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DETERMINATION OF ETHER-SOLUBLE FOOD PRESERVATIVES AFTER DIRECT EXTRACTION ON PAPER

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SUMMARY

A study was made of the determination of food preservatives by direct extraction and isolation on chromatographic paper. Recovery experiments from fruit juice (sorbic and benzoic acids) and from fish (benzoic acid and *p*-hydroxybenzoates) were carried out.

INTRODUCTION

In a previous paper[1], we described the ease with which ether-soluble food preservatives could be isolated from food material directly on to chromatographic paper. This method, with minor modifications, is also applicable to quantitative determinations. The procedure can be further simplified by making use of the differences in pH between the preservative compounds. A single run with diethyl ether as solvent is often adequate; two papers are prepared in case both benzoic and sorbic acids are present.

The acids are halted by an arresting zone of bicarbonate, and then *p*-hydroxybenzoates are arrested by a line of sodium hydroxide. Fats migrate up into the solvent front. The preservatives are thus isolated as narrow bands at the arresting zones, located with ultraviolet light, cut off and eluted for photometric or colorimetric measurement.

It is also practicable to extract *p*-hydroxybenzoates, and simultaneously to separate them from each other in a single run, with diethyl ether as solvent, on a paper impregnated with sodium hydroxide.

EXPERIMENTAL

Apparatus and materials

Extraction vessel. This consists of the lower part of a desiccator, covered with glass plates. A dish filled with diethyl ether is placed in the chamber at a suitable height.

Bromination chamber. A shallow dish (height about 4 cm) covered with glass plates is used.

UV lamp. A UV lamp with a radiation maximum at 254 nm is used.

Spectrophotometer. A UV/visible spectrophotometer is used.

Paper strips. Whatman 3 MM paper, or a corresponding thick paper, is cut into

strips of 5 by 20 cm, or possibly longer. These strips are washed with diethyl ether by adsorbing the solvent through a pile of strips in a closed container.

Weighing tubes. Glass tubes about 12 cm long and 2–3 mm I.D., depending on the consistency of the material to be analysed, are used. Both ends are open, and one end is narrowed by melting it in a flame.

Test-tubes. The test-tubes have a ground female joint and plastic stoppers.

Reagents. The following reagents are used: *Pro analysi* grade diethyl ether; buffer solution (pH 6.5) consisting of a mixture of monopotassium and disodium phosphates, according to SOERENSEN[2]; ethanol (spectroscopic quality) acidified with 1 N sulphuric acid (9:1); a saturated sodium bicarbonate solution; 2 N and 0.5 N aqueous solutions of sodium hydroxide; acetone mixed with 0.5 N sodium hydroxide solution (2:1) immediately before use.

Extraction

The food sample, either liquid or solid, is mixed with water (usually equal amounts of water and food material are mixed) and well homogenized. About 200 mg of homogenate is measured into a weighing tube. The tube is weighed on an analytical balance and the contents are streaked across a paper strip as a broad band or as a number of parallel transverse bands without any intermediate drying. The empty tube is re-weighed. Some fatty foods, such as margarine, may be streaked as such on the paper with a spatula.

Subsequently, three lines of solution are drawn in succession across the paper at intervals of about 2 cm; the first of these is a buffer solution (pH 6.5), the second saturated sodium bicarbonate solution and the third 2 N sodium hydroxide solution (see Fig. 1). The sample zone is then acidified by adsorbing 1 N hydrochloric acid through the lower edge of the paper, until the acid has moistened the sample zone. Neither the sample nor the arresting lines should be allowed to become too dry, as moisture is necessary for extraction. Thus, after a short equilibrating period (2 or 3 min), the strip is pressed between the edges of the cover plates of the extraction vessel, so that the sodium hydroxide zone is located below the cover plates, and the lower end of the strip reaches into the dish containing diethyl ether. The solvent is adsorbed through the paper and evaporates on the slit. Ether-soluble organic acids are concentrated at the lower edge of the bicarbonate line, and *p*-hydroxybenzoates similarly on the sodium hydroxide line. Fats and oils are transported up to the evaporation front between the glass plates. The extraction is completed in about 30 min. Up to 1 h may be required for foods with a high fat content.

When identification and isolation of the individual *p*-hydroxybenzoates is necessary, the paper is impregnated with an alkaline acetone solution. After the food has been applied to the paper and the sample zone is acidified, the strip is dipped into the freshly prepared impregnating solution. However, the starting line, which contains the sample, is not impregnated. The impregnated area begins 1–2 cm above the starting line.

The time of equilibration of the paper depends on the fat content of the sample. If this content is low or moderate, the paper is transferred into the extraction vessel 5–7 min after impregnation. For fatty foods, the waiting period is even shorter (1–3 min). The migration of the esters is slower on wet paper, which facilitates the separation from the fats.

