11.11.62 OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES 2645/62

COUNCIL DIRECTIVE

on the approximation of the rules of the Member States concerning the colouring matters authorised for use in foodstuffs intended for human consumption

THE COUNCIL OF THE EUROPEAN ECONOMIC COMMUNITY,

Having regard to the Treaty establishing the European Economic Community, and in particular Articles 100 and 227 (2) thereof;

Having regard to the proposal from the Commission;

Having regard to the Opinion of the European Parliament;

Having regard to the Opinion of the Economic and Social Committee;

Whereas all rules relating to the colouring matters which may be used in foodstuffs intended for human consumption must give priority to the protection of public health, but the protection of the consumer against falsification and the needs of the economy must also be taken into consideration;

Whereas differences between national rules concerning these colouring matters hinder the free movement of foodstuffs and may create conditions of unfair competition, thereby directly affecting the establishment or functioning of the common market;

Whereas the approximation of these rules is necessary for the free movement of foodstuffs;

Whereas the harmonisation of such rules must involve, as a first stage, the establishment of a single list of colouring matters whose use is authorised for colouring foodstuffs and the laying down of criteria of purity which those colouring matters must satisfy, while, during a second stage, the Council is to take decisions on the harmonisation of the conditions governing the colouring of foodstuffs;

Whereas, in order to take the economic needs of certain States into account, a period should be set during which such Member States may, in respect of certain colouring matters, retain their existing rules, it being understood that during that period the Council may, in the light of any scientific research carried out, take decisions as to the authorisation of such colouring matters;

HAS ADOPTED THIS DIRECTIVE:

Article 1

1. Save as otherwise provided in Article 2, 3, 4 or 13, Member States shall not authorise the use for colouring foodstuffs intended for human consumption (hereinafter called 'foodstuffs') of any colouring matters other than those listed in Annex I.

2. The use of such colouring matters for colouring foodstuffs shall not be subject to any general prohibition.

3. Where the use in foodstuffs of one of the colouring matters listed in Annex I might endanger human health, a Member State may, for a maximum period of one year, suspend the authorisation to use that colouring matter in foodstuffs. It shall inform the other Member States and the Commission of any such suspension within one month. The Council shall, acting unanimously on a proposal from the Commission and by directive, forthwith decide whether the list in Annex I should be amended and, if so, to what extent. The Council may, if necessary, extend the period set in the first sentence of this paragraph.

4. The provisions of this Directive shall also apply to imported products, whether or not processed, intended for consumption within the Community.

Article 2

1. For a period of three years following notification of this Directive, Member States may maintain the provisions of their existing national rules concerning the colouring matters listed in Annex II.

2. Before expiry of the period set in paragraph 1, the Council may, under Article 100 of the Treaty, act on a proposal for a directive authorising the use of these colouring matters. Authorisation may be granted only if, after scientific investigation, these colouring matters are proved harmless to health and if their use is necessary for economic reasons. Where the Council has not acted within the period set in paragraph 1, Article 12 shall apply.

Article 3

This Directive shall not affect national rules concerning natural substances which are used in the manufacture of certain foodstuffs because of their aromatic, sapid or nutritive properties but which also have a subsidiary colouring property, for example paprika, turmeric, saffron and sandal-wood in particular.

Article 4

This Directive shall not affect national rules concerning colouring matters authorised:

(a) for colouring the shells of hard boiled eggs, tobacco and manufactured tobacco;

(b) for stamping meat, citrus fruit, cheese-rinds, the shells of eggs and other external parts not usually consumed with the foodstuffs.

Article 5

This Directive shall not affect national rules specifying which foodstuffs may be coloured by means of the colouring matters listed in Annexes I and II or on what conditions they may be so treated.

Article 6

The Member States shall, for diluting or dissolving the colouring matters listed in Annex I, authorise the use of the following products only:

Sodium carbonate and sodium hydrogen carbonate
Sodium chloride
Sodium sulphate
Glucose
Lactose
Sucrose
Dextrins
Starches
Ethanol
Glycerol
Sorbitol
Edible oils and fats
Beeswax
Water.

Article 7

By way of derogation from Articles 5 and 6, Member States may authorise the use of pigment rubine and of burnt umber, whether or not mixed with paraffin wax or with other harmless substances, only for colouring cheese-rinds.

