%	p.p.m.	%
Prunes	28	-	-	-	-	26	93
Prunes in Syrup	29	56	193	30	103	32	110
Prunes in Syrup	57	78	137	57	100	54	95
Prunes	57	-	-	-	-	59	103
Prunes in Syrup	75	133	178	69	92	77	103
Prunes in Syrup	86	105	122	83	97	79	92
Prunes in Syrup	114	-	-	-	-	111	97
Prunes in Syrup	114	163	143	113	99	107	95
Prunes	129	137	106	136	105	137	106
Prunes	171	-	-	-	-	176	103
Prunes in Syrup	195	191	98	184	94	185	95
Prunes	281	-	-	-	-	288	102
Prunes	343	391	114	344	100	318	93
Prunes in Syrup	429	433	101	416	97	396	92
Prunes	500	-	-	-	-	488	98
Prunes	514	494	96	474	92	450	88

TABLE II

OCCURRENCE OF SORBIC ACID IN PRUNES AND PRUNES IN SYRUP

Sample	U.V. Method		Colorimetric Method
	Uncorrected	Corrected	
	p.p.m.	p.p.m.	p.p.m.
Prunes	28	Nil	Nil
Prunes	20	2	Nil
Prunes	26	7	Nil
Prunes	34	3	Nil
Prunes	147	120	121
Prunes	111	82	87
Prunes in Syrup	31	-8	Nil
Prunes in Syrup	31	2	Nil
Prunes in Syrup	81	24	31

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THE DETERMINATION OF RESERPINE IN TABLETS

by P. F. G. Boon
(Research Division, CIBA Laboratories, Ltd., Horsham, Sussex).

The official method for the determination of reserpine in tablets is shown to be inapplicable to "Serpasil" brand reserpine tablets. The extraction stage fails to achieve quantitative extraction of reserpine. A suitable method based on extraction with boiling methanol is described.

Complaints from several Public Analysts concerning the apparent low reserpine content of "Serpasil" brand reserpine tablets prompted an investigation into the general validity of the official method for the determination of reserpine in tablets. The B.P. 1958 directs the powdered sample to be extracted with dry chloroform and the reserpine content to be determined by measurement of the U.V. absorption of the extract. The B.P. 1963 modifies the method by substituting the more specific and sensitive colour reaction with nitrous acid for the quantitative measurement but retains the single phase chloroform extraction.

The reserpine contents of four samples of tablets described as "Reserpine Tablets B.P." and three typical batches of "Serpasil" tablets were determined by the method of the B.P. 1963.

Results.

		Apparent Reserpine Content per cent. of declared value (0.25 mg)
Manufacturer	A	101%
"	B	97%
"	C	101%
"	D	99%
Serpasil	1	75%
"	2	80%
"	3	75%

The reserpine contents of the Serpasil samples 1, 2 and 3

determined by the method currently used in this laboratory for production control purposes were 95%, 102% and 94% respectively of the declared value. The chloroform-insoluble residues from the determinations by the official method when submitted to this method yielded additional quantities of reserpine equivalent to 21%, 18% and 21% respectively of the declared value.

The official method is clearly not applicable to Serpasil tablets. This is due to the special manufacturing process that results in a product from which reserpine is not completely extracted by chloroform. There appears to be no difference between the extracting powers of "Analar" chloroform or Chloroform B.P in this respect. The use of boiling chloroform to extract the sample results in a somewhat improved recovery but this is still not complete. The presence of water during the extraction leads to quantitative recovery. The tablet excipients, which comprise more than 99% of the tablet weight, are essentially insoluble in chloroform but largely soluble in water. Whilst a suitable method based on the simultaneous extraction of the sample with chloroform and aqueous buffers is possible, (the U.S. Pharmacopoeia XVI uses such a method), the following procedure is shorter and more convenient.

Method for Serpasil Tablets 0.25 mg

Note Solutions of reserpine are photolabile: the exposure of solutions to light in this determination must be kept to a minimum.

