A New Benzene-Ethanol-Water Solvent System for TLC Separation of Aflatoxins

ABSTRACT

Gas liquid chromatography was used to determine the composition of the benzene-ethanol-water (BEW) solvent system frequently used in the separation of aflatoxins by thin layer chromatography. Investigation of a rapid procedure for preparing and using this solvent system led to the discovery of a new BEW solvent system with advantages for the routine determination of aflatoxins in roasted nut products, which consists of benzene-ethanol-water 40:6:3 for the trough solution and 4:27:20 for the bottom solution.

INTRODUCTION

The benzene-ethanol-water (BEW) solvent system first became part of the official procedure for the analysis of aflatoxin in nut products in 1966 (1) as a means for confirming the amount and identity of the green fluorescent G_1 and G_2 aflatoxins. It was to be used in place of the general solvent system of chloroform-acetone 9:1 whenever the proportions of G_1 and G_2 aflatoxins were found (singly or in combination) to be greater than 20% of the total aflatoxins. This rule remains in effect with the three current official procedures (2). However the preparation time and the cost of this solvent system has inhibited its application. Yet the distinct advantage of transporting background interferences on the thin layer chromatographic (TLC) plates away from the region of the aflatoxins would benefit routine analyses of roasted peanut products, which invariably posses compounds causing background interferences on the TLC plate.

EXPERIMENTAL PROCEDURES AND DISCUSSION

As directed in the official procedure, a mixture of benzene-ethanol-water 46:35:19 v/v was shaken in a separatory funnel and allowed to stand overnight at room temperature (not greater than 22 C). The upper and lower phases were carefully separated and their volumes carefully measured.

A 0.5 μl sample of an equal mixture by weight of the upper phase and methyl isobutyl ketone (MIBK) was injected into an F&M, Model 700 gas chromatograph equipped with a dual flame detector and matched columns under the following operating conditions: temperature–injection port 180 C, detector 200 C, column 70 C; column composition–6 ft x 1/8 in., 10% SE 30 on Diatoport S. The MIBK acted as a diluent as well as an internal standard. Triplicate analyses were made of this preparation, and area measurements were obtained with a compensating polar planimeter. Response factors were determined on prepared standards by separately relating the ratio of MIBK to both benzene and ethanol. Using concentrations that closely bracketed the components of the sample and the response factors, the benzene and ethanol contents (by volume) shown in Table I were calculated. The water content was obtained by difference.

A second BEW preparation was made with the same component proportions but was allowed to separate for only 1 hr. The upper phase was analyzed and found not to differ significantly in composition from that prepared by equilibration overnight.

Both preparations were repeated, and reasonable duplication of the values was obtained. These values are shown in Table I along with the calculated compositions of the bottom phases.

TLC plates were prepared using Adsorbosil No. 1 and spotted with extracts, obtained by the BF Method (3), of a contaminated raw peanut sample, a roasted peanut product and a mixed aflatoxin standard. One pair of plates was developed as described in the official procedure with the BEW solvent system prepared by overnight equilibration. A second pair of plates was developed with the solvent system obtained by the 1 hr equilibration. Resolution and clarity of the aflatoxins were identical, indicating that a 1 hr equilibration of the BEW solvent system was sufficient, as well as confirming the like composition shown for the 1 hr and overnight preparations by the gas liquid chromatographic (GLC) results.

Finally, a BEW solvent system was made by merely combining in separate Ehrlenmeyer flasks the individual

TABLE I

Composition of Benzene-Ethanol-Water Solvent System

Equilibration time		Top layer[a]			Bottom layer[a]		
		Benzene	Ethanol	Water	Benzene	Ethanol	Water
Overnight	1st set	40.6	6.6	1.8	5.4	28.4	17.2
1 hr	1st set	40.7	6.3	2.0	5.3	28.7	17.0
Overnight	2nd set	41.6	6.9	0.5	4.4	28.1	18.5
1 hr	2nd set	41.6	6.9	0.5	4.4	28.1	18.5
Average		41.1	6.7	1.2	4.9	28.3	18.0
Direct preparation composition		41	7	1	5	28	18
Recommended improved composition		40	6	3	4	27	20

[a] All data are on % v/v basis.

components in the proportions determined by the GLC study for the top (benzene-ethanol-water 41:7:1) and the bottom (5:28:18) phases of the BEW system. The flasks were shaken and the solvent systems used immediately for the development of another set of plates. No difference could be detected in the resolution or clarity of the aflatoxins on the plates prepared using this instant BEW preparation from that obtained with BEW systems using overnight equilibration.

