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Gas chromatographic determination of preservatives in food

The determination of preservatives in food involves the extraction of the preservative from the food. Two methods of isolation are generally used, depending on the properties of the preservative, *viz.* steam distillation, where the preservative is separated from the food sample and then extracted from the distillate by an organic solvent[1–3], and direct extraction of the preservative from the food sample by an organic solvent[4–9].

The procedures used for the subsequent determination of the preservatives vary from spectrophotometric methods in the visible, UV and IR regions to thin-layer and gas chromatographic methods. As the gas chromatographic methods[3,7,9,10] seem to be the most practical, we have used these methods for some years. Here again we must differentiate between injection of the preservative without any modification on to polar columns[8,9] and injection after conversion into an ether or an ester[4,6,11,12]. We have tried injecting the free acids on to polar columns[3], as NISHIMOTO[7] did, but found the method rather difficult because the standard curves varied with time, as was also reported by NOBE AND STANLEY[8]. For that reason we switched over to conversion to silyl ethers[10].

Experimental

Preparation of samples. For the preparation of samples of marmalades, mustard, mayonnaise, sardines in oil, canned tunny and margarine 10 g of the food and 60 g sand were thoroughly mixed in an erlenmeyer flask. After the addition of 3 ml 25% H_2SO_4, the mixture was extracted three times with 30 ml ether by agitating the ground-glass stoppered flask vigorously for at least 1 min. The preservatives were then extracted from the ether by two extractions with 20 ml 0.1 *N* NaOH. (After each NaOH addition 10 ml saturated NaCl solution were added.) The aqueous layer was neutralised with HCl (1:3) to the end point of the Methyl Red indicator. The preservatives were then extracted from the acid aqueous phase with one 100-ml and four 50-ml portions of chloroform. (After the first addition of chloroform another 10 ml saturated NaCl solution were added.) The extracts thus obtained were passed through a funnel containing anhydrous Na_2SO_4. A washing procedure as described below for beer and wine can also be recommended. After concentration of the combined chloroform extracts in a Kuderna–Danish evaporator on a water-bath to approximately 5 ml, the lower receiving tube of the Kuderna–Danish evaporator was directly heated on a water-bath at 60° and concentrated under a current of air to approximately 1 ml. One millilitre of internal standard (4 mg/ml methyl gallate in pyridine) was then added. The 2 ml were concentrated to approximately 1 ml and 0.2 ml *N,O*-bis(trimethylsilyl)acetamide (BSA) was added. This mixture was heated under reflux for 15 min at 60°. The solutions were then ready for gas chromatographic analysis.

For the preparation of beer and wine samples 3 ml 25% H_2SO_4 were added to 20 ml beer or wine and the mixture was extracted three times with 30 ml ether by agitating the stoppered flask vigorously for at least 1 min. The ethereal layer was then washed with 50 ml water. After the washing step the preservatives were extracted as described above.

Silanisation. The silanisation methods tested were: (a) the method of SWEELEY *et al.*[13] — hexamethyldisilazane and trimethylchlorosilane; (b) the method of BROBST AND LOTT[14] — hexamethyldisilazane and trifluoroacetic acid; (c) trimethylsilylimidazole; and (d) N,O-bis(trimethylsilyl)acetamide (BSA)[15,16]. Some difficulties were experienced owing to the amount of water in the final extract. Therefore, under the conditions used, the best silanisation reagents were those described in the method of BROBST AND LOTT[14] and BSA. Trifluoroacetic acid also causes trouble as it corrodes the detector electrodes. With some detectors, for a fortnight after sample injection the electrodes were completely corroded. The best results were obtained with BSA.

The most attractive points of the silanisation process were: (a) the ease with which derivatives of all the preservatives examined (benzoic and sorbic acid and the propyl, ethyl and methyl esters of *p*-hydroxybenzoic acid) were obtained; (b) the very reproducible standard curves obtained day after day; and (c) the high stability of the silyl ethers (the same solution injected the following day giving nearly the same results).

Isolation of the preservatives. The esters of *p*-hydroxybenzoic acid are not very steam volatile, so the steam distillation method can be used only for the quantitative isolation of sorbic and benzoic acid (Fig. 1). Fig. 1 shows that the amount of ester left in the residue in the flask is nearly equal to the amount in the distillate. On the other hand, several methods based on extraction with an organic solvent[4-9] give good results on some kinds of food but very bad recoveries on other kinds of food. The extraction with ether as proposed by GROEBEL[6] is indeed satisfactory for fatty food, but not for food such as marmalade. Another problem is caused by the extraction of the preservatives from the ether solution with 1 *N* NaOH solution. This concentration of NaOH causes hydrolysis of the esters, especially the propyl and ethyl esters of *p*-hydroxybenzoic acid. This was easily demonstrated by the supplementary peak