Reagents

1. Methanol, Analytical Grade.
2. N sulphuric acid
3. 0.6% aqueous sodium nitrite solution.
4. 5% aqueous sulphamic acid solution.

Procedure

Powder 20 tablets in a mortar. Weigh accurately about 800 mg (W) of powdered tablets into a 150 ml beaker, add 40 ml of methanol, cover the beaker with a watch-glass and boil on the steam bath for 30 min. replacing losses due to evaporation. Cool, filter quantitatively with the aid of more methanol into a 50 ml volumetric flask, dilute to 50 ml with methanol and mix. Transfer two 10.0 ml aliquots of this solution to two 20 ml volumetric flasks and add 10.0 ml of methanol to a third 20 ml

flask. Add 1.0 ml of N sulphuric acid to each flask and 1.0 ml of sodium nitrite solution to one sample flask and to the flask containing methanol. Mix and maintain all flasks at 55 ± 1° C for 30 min. Cool, add 1.0 ml of sulphamic acid solution to each flask, dilute to 20 ml with methanol and mix. Set aside for 10 min. and then determine the absorbances at 390 mμ in 1 cm cells of the (sample + nitrite) solution against the (methanol + nitrite) solution (E_1) and the sample solution without nitrite against methanol (E_2).

$$\text{Reserpine mg per tablet} = \frac{1000\ (E_1 - E_2)}{E_{Ref}} \times \left(\frac{Wa}{W}\right),$$

Where Wa is the average weight of the tablet in mg and E_{Ref} is the $E^{1\%}_{1cm}$ of the reserpine-nitrite reaction product. In practice this need not be re-determined on every occasion, the value 419 having been established in this laboratory.

LAW REPORT

IMPORTED TOMATO PRODUCTS

Report of a successful application for an Order to condemn a shipment of tomato concentrate as "unsound" on the evidence of analysis by the Howard Mould Count procedure.

A test case was recently taken by the Corporation of London which is of interest to all those concerned in the creation and maintenance of standards for foodstuffs in this country.

Late in 1962, Dr. H. Amphlett Williams, F.R.I.C., Public Analyst to the Corporation, reported that samples of tomato concentrates taken in the Port of London were showing an excessively high Howard Mould Count and an associated high level of microscopic fragments of rotten tomato. Some samples showed mould counts as high as 90–100%. Although there is no proven absolute correlation between the Howard Mould Count and the amount of rot in the fresh fruit, experience shows that