Article 8

The Member States shall take all measures necessary:

— to ensure that the colouring matters listed in Annex I, where these are used to colour foodstuffs, satisfy the criteria, both general and specific, laid down in Annex III;

— to ensure that the products listed in Article 6, where these are used to dilute or dissolve the colouring matters listed in Annex I, satisfy the general criteria of purity laid down in Annex III, Section A (1) and (2) (b).

Article 9

1. The Member States shall take all measures necessary to ensure that the colouring matters listed in Annex I are placed on the market only if their packagings or containers bear:

(a) the name and address of the manufacturer or of the seller established within the European Economic Community;

(b) the number of colouring matter or matters according to the European Economic Community numbering system given in Annex I;

(c) the words 'colouring matter for foodstuffs'.

2. If the information required under paragraph 1 appears on the packagings or containers and if the words required under paragraph 1 (c) are given in two of the official languages of the Community, one of Germanic and the other of Latin origin, Member States shall not refuse to allow the importation of colouring matters listed in Annex I solely on the grounds that they consider the labelling inadequate.

Article 10

This Directive shall apply to chewing gum in so far as the latter contains any colouring matter.

Article 11

1. The Council, acting unanimously on a proposal from the Commission, may amend by directive the

criteria of purity laid down in Annex III if it becomes evident, in particular in the light of scientific research, that this is necessary for the protection of public health.

2. After consulting the Member States, the Commission shall establish by directive the methods of analysis needed to verify that the criteria of purity laid down in Annex III are satisfied.

Article 12

1. Member States shall, within a period of one month following notification of this Directive, amend their rules in accordance with the above provisions. The rules thus amended shall apply to products placed on the market in Member States not later than two years after that notification.

2. Where the last sentence of Article 2 (2) is applicable, the date of expiry of the period set in that Article shall be substituted for the date of notification referred to in the preceding paragraph.

Article 13

This Directive shall not affect the provisions of national rules concerning products intended for exportation from the Community.

Article 14

This Directive shall also apply in the French overseas departments.

Article 15

This Directive is addressed to the Member States.

Done at Brussels, 23 October 1962.

For the Council

The President

E. GOLOMBO

ANNEX I

The colouring matters referred to in Article 1 of this Directive are listed in the three sections below.

The chemical name given is usually that of the colouring matter when combined with sodium. Except as provided in respect of No E 180, pigment rubine, the use is authorised of the acid itself, of the colouring matter combined with sodium, calcium, potassium and aluminium, whether these combinations are mentioned or not, and of other combinations where stated.

Synthetic chemical products which are identical to the natural colouring matters listed below are also authorised.

Colour	EEC No.	Common name[1]	Schultz	CI	DFG[2]	Chemical formula or description
		I. Colouring matter for both mass and surface colouring				
Yellow	E 100	Curcumin	1 374	(1 238) 75 300	139	1, 1, 7-di-(4-hydroxy-3-methoxyphenyl) hepta-1, 6-diene-3,5 dione
	E 101	Lactoflavin (Riboflavin)	—	—	111	6, 7-dimethyl-9-(D'-1'-ribityl) isoalloxazine: 7, 8-dimethyl-10-(2, 3, 4, 5-tetrahydroxy-pentyl) isoalloxazine.
	E 102	Tartrazine	737	(640) 19 140	64	Trisodium salt of 5-hydroxy-1-p-sulphophenyl 1-4-p-sulphophenyl-azopyrazole-3-carboxylic acid
	E 103	Chrysoine S	186	(148) 14 270	26	Sodium salt of p-sulphobenzene azoresoroinol, or 2, 4-dihydroxyazobenzene-4-sulphonic acid
	E 104	Quinoline yellow	918	(801)[3] (47 005)[3]	97	Sodium salt of a mixture of the mono- and disulphonic acids (mainly the latter) of quinophthalone or 2-quinolylindandione
	E 105	Fast Yellow AB	172	(16) 13 015	23	Disodium salt of 1-(4-sulpho-1-phenylazo)-4-aminobenzene-5-sulphonic acid
Orange	E 110	Orange yellow S sunset yellow FCF	—	15 985	29	Disodium salt of 1-p-sulphophenylazo-2-naphthol-6-sulphonic acid
	E 111	Orange GGN	—	15 980	32	Disodium salt of 2-(4-sulpho-1-naphthylazo)-1-naphthol-4-sulphonic acid
Red	E 120	Cochineal carminic acid	1 381	(1 239) 75 470	107	extract of *Coccus cacti* (including ammonium salts
	E 121	Orchil, orcein	1 386	(1 242)	141	extract obtained with ammonia solution in air, of the red colouring matter of the species *Roccella, Lichanora* and *Orchella*
	E 122	Azorubine Carmoisine	208	(179) 14-720	38	Disodium salt of 2-(4-sulpho-1-naphthylazo)-1-naphthol-4-sulphonic acid
	E 123	Amaranth	212	(184) 16 185	40	Trisodium salt of 1-(4-sulpho-1-naphthylazo)-2-naphthol-3, 6-disulphonic acid
	E 124	Cochineal Red A Ponceau 4R	213	(185) 16 255	41	Trisodium salt of 1-(4-sulpho-1-naphthylazo)-2-naphthol-6, 8-disulphonic acid
	E 125	Scarlet GN	—	14 815	34	Disodium salt of 2-(6'-sulpho-2' + 4'-xylylazo)-1-naphthol-3, 6, 8-trisulphonic acid

