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Note

Gas chromatographic determination of preservatives in rennet

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Sorbic acid and benzoic acid are frequently added to food products as preservatives to prevent the development of moulds and bacteria. We have tried to develop a rapid and accurate procedure for evaluating these two preservatives in rennet, using gas chromatography. Already published methods relied either on direct injection[1–5] or indirect injection, either after silylation[6] or methylation with methanol–hydrochloric acid[7], methanol–boron trifluoride[8] or diazomethane[9–11]. We found that the best results were obtained by conversion into methyl esters with diazomethane, and by adding an internal standard (undecanoic acid)[1].

Prior to the gas chromatographic analysis, the acids were isolated by extraction of the rennet sample with diethyl ether in acidic media.

EXPERIMENTAL

Apparatus

A gas chromatograph (Fractovap 2350, Carlo Erba, Milan, Italy) with a flame-ionization detector was used. The carrier gas was nitrogen at a flow-rate of 30 ml/min. Glass columns (2 m × 2 mm I.D.) were packed with 15% EGA coated on 80–100-mesh Chromosorb W AW DMCS. The column temperature was 130° and the injector temperature 250°. A Hewlett-Packard 3380A recorder–integrator was employed.

Reagents

Analytical-reagent grade benzoic, sorbic and undecanoic acid (Fluka, Buchs, Switzerland) were used as standards. Standard solutions of 1 g/l of benzoic and sorbic acid in diethyl ether were prepared. The internal standard solution was a 1 g/l solution of undecanoic acid in diethyl ether.

Analytical-reagent grade methanol and diethyl ether were employed, and analytical-reagent grade concentrated hydrochloric acid was used to prepare a 0.1 *N* solution.

The methylation reagent (diazomethane) was prepared by adding to 1 g of N-nitrosotoluene-4-sulphomethylamide (Fluka) 30 ml of diethyl ether, 6 ml of methanol and 10 ml of 6% potassium hydroxide solution. The supernatant fraction was introduced into a twonecked flask from which diazomethane was transferred into a second flask using nitrogen as carrier gas.

Preparation of methylated standards

To 1 ml each of the benzoic and sorbic acid solutions are added 1 ml of the undecanoic acid internal standard solution and a mixture of 0.3 ml of methanol and 1 ml of diethyl ether. Methylation is then effected by treatment with gaseous diazomethane until the development of a yellow colour. The final volume is made up to 10 ml with diethyl ether. A 1-μl volume of the solution corresponds to 100-ng aliquots of benzoic, sorbic and undecanoic acid.

Isolation and methylation of the preservatives

To 0.5 ml of rennet in a ground-glass stoppered test-tube are added 2 ml of 0.1 *N* hydrochloric acid and the mixture is extracted three times with 5 ml of diethyl ether with vigorous agitation. The ethereal layers are concentrated to approximately 3 ml by heating at 30° under a flow of nitrogen. A 1-ml aliquot of the undecanoic acid internal standard solution and 0.3 ml of methanol are added. Methylation of the sample is effected as described for the standards, followed by injection of 1 μl into the gas chromatograph.

RESULTS AND DISCUSSION

Peak areas were calculated for benzoic acid and sorbic acid, and the relative response for each preservative was compared with that of the internal standard (undecanoic acid) by means of the integrator.

Fig. 1 illustrates the good separation of the methylated acids, with retention

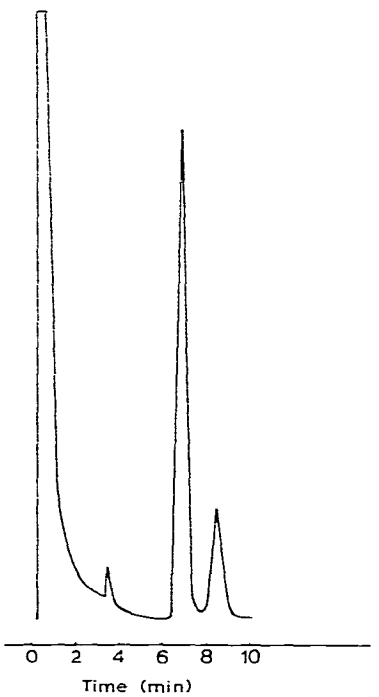

Fig. 1. Gas chromatogram of methyl esters of sorbic acid (retention time 3.52 min), benzoic acid (6.99 min) and undecanoic acid (8.56 min). Each peak is equivalent to 100 ng.

times of 3.52 min for sorbic acid, 6.99 min for benzoic acid and 8.56 min for undecanoic acid.

The smallest amount of preservative detectable is 20 ng/μl in the actual extract, equivalent to 400 mg/l in the rennet sample. The recovery was checked by extraction of known amounts of preservatives from aqueous solutions according to the procedure previously described, followed by UV spectrometry. It was found to be above 95% for concentrations as low as 20 mg/l.

Hence this method permits the specific and rapid (about 30 min) quantitative determination of benzoic and sorbic acid in rennet, with very satisfactory recoveries.

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