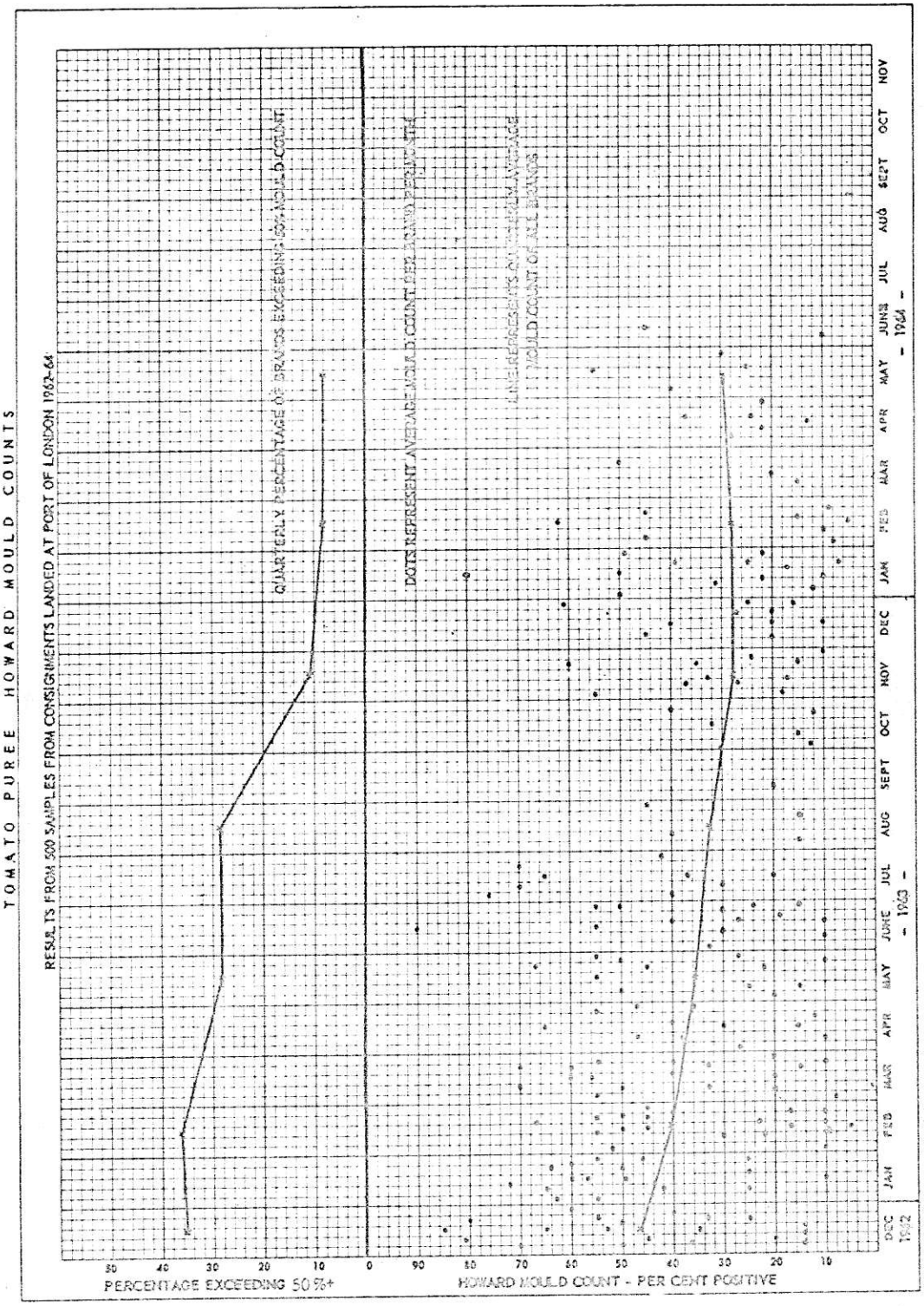
microscopic fragments of rotten fruit may be expected in any sample of concentrate where the mould count exceeds 50% positive fields.

In the opinion of the Public Analyst these figures were high enough to indicate that the concentrates had been made from partly rotten tomatoes, and this raised doubts as to their soundness. There is no standard laid down in this country for the control of these foods, but in the U.S.A. and Canada an upper limit is imposed of 40% and 50% (Howard Mould Count) respectively.

The Public Health (Imported Food) Regulations, 1937, and amendments lay on the Port Medical Officer the responsibility of ensuring that food coming in through his port is not "diseased or unsound or unwholesome or unfit for human consumption". It will be noted that these words are disjunctive i.e. a foodstuff can be fit for human consumption and yet "unsound" within the meaning of the Regulations and, therefore, may not be imported. Further, the sale of such a product would raise the question of offence against Section 2 of the Food and Drugs Act, 1955, in that the product would presumably not be of the nature, substance or quality demanded by the purchaser. Its use for the manufacture of tomato sauce would also contravene the Food Standards (Tomato Ketchup) Order, 1949, which requires that tomato suaces shall be made from clean and wholesome tomatoes.