Colour	EEC No	Common name[1]	Schultz	CI	DFG[2]	Chemical formula or description
Red (Cont'd)	E 126	Ponceau 6R	215	(186) 16 290	42	-Tetrasodium salt of 1-(4-sulpho-1--naphthylazo)-2-naphthol-3, 6, 8-trisulphonic acid
Blue	E 130	Anthraquinone blue (solanthrene blue RS)	1 228	(1 106) 69 800	104	-N:N'-dihydro-1,2,1,2'-anthraquinoneazine (Indanthrone)
	E 131	Patent Blue V	826	(712) 42 051	85	Calcium salt of the disulphonic acid of m-hydroxytetraethyl diaminotriphenyl -carbinol anhydride
	E 132	Indigotin (indigo carmine)	1 309	(1 180) 73 015	105	Disodium salt of indigotin-5, 5-disulphonic acid
Green	E 140	chlorophylls	1 403	(12 49a) 75 810	110	*Chlorophyll a:* magnesium complex of 1, 3, 5, 8-tetramethyl-4-ethyl-2-vinyl-9-oxo-10-methoxycarbonylphorbin-7-propionic acid phytyl ester *Chlorophyll b:* magnesium complex of 1,5,8-trimethyl-4-ethyl-2-vinyl-3-formyl-9-oxo-10-methoxycarbonylphorbin-7-propionic acid phytyl ester
	E 141	Copper complexes of chlorophylls and chlorophyllins	—	—	110	Copper chlorophyll complex and copper chlorophyllin complex
Brown	E 150	Caramel[4]	—	—	—	Product obtained exclusively by heating saccharose or other sugars
Black	E 151	Brilliant Black BN, Black PN	—	28 440	58	Tetrasodium salt of 8-acetamido-2-(7-sulpho-4-p-sulphophenylazo-1-naphthylazo)-1-naphthol-3, 5-disulphonic acid
	E 152	Black 7984	—	—	—	Tetrasodium salt of 1[4-(4-sulpho-1-phenylazo)7-sulpho-1-naphthylazo]-1-hydroxy-7-amino-naphthalene-3, 6-disulphonic acid
	E 153	Carbo medicinalis vegetalis (charcoal)	—	—	—	vegetable charcoal having the same properties as medicinal charcoal
Various shades	E 160	*Carotenoids:*				
		(a) alpha-, beta-, gamma-carotene	—	—	—	All the *trans* forms
		(b) Bixin, Norbixin (Roucou, Annatto)	—	—	—	Bixin, a carotenoid colour, is the principal colouring of oil extracts of Annatto: it is the monomethyl ester of Norbixin Norbixin is a symmetrical dicarboxylic acid: its alkali salt is the principal colouring of aqueous extracts of Annatto
		(c) Capsanthhein Capsantin, Capsorbin	—	—	—	paprika extract
		(d) Lycopene	—	—	—	All the *trans* forms

