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Council Directive 67/427/EEC of 27 June 1967 on the use of certain preservatives for the surface treatment of citrus fruit and on the control measures to be used for the qualitative and quantitative analysis of preservatives in and on citrus fruit

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COUNCIL DIRECTIVE of 27 June 1967 on the use of certain preservatives for the surface treatment of citrus fruit and on the control measures to be used for the qualitative and quantitative analysis of preservatives in and on citrus fruit (67/427/EEC)

THE COUNCIL OF THE EUROPEAN ECONOMIC COMMUNITY,

Having regard to the Treaty establishing the European Economic Community, and in particular Article 100 thereof;

Having regard to the proposal from the Commission;

Having regard to the Opinion of the European Parliament (1);

Having regard to the Opinion of the Economic and Social Committee (2);

Whereas, under Article 5 of the Council Directive of 5 November 1963 (3) on the approximation of the laws of the Member States concerning the preservatives authorised for use in foodstuffs intended for human consumption, as last amended by Article 1 of the Council Directive of 14 December 1966, (4) Member States may, until 30 June 1967, maintain in force the provisions of their national laws relating to the surface treatment of citrus fruit with biphenyl (diphenyl), orthophenylphenol and sodium orthophenylphenate;

Whereas the use of those substances for the surface treatment of citrus fruit does not constitute a danger to health where the residual amount per kilogramme of whole fruit does not exceed 70 milligrammes of biphenyl and 12 milligrammes of orthophenylphenol and sodium orthophenylphenate, expressed as orthophenylphenol;

Whereas, moreover, the treatment carried out should be indicated in an appropriate manner at all stages of marketing;

Whereas, if the three substances in question are to be authorised at Community level, common rules must be laid down for the official control of treated citrus fruit;

Whereas a transitional period is necessary before the provisions of this Directive can be implemented by the Member States ; whereas the provisions of national laws relating to the surface treatment of citrus fruit with the three preservatives in question should therefore be maintained in force until the end of that period;

Whereas a Member State should not be required to authorise the use of a preservative in foodstuffs produced and consumed in its own territory when there is no technological reason to justify such use;

HAS ADOPTED THIS DIRECTIVE:

Article 1

The Council Directive of 5 November 1963 on the approximation of the laws of the Member States Concerning the preservatives authorised for use in foodstuffs for human consumption is hereby amended as follows: 1. In Article 2 (2), for the second sentence there shall be substituted the following:

"However, the laws of a Member State may totally exclude the use of any of the preservatives listed in the Annex only where there is no technological reason for using such preservative foodstuffs produced and consumed in its own territory."

2. The following preservatives shall be added to those listed in Section I of the Annex: (1) OJ No 63, 3.4.1967, p. 990/67. (2) OJ No 64, 5.4.1967, p. 1005/67. (3) OJ No 12, 27.1.1964, p. 161/64. (4) OJ No 233,20.12.1966, p. 3947/66 >PIC FILE= "T0001696">

3. Subparagraph (b) of Article 5 shall be deleted.

Article 2

The Member States shall take all measures necessary to ensure that the taking of samples and the qualitative and quantitative analysis of biphenyl, orthophenylphenol and sodium orthophenylphenate in and on citrus fruit are carried out in accordance with the provisions of Annexes I, II, III and IV to this Directive.

Article 3

1. Member States shall, not later than 1 July 1968, bring into force the measures necessary to comply with this Directive and shall forthwith inform the Commission thereof.

2. Until 1 July 1968 Member States may maintain in force the provisions of their national laws relating to the surface treatment of citrus fruit with biphenyl, orthophenylphenol and sodium orthophenylphenate.

Article 4

This Directive is addressed to the Member States.

Done at Brussels, 27 June 1967.

For the Council

The President

R. VAN ELSLANDE

ANNEX I PROCEDURE FOR TAKING SAMPLES OF CITRUS FRUIT FOR PRESERVATIVE CONTROL

A. Taking of samples

I. Samples shall be taken using scientific methods which ensure that the samples are representative of the lot to be analysed.

II. The samples must satisfy at least the following requirements: 1. Packaged goods (crates, paperboard boxes, and similar containers) >PIC FILE= "T0001697">

2. Goods in bulk >PIC FILE= "T0001698">

III. By "lot" is meant : a part of a consignment, which part has the same characteristics, such as variety, degree of ripeness, type of packaging.