The paper strip is lowered into the extraction vessel more deeply than in the extraction procedure described above. The paper is immersed about 15 cm or more into the diethyl ether at the bottom of the vessel so as to ensure that there is a sufficient length of paper for the separation of the esters. The fats migrate with diethyl ether up to the evaporation front and acids are arrested at the low border of the alkaline region. The esters are separated from each other as distinct lines on the impregnated area, so that those with a shorter side-chain migrate more slowly. Detection is effected by means of short-wave ultraviolet illumination.
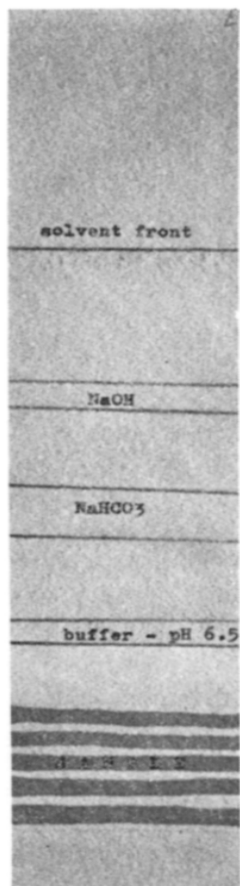

Fig. 1. Chromatographic paper with zones of different solutions.

Elution

The separated preservatives are outlined under short-wave ultraviolet illumination, and the dark lines marked are cut off. The cut-off lines must be as narrow as possible so as to avoid contamination. The cuttings are transferred into stoppered test-tubes containing 20 ml of acidified ethanol (for benzoic acid and *p*-hydroxybenzoates) or distilled water (for sorbic acid). During the elution, which is completed within about half an hour, the tubes are shaken occasionally.

Spectrophotometric determination

The concentration of the aromatic compounds used as preservatives can be measured directly from the eluate in the ultraviolet region. Sorbic acid also has an absorption maximum in the ultraviolet region, but there is less interference with a colorimetric determination.

Benzoic acid. FORD[3] derived an equation for the elimination of interference in the spectrophotometric determination of benzoic acid. This equation was derived from a general equation proposed by MORTON AND STUBBS[4], and expanded by ALLEN[5]. FORD's equation was based on the maximum absorption of benzoic acid at 230 nm. However, at the acidity of the eluate used in the present study, the maximum absorption is found to be 228 nm, and the equation was accordingly modified to

$$E_{228}^{corr} = E_{228} - (0.6\,E_{218} + 0.4\,E_{243}) \qquad \cdots \qquad (1)$$

where E_{218}, E_{228} and E_{243} represent the absorbances observed at wavelengths of 218, 228 and 243 nm, respectively, and E_{228}^{corr} is the corrected absorbance at 228 nm.

p-Hydroxybenzoates. The maximum absorbance at 260 nm is corrected by analogy with the formula used for benzoic acid. When 230 and 280 nm are chosen as the bases for correction, the equation becomes

$$E_{260}^{corr} = E_{260} - (0.4\,E_{230} + 0.6\,E_{280}) \qquad (2)$$

Colorimetric determination of sorbic acid

The reaction with thiobarbituric acid is used for the determination of sorbic acid in aqueous eluates at concentrations of 1–8 μg/ml. When about 200 mg of 1:1 diluted food material is extracted and the aqueous eluate is made up to 20 ml with distilled water, in practice the content of sorbic acid usually falls within this range. The UV absorption provides a basis for the evaluation of the approximate concentration, and hence the appropriate dilution to be used.

A 2-ml volume of the eluate is measured into a reaction tube, in which the colorimetric reaction is carried out by application of the method of SCHMIDT[6]. The presence of benzoic acid does not influence the reaction to any appreciable extent.

Recovery tests

Samples of blackcurrant and orange juice, with sugar contents of about 10 %, were used to study the recovery from liquid foods. Either sorbic or benzoic acid alone (0.12 %), or both acids together (0.06 % of each) were added to the juice. About 200 mg of water-diluted (1:1) juice was used for each determination.

Herring was used as a representative of solid foods. An equal weight of water was added to the fish, and the mixture was homogenised. Benzoic acid was added at two concentrations, 0.05 and 0.1 %, calculated on the basis of the diluted homogenate. Fish material free from benzoic acid was used to determine the background interference. The amounts of fish homogenate applied to an extraction strip ranged from 100 to 200 mg.

RESULTS AND DISCUSSION

Benzoic acid

The purity of the benzoic acid isolated from the eluate was studied with a recording spectrophotometer. With fish, the biological material caused a high background absorption in the zero-concentration experiments. In a series of five experiments with 10 ml of eluate and 100 mg of homogenate, absorbances of between 0.050 and 0.103 were found. Without the introduction of a correction factor, this would give a positive error of 10–20 % with the concentrations of benzoic acid that are commonly used for preservation purposes. Nevertheless, the interference was completely eliminated by the correction calculation based on the absorbances at 218, 228 and 243 nm. The correction resulted in slight overcompensation, the resulting absorbances being between − 0.004 and −0.007.