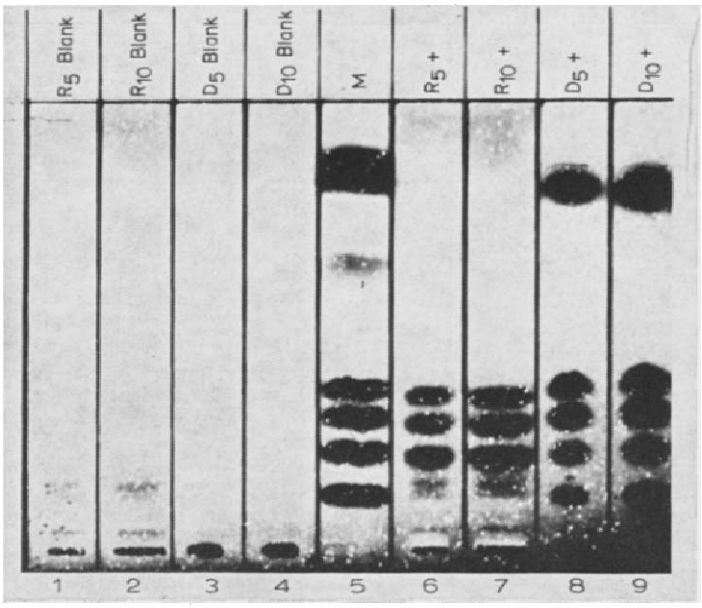

Fig. 1. Thin-layer chromatogram of a mixture of sorbic and benzoic acid and esters of *p*-hydroxy benzoic acid. D_5 and D_{10} refer to 5 and 10 µl of the distillate, and R_5 and R_{10} to 5 and 10 µl of the residue, respectively. + = Sample spiked with mixture M, a mixture of nine preservatives added to the sample before steam distillation.

of *p*-hydroxybenzoic acid, and the diminishing peak area of the propyl and ethyl esters.

Gas chromatography. A 1-ml standard solution containing 0.5, 2, 4, 6 and 8 mg/ml of benzoic and sorbic acid and the propyl, ethyl and methyl esters of *p*-hydroxybenzoic acid, respectively, was prepared, to which 1 ml of internal standard was added. This mixture was subjected to gas chromatography (Fig. 2).

Fig. 3 shows a standard curve for sorbic acid from which it is possible to read the amount of preservative in 10 g of sample directly.

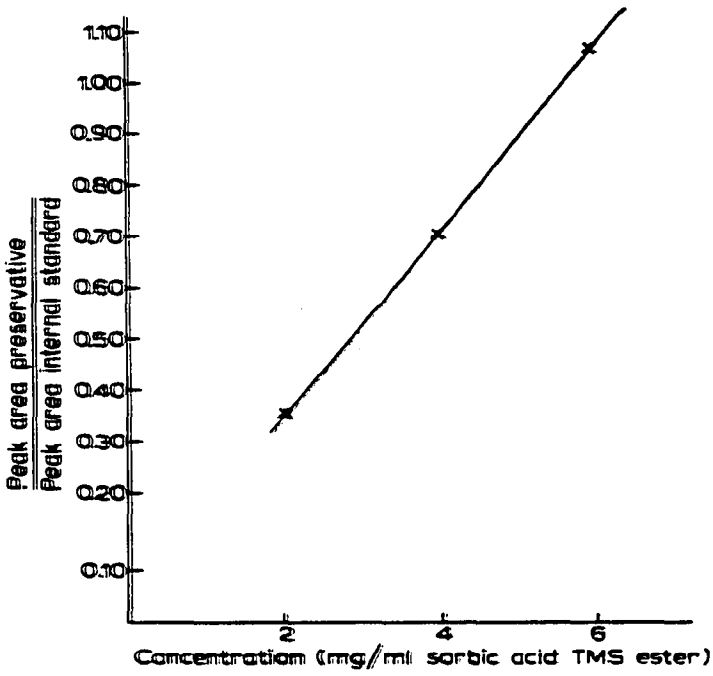

Fig. 2. Gas chromatogram of the TMS derivatives of five preservatives. Column: 3 m 3% SE-30 on 100–120-mesh Aeropak. Temperature programmed from 90° to 290° at a rate of 8°/min. Detector and injector temperatures: 300° and 290°, respectively. Carrier gas: nitrogen, flow-rate approximately 30 ml/min. FID detector. Quantitation with a digital integrator. 1 = Sorbic acid; 2 = benzoic acid; 3 = methyl, 4 = ethyl and 5 = propyl ester of *p*-hydroxybenzoic acid; 6 = internal standard (the TMS derivative of methyl gallate).

Fig. 3. Standard curve for sorbic acid.

Results

The recoveries for the preservatives in the samples tested were very good, *i.e.* at least 85 %, except for the propyl and ethyl esters of *p*-hydroxybenzoic acid in mustard, which gave lower recoveries (57 and 76%, respectively). Even the addition of 80 or 100 g of sand did not increase the recovery. The best results were in general obtained with a sample of 10 g, 60 g sand and three extractions with 30 ml ether. Without the addition of sand, samples such as marmalade gave very low recoveries because of the bad penetration of the solvent in the sample.

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