B. Packaging and delivery of samples

1. The samples shall be placed in air-tight containers;

2. The containers shall be sealed;

3. The samples thus packaged shall be delivered as quickly as possible to the test laboratories.

ANNEX II QUALITATIVE ANALYSIS FOR RESIDUES OF BIPHENYL, ORTHOPHENYLPHENOL AND SODIUM ORTHOPHENYLPHENATE IN CITRUS FRUIT PEEL

1. Purpose and scope

The method described below enables the presence of residues of biphenyl, orthophenylphenol (OPP) or sodium orthophenylphenate in the peel of citrus fruit to be detected. The sensitivity limit of this method, in absolute terms, is approximately 5 ¶g for biphenyl and 1 milligramme for OPP or sodium orthophenylphenate, which is the equivalent of 5 ¶g of biphenyl (5 ppm) and 1 milligramme of OPP (1 ppm) respectively in the peel of 1 kilogramme of citrus fruit.

When citrus fruit is treated with the above-mentioned products the residues deposited are found largely in the peel of the fruit. Quantitative analysis of such residues in the whole fruit therefore seems necessary only if they are found in the peel.

2. Principle

An extract is prepared from the peel using dichloromethane in an acid medium. The extract is concentrated and separated by thin layer chromatography using silica gel. The presence of biphenyl, orthophenylphenol or sodium orthophenylphenate is shown by fluorescence and colour tests.

3. Reagents

cyclohexane A.R.

dichloromethane A.R.

hydrochloric acid 25 % (w/v)

silica gel GF 254 Merck or equivalent

0.5 % solution of 2,4,7-trinitrofluorenone (Fluka, B.D.H or equivalent) (TNF) in acetone

0.1 % solution of 2,6-dibromo-benzoquinone-4-chloroimide in ethanol (stable for up to one week if kept in the refrigerator)

concentrated solution of ammonia, S.G. : 0.9

standard 1 % solution of pure biphenyl in cyclohexane

standard 1 % solution of pure orthophenylphenol in cyclohexane

4. Apparatus

mixer

250 ml flask with ground glass joint and with a cooled reflux condenser

reduced pressure evaporator

micropipettes

thin layer chromatographic apparatus with plates measuring 20 X 20 cm

U.V. lamp (254 nm) : the intensity should be such that a spot of 5 ¶g of biphenyl is visible

equipment for pulverising reagents

oven

5. Method

(a) Preparation of the sample and extraction

All the fruit in the sample for testing is cut in half. Half of each piece of fruit is kept for quantitative determination of residues of biphenyl and/or orthophenylphenol. Pieces of peel are taken from the other halves to give a sample of about 80 grammes. These pieces are chopped, crushed in the mixer and placed in the 250 ml flask ; to this is added 1 ml of 25 % hydrochloric acid and 100 ml of dichloromethane. The mixture is heated under reflux for ten minutes. After cooling and rinsing of the condenser with about 5 ml of dichloromethane, the mixture is filtered through a fluted filter. The solution is transferred to the evaporator and some porous granules are added. The solution is concentrated at reduced pressure at a temperature of 60 ºC to a final volume of about 10 ml. If a rotary evaporator is used, the flask should be kept in a fixed position to avoid loss of biphenyl through the formation of a film of the product on the upper wall of the flask.

(b) Chromatography

30 grammes of silica gel and 60 ml of water are placed in a mixer and mixed for one minute. The mixture is then poured on to 5 chromatographic plates and spread to form a layer approximately 0.250 mm thick. The plates covered with this layer are subjected for fifteen minutes to a stream of hot air and then placed in an oven where they are kept for thirty minutes at a temperature of 110 ºC.

After cooling each plate is divided into strips 2 cm wide, by parallel lines penetrating the covering layer down to the surface of the plate. 50 ¶l of the extract to be analysed are put, as a row of drops, close together on to each strip, approximately 1.5 cm from the edge. At least one strip is kept for the controls consisting of a deposit of 1 ¶l (that is, 10 ¶g) of the standard solutions of biphenyl and orthophenylphenol.

The chromatographic plates are developed in a mixture of cyclohexane and dichloromethane (25 : 95) in dishes previously lined with filter paper.

(c) Detection and identification

The presence of biphenyl and orthophenylphenol is shown by the appearance of spots in U.V. light (254 nm). The sodium orthophenylphenate has changed into orthophenylphenol during the extraction in an acid medium, and its presence can not therefore be distinguished from that of orthophenylphenol. The products are identified in the following manner: (i) biphenyl gives a yellow spot in daylight when sprayed with the TNF solution;

(ii) orthophenylphenol gives a blue spot when sprayed with the solution of 2,6-dibromobentoquinone-4-chlorimide, followed by rapid passage through a stream of hot air and exposure to an ammonia-saturated atmosphere.

ANNEX III QUANTITATIVE ANALYSIS OF THE RESIDUES OF BIPHENYL IN CITRUS FRUIT

1. Purpose and scope

The method described below gives a quantitative analysis of the residues of biphenyl in citrus fruit (whole fruit). The accuracy of the method is ± 10 % for a biphenyl content greater than 10 milligrammes per kilogramme of fruit (10 ppm).