Colour	EEC No	Common name[1]	Schultz	CI	DFG[2]	Chemical formula or description
Various shades (Cont'd)	E 161	*Xanthophylls:* (a) Flavoxanthein (b) Lutein (c) Kryptoxanthein (d) Rubixanthein (e) Violoxanthein (f) Rhodoxanthein	1.403	(1 249a)	144	The xanthophylls are ketonic and/or hydroxylic derivatives of carotene
	E 162	Beetroot red Betanin	—	—	—	Aqueous extract of the root of the red beetroot
	E 163	*Anthocyanins*	1 394 1 400	—	112	The anthocyanins are glucosides of salts of 2-phenylbenzopyrylium; most of them are hydroxyl derivatives They include the following, non-glucosated anthocyanidins: pelargonidine, cyanidine, peonidine, delphinidine, petunidine, malvidine The anthocyanins may be obtained only from edible fruit or vegetables such as strawberries, mulberries, cherries, plums raspberries, blackberries, blackcurrants, red currants, red cabbage, red onions, cranberries, bilberries, aubergines, (egg plants), grapes and elderberries

II. Colouring matter for surface colouring only

	E 170	Calcium carbonate	1 405	(1 261) 77 220	—
	E 171	Titanium dioxide	1 418	(1 264) 77 891	—
	E 172	Iron oxides, and hydroxides	1 276 1 311 1 428 1 429 1 470	77 489 77 491 77 492 77 499	— — — —
	E 173	Aluminium	—	77 000	—
	E 174	Silver	—	—	—
	E 175	Gold	—	—	—

Colour	EEC No	Common name[1]	Schultz	CI	DFG[2]	Chemical formula or description
		III. Colouring matter for certain uses only				
Various Shades (Cont'd)	E 180	Pigment rubine lithol-rubin BK (for colouring cheese-rind)	194	(163) 15 850	147	Only the calcium and aluminium salts of 3-carboxy-1-p-lotylazo-2-naphthol-2-sulphonic acid
	E 181	Burnt umber (for colouring cheese-rind)	—	—	—	Product obtained by roasting in air a mixture consisting essentially of iron and manganese oxides, and calcium and aluminium silicates, carbonates and sulphates

[1] These names are given for information only.

[2] The abbreviations mean:

Schultz — G. Schultz, *Farbstofftabellen*, 7th Edition, Leipzig 1931;

CI — Figures in brackets: Rowe Colour Index 1924. Other figures Rowe Colour Index, Second Edition, Bradford, England, 1956;

DFG — Toxikologische Daten von Farbstoffen und ihre Zulassung für Lebensmittel in verschiedenen Ländern, compiled on behalf of the Commission by Professor Dr. G. Hecht, Wuppertal-Elberfeld, 6th Communication by the German Research Institute's Commission on Colouring Matters, 2nd Edition, Wiesebaden, 1957.

[3] This relates only to the colouring matter 'early dye' which is identical to that dealt with under Nos 918 Schultz and 97 DFG.

[4] The name 'caramel' relates to products of a more or less intense brown colour which are intended for colouring. It does not correspond to the German expression 'Karamell', which is the term used for the sugary aromatic product obtained by heating sugar and which is used for confectionery and pastry.

ANNEX II

Common name[1]	Schultz	CI	DFG[2]	Chemical formula or description
		I. Colouring matter for both mass and surface colouring		
Extracts from Persian berries	1 369	(1 234) 75 640	138	Extracts from the berries of various *Rhamnus* species, particularly *infectorius, amygdalinus* and *sexatalis*
Orcanet, alkanet, alkannin	1 382	(1 240) 75 520 75 530	140	Extract from the root of *Alkanna tinctoria*
Vegetable carameline	—	—	—	Extract from Cassel's earth obtained by specially treating certain peats and lignites
Erythrosine	887	(773) 45 430	93	Disodium or dipotassium salt of 2, 4, 5, 7-tetra-iodofluoroscein
Acid brilliant green BS (Lissamine green, Green S)	836	(737) 44 090	86	Sodium salt of 4,4-bis (dimethyl-amino)-diphenyl-methylene-2-naphthol-3, 6-disulphonic acid
		II. Colouring matter for certain uses only		
Ultramarine Blue (for colouring sugar blue)	1 435	(1 290) 77 007	—	Combination of aluminium, sodium silicon and sulphur

[1] and [2] See notes to Annex I.