Accordingly, Dr. J. Greenwood Wilson, M.D., F.R.C.P., the Medical Officer of Health, Port and City of London, decided that action should be taken. The first problem was the determination of a provisional standard, below which food would be regarded as unsound. Account was taken of the standards used in the U.S.A. and Canada, but the most telling factor was that already discussed, namely, the apparent correlation between a Howard Mould Count of 50% and the presence of microscopic fragments of rotten tomato. As a result of consultations between Dr. Amphlett Williams and the Riparian Medical Officers, it was decided that a maximum count of 50% should be the ultimate objective, but because an immediate application of this standard might cause difficulties for importers, a provisional standard of 60% was decided upon, this standard to apply until September, 1963, when the lower permanent standard would come into force. In the meantime, it would be permissible to re-export shipments which did not meet the requirements.

A considerable amount of discussion followed this decision. Articles were published on the subject, and a meeting took place


TOMATO PUREE HOWARD MOULD COUNTS
RESULTS FROM 500 SAMPLES FROM CONSIGNMENTS LANDED AT PORT OF LONDON 1962-64
QUARTERLY PERCENTAGE OF DRAWS EXCEEDING 50% MOULD COUNT
DOTS REPRESENT AVERAGE MOULD COUNT PER LAND PARCEL
LINE REPRESENTS AN ARITHMETIC AVERAGE MOULD COUNT OF ALL DRAWS
PERCENTAGE EXCEEDING 50 %+
HOWARD MOULD COUNT - PER CENT POSITIVE
DEC 1962
JAN FEB MAR APR MAY JUNE JUL AUG SEPT OCT NOV DEC
- 1963 -
JAN FEB MAR APR MAY JUNE JUL AUG SEPT OCT NOV
- 1964 -

between Corporation officers and tomato producers from Italy, Portugal and Greece, London importers, soup and sauce manufacturers, Public Analysts, and others engaged in the trade. The M.O.H. stood firm, however, and the majority of importers, recognising the need for a standard, took advantage of the offer to allow re-exportation where necessary.

One Italian firm of importers, however, decided to challenge the decision of the M.O.H., and a letter was received by the Corporation from solicitors acting for the firm, stating that, unless the matter was reconsidered, their clients would seek a Declaration of the High Court to the effect that the prohibition by the M.O.H. of the importation of a shipment of tomato concentrate, (which arrived in the Port of London in December, 1962), was unlawful, and that the question of compensation would have to be considered. This shipment had been sampled by the Corporation's Inspector on three occasions and Dr. Amphlett Williams had reported Howard Mould Counts of 70%, 75% and 70%, with, in all three cases, the presence of "numerous microscopic fragments of rotten tomato". A considerable length of time had been allowed by the Corporation to importers to decide whether they would re-export goods which did not come up to the standard, but on receipt of the above communication, it was obvious that this firm was not going to avail itself of this opportunity and the M.O.H. ordered the formal seizure of the goods and their conveyance before a Justice under Regulation 15 of the Public Health (Imported Food) Regulations, 1937. This Regulation empowers a Justice to "condemn the article of food and order it to be destroyed or disposed of under the supervision of the M.O.H. by such means and in such manner as to prevent it being used for human consumption" if he is satisfied that the article is diseased, or unsound, or unwholesome, or unfit for human consumption.

Application for an Order to this effect was made at Thames Magistrates Court on 20th December, 1963, when the case was adjourned part-heard. It was again adjourned part-heard on the 30th January, 1964, but completed on 20th February at Bow Street Magistrates Court. Both sides were represented by Counsel, and both called expert witnesses in their support. The applicants relied on their contention that the high Howard Mould Count figures indicated that a substantial proportion of rotten fruit had been used in the preparation of the concentrate, and that this meant that the product, even though it was now sterile and fit for human consumption, was unsound. The importers contended that the Howard Mould Count was of so empirical a nature as to render it unusable as a basis on which to establish a

standard and supported this by producing mould counts for the shipment in question which were appreciably lower than those given by Dr. Amphlett Williams. They argued further that the Imported Food Regulations, originally made under the Public Health Act of 1875, had been transferred to the Food and Drugs Act in 1938, under which the offence was confined to the sale of food "unfit for human consumption", and that this interpretation was confirmed in the more recent Act of 1955. Further legal submission was made to the effect that the application was out of time, having been issued more than six months after the date on which the offence was alleged, i.e. the date of the Public Analyst's certificate.