2. Principle

After distillation in an acid medium and extraction by cyclohexane, the extract is chromatographed in a thin layer on silica gel. The chromatogram is developed and the biphenyl is eluted and determined spectrophotometrically at 248 nm.

3. Reagents

concentrated sulphuric acid solution

silicone-based anti-foaming emulsion

cyclohexane A.R.

hexane A.R.

ethanol A.R.

anhydrous sodium sulphate

silica gel GF 254 Merck or equivalent

standard 1 % solution of pure biphenyl in cyclohexane : dilute with cyclohexane to obtain the following three solutions:

(a) 0.6 ¶g/¶l (b) 1 ¶g/¶l (c) 1.4 ¶g/¶l

4. Apparatus

1 mixer (1 litre capacity)

2-litre distillation flask with modified Clevenger-type separator (1) and cooled reflux condenser

10 ml graduated flask (1) See figure on page 180.

50 ¶l micropipettes

thin layer chromatographic apparatus with 20 X 20 cm plates

oven

centrifuge with 15 ml conical tubes

U.V. spectrophotometer

5. Method (a) Preparation of the sample and extraction

All the fruit in the sample for testing is cut in half.

Half of each piece of fruit is kept for qualitative analysis for residues of biphenyl, OPP or sodium orthophenylphenate. The other halves are put all together and shredded in a mill or crushed until a homogeneous mixture is obtained. From this at least two subsamples of 200 grammes are taken for analysis in the following manner. Each subsample is placed in a mixer with 100 ml of water and mixed at slow speed for several seconds. Water is added until the volume of the mixture reaches three-quarters of the capacity of the mixer, and the mixture is then mixed for five minutes at full speed. The resulting purée is transferred to the 2-litre distillation flask. The mixer is rinsed with water and the rinsings added to the contents of the flask. (The total quantity of water to be used in the mixing and rinsing is 1 l). To the mixture are added 2 ml sulphuric acid, 1 ml anti-foaming emulsion and several porous granules. The separator and reflux condenser are fitted on to the flask. Distilled water is poured into the separator until the water level is well past the lower arm of the lateral return tube, followed by 7 ml of cyclohexane. This is distilled for about two hours. The contents of the separator are then collected in the 10 ml graduated flask, the separator is rinsed with about 1.5 ml of cyclohexane and the rinsings added to the contents of the flask, which are then brought up to volume with cyclohexane. Finally a little anhydrous socium sulphate is added and the mixture is shaken.

(b) Chromatography

30 grammes of silica gel and 60 ml of water are placed in a mixer and mixed for one minute. The mixture is then poured on to 5 chromatographic plates and spread to form a layer approximately 0.250 mm thick. The plates covered with this layer are subjected for fifteen minutes to a stream of hot air and then placed in an oven where they are kept for thirty minutes at a temperature of 110 ºC. After cooling each plate is divided into four strips 4.5 cm wide, by parallel lines penetrating the covering layer down to the surface of the plate. 50 ¶l of the extract to be analysed are put, as a row of drops, close together on to one of the strips approximately 1.5 cm from the egde of the plate. On to each of the three other strips are put in the same way 50 ¶l of the standard solutions (a), (b) and (c), corresponding respectively to 30, 50 and 70 ¶l levels of biphenyl.

If the analyses are made in series, standard solutions need not be put on to each plate and a standard curve may be produced from the average of the values obtained from at least five plates, with the same standard amounts.

(c) Development of chromatograms and elution

The chromatograms are developed with hexane to a height of 17 cm in dishes previously lined with filter paper. The plates are air dried. The areas in which the biphenyl is localised are picked out in U.V. light (254 nm), and marked off in rectangles of equal areas.

The areas thus marked off are immediately scraped clean with a spatula, through the full thickness of the supporting layer. The biphenyl is extracted from this by 10 ml of ethanol, for ten minutes, shaking several times. The mixture is transferred to the centrifuge tubes and centrifuged for five minutes at 2500 r.p.m.

A sample control area of the same size is taken by the same method. If the analyses are made in series, this control area is taken from an unused strip of the plate ; if the analyses are made individually, it is taken from one of the strips containing a standard solution located below the area containing the biphenyl.

(d) Spectrophotometric determination

The supernatant liquid is decanted into the spectrophotometer cells and the extinction determined at 248 nm and compared with a control extract from a chromatographic area free from biphenyl.

6. Calculation of results

A standard curve is drawn, plotting the biphenyl values of 30, 50 and 70 ¶g against the corresponding extinctions, as determined on the spectrophotometer. This gives a straight line which passes through the origin. This graph allows the biphenyl content of the samples to be read directly in ppm from the extinction value of their extracts.