ANNEX III

Criteria of Purity

A. GENERAL CRITERIA OF PURITY

Unless otherwise provided in the specific criteria in Section B the colouring matters referred to in Annex I are required to satisfy the following criteria of purity, quantities and percentages being calculated on the pure colour.

1. *Inorganic impurities*

   (a) They should contain not more than 5 mg/kg of arsenic and not more than 20 mg/kg of lead;

   (b) They should contain not more than 100 mg/kg of the following substances, taken separately: antimony, copper, chromium, zinc, barium sulphate; and not more than 200 mg/kg of these products taken together;

   (c) They should not contain cadmium, mercury, selenium, tellurium, thallium, uranium or chromates, or soluble combinations of barium in detectable quantities.

2. *Organic impurities*

   (a) They should not contain 2-naphthylamine, benzidine, amino-4-diphenyl (or xenylamine) or their derivatives;

   (b) They should not contain polycyclic aromatic hydrocarbons;

   (c) Synthetic organic colouring matters should contain not more than 0·01% of free aromatic amines;

   (d) Synthetic organic colouring matters should contain not more than 0·5% of intermediate synthetic products other than free aromatic amines;

   (e) Synthetic organic colouring matter should contain not more than 4% of accessory colouring matters (isomers, homologues etc.);

   (f) Sulphonated organic colouring matters should contain not more than 0·2% of substances extractable by diethyl ether.

B. SPECIFIC CRITERIA OF PURITY

E 101 — Lactoflavin (Riboflavin)

Lumiflavin: Prepare ethanol-free chloroform as follows: Shake 20 ml of chloroforn with 20 ml of water gently but carefully for three minutes and allow time to separate. Draw off the chloroform layer and repeat the operation twice using 20 ml each time. Finally, filter the chloroform through dry filter paper, shake the filtrate well for five minutes with 5 g of powdered anhydrous sodium sulphate, leave the mixture to settle for two hours, then decant or filter the clear chloroform. Shake 25 mg of riboflavin with 10 ml of ethanol-free chloroform for five minutes, then filter: the colour of the filtrate should not be more intense than that of an aqueous solution obtained by diluting 3 ml of 0·1 N potassium dichromate to 1000 ml.

E 102 — Tartazine

Products insoluble in water: not more than 0·2%

Accessory colourings: not more than 1%

E 103 — Chrysoine S

Products insoluble in water: not more than 0·2%

E 104 — Quinoline Yellow

Products insoluble in water: not more than 0·2%

E 105 — Fast Yellow AB

Products insoluble in water: not more than 0·2%

Accessory colourings: not more than 3%

Unsulphonated aromatic amines and aniline: not more than 10 mg/kg

(a) *Determination of 2-aminoazobenzene and 4-aminoazobenzene:* Dissolve 20·0 g of Fast Yellow AB in 400 ml of water and add 5 ml of N sodium hydroxide. Shake in a separating funnel with four successive portions of 50 ml of chlorobenzene, for five minutes each time. Wash the combined chlorobenzene extracts with successive amounts of 400 ml of 0·1 N sodium hydroxide until the upper aqueous layer remains colourless. Filter the chlorobenzene solution through a thickly-folded filter paper and measure the extinction (E_1) in a spectrophotometer against chlorobenzene contained in cells of suitable thickness (d_1) at 414 mμ.

Calculation:

$$\text{Content of 2 and 4-aminoazobenzene (mg/kg)} = \frac{E_1 \times 100}{0{\cdot}397 \times d_1}.$$

Note:

$$E\,{}^{1\ \text{mg/ml}}_{1\ \text{cm}} \text{ at } 414\ m\mu \begin{cases} \text{for 2-aminoazobenzene} = 39{\cdot}7 \\ \text{for 4-aminoazobenzene} = 35{\cdot}2 \end{cases}$$