The defendants called Dr. A.J. Amos, F.R.I.C., who stated that he had analysed samples drawn from the consignment in question and while his results on the Howard Mould Count were in agreement with those of the Public Analyst, he did not consider the purée to be unwholesome or in any way unfit for human consumption. On cross examination he agreed that one would have to draw a line somewhere regarding the use of rotten fruit but that he would not be concerned unless the Mould Count was 80 or 85% positive. Mr. Zibana said the purée was of excellent quality, as could be seen from its colour, consistency and taste and that a certain proportion of mould, which was quite harmless, was natural to tomatoes grown in the Po valley where the purée was manufactured.

At the termination of the case, the Magistrate declared that he was satisfied that the product was unsound, and granted the application. An appeal from this decision to the Divisional Court was at first considered by the importers, but this was later abandoned and an application to the M.O.H. for permission to re-export was granted.

This case is of interest in two ways :-

A. The principle has been established in the Magistrate's Court that a product made from unsound fruit is itself unsound, although it may have been sterilised.

B. The Howard Mould Count has been accepted for the first time in this country as evidence of such unsoundness.

BOOK REVIEW

BELL'S 'Sale of Food and Drugs'. Service Volume Issue No. 8. Butterworth and Co. Ltd., London. 1964. Including Binder, 45s. 0d. Complete work £7. 7s. 0d. (+ 3/- postage).

Service Volume No. 7 (see J. Ass. Publ. Analysts, 1963, 1, 108) brought 'Bell' up-to-date to the 1st August, 1963. Issue No. 8 follows closely on its heels and covers the period up to the 1st January, 1964. The number of pages in the combined Service Issues is now too much for the original binder, and a new one is provided with the No. 8 issue.

The principal Statutory Instruments covered since Issue No. 7 are the Bread and Flour Regulations; the Liquid Egg (Pasteurisation) Regulations; and the Milk (Special Designation) Regulations, all of 1963. As usual, the 'noter-up' to the main volume contains a number of new entries, including High Court Judgments in appeal cases and the three new Codes of Practice recently agreed between trade interests and the Local Authorities' Joint Advisory Committee on Food Standards, relating respectively to 'chocolate' flour confectionery, crab products, and brandy.

The law relating to Foods and Drugs is constantly being amended in one way or another, and all who use 'Bell' must be very thankful for the prompt and efficient way in which it is ensured that it is never more than a few months out-of-date. The new Issue can be unreservedly commended.

Eric C. Wood.

LETTER TO THE EDITOR

Dear Sir,

POLENSKE FLASKS

Resulting from the note on the Availability of Polenske flasks (J. Ass. Publ. Analysts. 1963, 1, 24) we ordered a number of such flasks from Griffin and George Ltd. against the quoted catalogue number. On receipt the capacity and dimensions were measured and compared with the relevant British Standard Specification. All exceeded the maximum permissible volume and neck length, and some were also out of specification in internal neck diameter.

As a result the matter was taken up with Griffin and George who agreed very largely with our findings. They pointed out however, that 2 to 3 years ago when Joblings ceased making this size of flask, the only flask of similar size left on the market was being produced by Wood Brothers and, since the geometric dimensions were somewhat different, Griffin and George advised the B.S.I. that either the specification would need to be relaxed, or if not, then the only alternative would be bench blown flasks which would be more expensive.

Unfortunately, the position was never resolved. Consequently we approached the B.S.I. who replied stating that they had no information to assist us in the matter, but they put us in contact with the Glass Manufacturers' Federation.

This latter body has since written to us stating categorically that Polenske flasks are not stock items but that Gallenkamp are willing to quote for their manufacture.

The position therefore is not as reported to have been stated by the B.S.I. in the reference quoted above.

Yours etc.

A. Turner.

Cadbury Bros.

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