The recovery from fruit juice also exceeded 100 % if the results were uncorrected, which is an indication of some impurity in the eluate. A correction was found to be particularly necessary when bromination was carried out. The recoveries listed in Table I have been corrected in accordance with eqn. 1. However, in the routine analysis of fruit juice, time-consuming calculations are unnecessary, except when bromination is carried out. A simple correction factor, based on the average recovery, should be adequate. With an unfamiliar material, the purity of the isolated material should be determined by studying the spectrum. A recording spectrophotometer is therefore preferable.

TABLE I

RECOVERY OF BENZOIC ACID

Material analysed	Number of experiments	Mean recovery (%)	Relative standard deviation	Extreme values of recovery (%)
Aqueous solution, 0.1%	10	100.6	1.5	97.8–102.6
Orange juice, 0.12%	18	95.9	4.1	88.6–104.2
Orange juice, brominated[a], 0.06%	19	91.6	9.1	79.3–103.9
Blackcurrant juice, 0.12%	17	98.4	4.0	92.6–108.4
Blackcurrant juice, brominated[a], 0.06%	13	89.1	6.4	80.0–101.3
Fish, 0.05%	10	92.6	3.0	87.4–96.8
Fish, 0.1%	10	96.1	1.4	93.6–97.6

[a] The brominated juices also contained sorbic acid (0.06%).

Sorbic acid

The recovery of sorbic acid is considerably greater than that achieved by conventional paper chromatography, in which the losses often amount to 30–40%, probably owing to oxidation. During a rapid extraction on paper with diethyl ether, the recovery approaches 100%. Typical results are given in Table II.

It should be noted that sorbic acid is relatively unstable and may suffer losses in the food material and in the diluted reference solutions. At room temperature, changes may occur rapidly and recovery experiments should therefore be carried

TABLE II

RECOVERY OF SORBIC ACID

Material analysed	Number of experiments	Mean recovery (%)	Relative standard deviation	Extreme values of recovery (%)
Aqueous solution, 0.1%	12	99.1	1.5	95.6–101.2
Aqueous solution[a], 0.1%	11	98.9	1.9	97.2–102.0
Orange juice[a], 0.06%	6	99.1	1.0	97.8–100.8
Blackcurrant juice[a], 0.06%	6	99.0	1.0	97.5–100.3

[a] Benzoic acid also present.

out with freshly prepared material, and if the samples are stored overnight, they should be kept cool.

In old fish semi-preserves, the oxidation products of fats resulted in a high background. This was identifiable in the spectrum of the colour produced by the thiobarbituric acid reaction, and could consequently be deducted from the extinction. Benzoic acid did not interfere with the colour reaction.

p-Hydroxybenzoates

The biological material causes considerable interference. As with benzoic acid, the correction with eqn. 2 leads to overcompensation, and consequently the results obtained are too low. The procedure of calculation does not decrease the variation between the recovery values from the individual determinations, as compared with the uncorrected results. It might therefore be more practical in routine analysis to apply a simple correction factor based on the mean recovery.

All the analyses reported in Table III were carried out on impregnated paper, so that some loss attributable to hydrolysis might occur during the migration on the alkaline paper. If a study of the nature of the ester is unnecessary, direct elution from the sodium hydroxide line is adequate, as is explained above in the section on extraction.

TABLE III

RECOVERY OF *p*-HYDROXYBENZOATES

Material analysed	Number of experiments	Mean recovery (%)	Relative standard deviation	Extreme values of recovery (%)
Methyl ester				
Ethanol solution, 0.1%	10	93.3	5.6	81.2–98.0
Herring, 0.1%	12	89.9	6.0	79.2–98.0
Herring[a], 0.05%	14	93.5	6.9	76.0–100.4
Propyl ester				
Ethanol solution, 0.1%	10	92.8	3.9	85.8–97.2
Herring, 0.1%	14	89.1	9.5	72.8–106.4
Herring[a], 0.05%	13	86.4	8.3	78.0–105.2

[a] Both methyl and propyl esters present together.

CONCLUSIONS

The method outlined seems to be applicable to the analysis of preservatives in almost any type of food or biological material. In practical analyses, direct extraction on paper has provided reproducible results with a number of different materials, such as berries, fruit and berry jams and juices, margarine, fish semi-preserves, mustard preparations and food additive mixtures. Slight modifications are sometimes necessary, depending on the type of material. The results of extraction on paper are comparable with those of extraction in glass funnels.

The main disadvantage of the method compared with extraction in glass funnels is the smaller size of sample. Measurement of the small sample on an analytical balance is sufficiently accurate, and the size is adequate for the determination of the concentrations of preservatives found in foods in practice, although the homogeneity of the sample might introduce a problem. In particular, solid or semi-solid materials must be homogenised very carefully. However, the extra time that this may require will be well compensated for by the greater speed of analysis.

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