The aminoazobenzene content can be determined only up to 90%. It is possible to separate the 2- and 4- compounds by the following method. Concentrate 100 ml of chlorobenzene extract to about 20 ml by heating in a water bath in a current of warm air. Pour the concentrated solution on a column of alumina (of appropriate size). Elute with chlorobenzene. The first 100 ml of the chlorobenzene eluate contains the 2-aminoazobenzene. The *para* compound of the chlorobenzene is then eluted. Dilute the two solutions to 100 ml. Measure the extinction of the *ortho*-compound at 414 mμ (E_2), and that of the *para*-compound at 376 mμ (E_3).

$$E\,{}^{1\ \text{mg/ml}}_{1\ \text{cm}} \text{ 414 } m\mu \text{ for the 2-aminoazobenzene} = 39{\cdot}7$$

$$E\,{}^{1\ \text{mg/ml}}_{1\ \text{cm}} \text{ 376 } m\mu \text{ for the 4-aminoazobenzene} = 110$$

$$\text{2-aminoazobenzene content (mg/kg)} = \frac{E_2 \times 100}{0{\cdot}397 \times d_2}.$$

$$\text{4-aminoazobenzene content (mg/kg)} = \frac{E_3 \times 100}{1{\cdot}10 \times d_3}.$$

(b) *Determination of aniline:* Shake 75 ml of the remaining chlorobenzene extract with two successive portions of 50 ml of 0·5 N hydrochloric acid, then with two successive portions of 25 ml of water. Neutralise the combined aqueous extracts with a 30% solution of sodium hydroxide, then acidify with 10 ml of 0·5 N hydrochloric acid. Dissolve 1–2 g of potassium bromide in this solution. After cooling in iced water, add about 20 drops of 0·1 N sodium nitrate and leave to settle for ten minutes. Remove the excess nitrite by the addition of sulphamic acid. Pour the solution into about 5 ml of a 3% solution of R salt (disodium salt of 2-naphthol-3, 6-disulphonic acid) added to 10 ml of 2 N sodium hydroxide. Leave to settle for fifteen minutes. Acidify the solution of the dyestuff with Congo Red TS (indicator) until the latter turns blue, and filter. The aminoazobenzene dyestuff will remain on the filter. Dilute the filtrate to 200 ml, then measure the extinction at 490 mμ or E_4.

Calculation:

$$\text{Aniline content (mg/kg)} \frac{E_4 \times 266}{2{\cdot}26 \times d_4}.$$

$$E\,{}^{1\ \text{mg/ml}}_{1\ \text{cm}} \text{ 490 } m\mu \text{ for aniline} = 226$$

E 110 — Orange Yellow S, Sunset Yellow FCF

Products insoluble in water: not more than 0·2%

E 111 — Orange GGN

Products insoluble in water: not more than 0·2%

E 120 — Cochineal, carminic acid

Paper chromatography: with a solution of 2 g of trisodium citrate in 100 ml 5% ammonium hydroxide, cochineal gives only a single stain in the alkaline zone.

E 122 — Azorubin, Carmoisine

Products insoluble in water: not more than 0·2%

Accessory colourings: not more than 1%

E 123 — Amaranth

Products insoluble in water: not more than 0·2%

E 124 — Cochineal Red A, Ponceau 4 R

Products insoluble in water: not more than 0·2%

E 125 — Scarlet GN

Products insoluble in water: not more than 0·2%

E 126 — Ponceau 6 R

Products insoluble in water: not more than 0·2%

Accessory colourings: not more than 3%

E 131 — Patent Blue V

Products insoluble in water: not more than 0·5%

Chromium (estimated as CR): not more than 20 mg/kg

Accessory colourings: not more than 1%

E 132 — Indigotin indigo carmine

Products insoluble in water: not more than 0·2%

Accessory colourings: not more than 1%

Isatinsulphonic acid: not more than 1%

E 141 — Copper complexes of chlorophylls and chlorophyllins

A 1% solution of copper chlorophyll complex in turpentine should not be turbid and should not form a sediment.

Copper (free ionisable Cu): not more than 200 mg/kg

E 151 — Brilliant Black BN, Black PN

Products insoluble in water: not more than 0·2%

Accessory colourings: not more than 15%. (The presence of accessory colourings among which the diacetylised compound has been identified is essential in order to obtain the precise shade.)