ANNEX IV QUANTITATIVE ANALYSIS OF THE RESIDUES ORTHOPHENYLPHENOL AND SODIUM ORTHOPHENYLPHENATE IN CITRUS FRUIT

1.Purpose and scope

The method described below enables a quantitative analysis of the residues of orthophenylphenol (OPP) and sodium orthophenylphenate in citrus fruit (whole fruit) to be made. The method gives results which for an OPP or sodium orthophenylphenate content of the order of 12 ppm are low by an average value of between 10 % and 20 %.

2. Principle

After distillation in an acid medium and extraction by di-isopentyl ether, the extract is purified and treated with a solution of 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (= 4-aminoantipyrine). A red colour develops the intensity of which is measured by spectrophotometry at 510 nm.

3. Reagents

70 % orthophosphoric acid

silicone-based anti-foaming emulsion

di-isopentyl ether A.R.

purified cyclohexane ; shake three times with a 4 % solution of sodium hydroxide, wash three times with distilled water

4 % sodium hydroxide solution

buffer solution at pH 10.4 : into a 2-litre graduated flask put 6.64 grammes of boric acid, 8.00 grammes of potassium chloride and 93.1 ml of N sodium hydroxide solution : mix and bring up to calibration mark with distilled water

reagent I : dissolve 1.0 grammes of 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (= 4-aminotipyrine) in 100 ml of distilled water reagent II : dissolve 2.0 grammes of potassium ferrocyanide in 100 ml of distilled water. Reagents I and II must be kept in brown glass flasks and are only stable for approximately fourteen days

silica gel

standard solution : dissolve 10 milligrammes of pure OPP in 1 ml of 0.1 N NaOH ; dilute to 100 ml with a 0.2 m sodium borate solution (1 ml = 100 ¶g). For the standard solution, dilute 1 : 10 with the buffer solution.

4. Apparatus

shredding or crushing mill

mixer

1-litre distillation flask with modified Clevenger type separator (1) and reflux condenser

infra-red bath

200 ml separating funnel

graduated cylinders of 25 and 100 ml

graduated flasks of 25 and 100 ml

1 to 10 ml pipettes

0.5 ml graduated pipettes

spectrophotometer with 5 cm cells

5. Method

All the fruit in the sample for checking is cut in half. Half of each piece of fruit is kept for qualitative analysis for residues of biphenyl, OPP or sodium orthophenylphenate. The other halves are put all together and shredded in a mill or crushed until a homogeneous mixture is obtained. From this at least two sub-samples of 250 grammes are taken for analysis in the following manner.

Each sub-sample is placed in a mixer with 500 ml of water and mixed until a very fine homogeneous mixture is obtained in which the oily cells are no longer perceptible. A sample of 150 to 300 grammes of the purée is taken, depending on the presumed OPP content and placed in the 1-litre distillation flask with a quantity of water sufficient to dilute the mixture to 500 grammes in the flask. After the addition of 10 ml of 70 % orthophosphoric acid, several porous granules and 0.5 ml of anti-foaming emulsion, the separator and the reflux condenser are fitted on to the flask. 10 ml of di-isopentyl ether is put into the separator and the flask is heated gently in the infra-red bath, without allowing the purée to boil or foam. After distilling for about six hours, the contents of the separator are poured into the 200 ml separating funnel, and the separator and the condenser are rinsed with 60 ml of cyclohexane and then with 60 ml of water. The rinsings are added to the contents of the separating funnel. The mixture is shaken vigorously and when the phases have separated the aqueous phase is discarded.

To extract the OPP, the organic phase is shaken vigorously five times, each time for three minutes, with 10 ml of 4 % sodium hydroxide. The alkaline solutions are combined, neutralised to pII 9-10 with orthophosphoric acid in the presence of phenolphthalein paper, and diluted to 100 ml with distilled water. A pinch of silica gel is added in order to clarify the solution which will have a slightly cloudy appearance. The solution is then shaken and filtered through a dry, fine-grain filter. Since the colouring is developed with the maximum of accuracy and precision using quantities of OPP of between 10 and 70 ¶g, an aliquot sample of between 0.5 and 10 ml of solution is taken with a pipette, taking into account the quantities of OPP which might be expected to be found. The sample is placed in a 25 ml graduated flask ; to this are added 0.5 ml of reagent I, 10 ml of the buffer solution and then 0.5 ml of reagent II. The mixture is made up to the calibration mark with the buffer solution and shaken vigorously.

After five minutes the extinction of the red colouring at 510 nm is measured with the spectrophotometer in comparison with a control containing no extract. The colour does not lose intensity within thirty minutes. Evaluation is made by referring to a standard curve drawn under the same conditions using the standard OPP solution.

6. Observations

For each analysis it is recommended that the spectrophotometric determination be made in duplicate with different volumes of the neutralised alkaline extract.

Non-treated citrus fruit give by this method a "blank" reading of up to 0.5 ppm for oranges and 0.8 ppm for lemons. (1) See figure on page 180.

CLEVENGER

(Annex III, Chapter 4;

Annex IV, Chapter 4)

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