Intermediate products: not more than 1%

E 152 — Black 7984

Products insoluble in water: not more than 0·2%

Lead: not more than 10 mg/kg

Arsenic: not more than 2 mg/kg

E 153 — Carbo medicinalis vegetalis (charcoal)

Higher aromatic hydrocarbons: Extract 1 g of carbon black with 10 g of pure cyclohexane for two hours. The extract should be colourless. It should have little or no fluorescence in ultra-violet light; on evaporation it should leave no residue.

Tarry products: boil 2 g of carbon black with 20 ml of N sodium hydroxide, then filter. The filtrate should be colourless.

E 160 (a) — Alpha-, Beta-, Gamma-Carotene

Chromatography: By absorption on alumina or silica gel, pure Beta-carotene shows only one zone.

E 160 (b) — Bixin and Norbixin (Roucou, Annatto)

Chromatography:

(a) *Annatto:* Dissolve a sufficient quantity of Annatto in benzene or dilute a benzene solution of Annatto to obtain a solution of the same colour as a 1% solution of potassium dichromate. Pour 3 ml of the solution on the top of an alumina column; elute slowly. Wash the column three times with benzene. The bixin is very heavily absorbed on the surface of the alumina and forms a brilliant orange-red zone (as distinct from crocetin saffron). A very pale yellow zone usually moves very rapidly across the column, even with crystallised pure bixin. The bixin cannot be eluted in benzene, light petroleum, ether, chloroform, acetone, etharol or methanol. But the ethanol and methanol cause the orange tint to turn into an orange yellow.

Carr-Price reaction: Remove the benzene from the column by washing three times with chloroform previously dehydrated by means of potassium carbonate. After elution of the last chloroform wash, add 5 ml of the Carr-Price reagent to the top of the column. The bixin zone immediately turns to blue-green (as distinct from crocetin).

(b) *Bixin:* Dissolve 1 to 2 mg of crystallised bixin in 20 ml of chloroform. Add 5 ml of this solution to the top of the prepared column. Rinse the solution with chloroform previously dehydrated with sodium carbonate and proceed as for (a) (*Carr-Price reaction*).

(c) *Alkaline solutions of norbixin:* Place 2 ml of an aqueous solution of Annatto in a 50 ml separating funnel. Add sufficient 2 N sulphuric acid to obtain a highly acid reaction. The norbixin will separate out as a red precipitate. Add 50 ml of benzene, then shake vigorously. After separation discard the aqueous layer and wash the benzene solution with 100 ml of water until the solution is no longer acid. Centrifuge the solution (usually emulsified) of norbixin in benzene for ten minutes at 2500 revolutions per minute. Decant the clear norbixin solution and dehydrate by means of anhydrous sodium sulphate. Pour 3–5 ml of this solution on the top of the alumina column. Norbixin like bixin will form an orange-red zone on the surface of the alumina. When eluted as in (a), it will behave like bixin and will also give the Carr-Price reaction.

E 162 — Beetroot red, betanin

Paper Chromatography: With butanol saturated with 2 N hydrochloric acid as a solvent (ascending chromatography), betanin gives a single red spot with a brownish trail and little migration.

E 171 — Titanium dioxide

Substances soluble in hydrochloric acid: Suspend 5 g of titanium dioxide in 100 ml of 0·5 N hydrochloric acid and heat for thirty minutes in a water bath, stirring from time to time. Filter in a Gooch crucible on the bottom of which three layers have been placed, the first of coarse asbestos the second of filter paper reduced to a pulp and the third of fine asbestos. Wash with three successive portions of 0·5 N hydrochloric acid each of 10 ml. Evaporate the filtrate to dryness in a platinum evaporating dish, then heat to a dull red until the weight is constant. The weight of the residue should not exceed 0·0175 g.

Antimony: not more than 100 mg/kg

Zinc: not more than 50 mg/kg

Soluble barium compounds: not more than 5 mg/kg

E 172 — Iron oxides and hydroxides

Selenium: not more than 1 mg/kg

Mercury: not more than 1 mg/kg

E 181 — Burnt umber

Manganese oxides computed on the basis of Mn_3O_4: not more than 8%

Organic matters not completely burnt: Boil 2 g of burnt umber in 30 ml of a 20% solution of potassium hydroxide, then filter. The filtrate should be colourless.