Because ethanol is the most costly reagent in this solvent system and the bottom layer contains four times as much ethanol as the top layer, it is worthwhile to use the bottom layer repeatedly. This is possible because the bottom layer of solvent is in the bottom of the developing tank and does not come in contact with the TLC plate. Thereby the cost of using the BEW system is substantially reduced.

The instantly prepared BEW system containing benzene-ethanol-water 41:7:1 in the top layer and 5:28:18 in the bottom layer was used in our laboratory almost exclusively for over a year with repeatedly excellent resolution. The bottom layer (50 ml) was not replaced or supplemented during an entire week's operation, which ranged between 5 and 20 plates, while 25 ml of the top layer was pipetted into a clean trough for each run. However, after various laboratories around the country that had been asked to evaluate the system reported mixed success, apparently related to climatic conditions, a study was made to determine the effect of minor moisture differences on the system. Separate TLC plates were prepared using solutions containing the proportions indicated in Table I for set 1 and set 2, as well as the top layer of set 1 with the bottom layer of set 2, and the top layer of set 2 with the bottom layer of set 1. Improved separation of the aflatoxins was obtained in proportion to the amount of water in the system, with the best plate being the one prepared with the top layer of set 1 and the bottom layer of set 2. Additional plates were prepared varying the component proportions of the solution in the indicated direction until the optimal resolution, size and position of the aflatoxins on the plate were established. This system is composed of solutions of 40:6:3 for the top layer or trough solution and 4:27:20 for the bottom layer (% v/v benzene-ethanol-water, respectively). While the top layer preparation had a cloudy appearance after shaking and frequently separated into two phases with time, no adverse effect was found if the solution was thoroughly shaken just prior to use.

Evaluation of this system by six laboratories over the past year has shown it to be consistently superior to the previous BEW solvent system and less affected by climatic conditions. Optimum results are obtained by placement of the developing chamber in a constant temperature cabinet at 68-70 F.

ARTHUR E. WALTKING
GEORGE W. BLEFFERT
MARSHALL CHICK
ROSEMARY FOGERTY
Best Foods Research Center
Union, New Jersey 07083

REFERENCES

1. J. Ass. Off. Anal. Chem. 49:231 (1966).
2. "Official Methods of Analysis of the AOAC," 11th Edition, Ass. Off. Anal. Chem., Washington, D.C., 1970, p. 429.
3. Waltking, A.E., J. Ass. Off. Anal. Chem. 53:104 (1970).

[Received June 13, 1973]

Ethoxylated Glycerol and Propylene Glycol Glycoside Palmitates from Lactose

ABSTRACT

Polyoxyethylene polyol glycoside palmitates were prepared by the following successive reactions: transglycosylation of lactose by glycerol and propylene glycol to yield crude mixtures of the polyol glucosides and galactosides; alkoxylation with ethylene oxide; and tranesterfication by methyl palmitate. Almost all the solid waxy products exhibited low surface and interfacial tensions and good emulsion stability; they are expected to be effective food emulsifiers, cosmetic surfactants and biodegradable industrial surfactants.

Lactose is a crystalline sugar isolated from cheese whey (1). The potential supply of this sugar is estimated to be more than a billion pounds annually, based on the availability of whey solids in 1968 (2). Recovery and fractionation of whey are among the more encouraging proposals for the solution of pollution problems associated with whey disposal (3). An aspect of this approach would be the development of new industrial uses for lactose.

Lactose is a disaccharide composed of D-glucose and D-galactose in 1,4-linkage and certain of its derivatives, like those of glucose, (4-6) may have potential for use as food additives. Polyoxyethylene ethers derived from carbohydrates and long chain fatty acids, such as sorbitan polyoxyethylene monostearate, have been approved by the FDA for addition to food products in small amounts. Presumedly polyoxyethylene ethers of glycosides prepared from lactose and glycerol or propylene glycol would also be of interest for similar addition to foods. Proof of nontoxicity would be required, however, for FDA approval of such products for food additive use. We therefore prepared a series of polyoxyethylene glycerol and propylene glycol glycoside palmitates from lactose as the base sugar. Lactose was reacted separately with glycerol and propylene glycol by transglycosylation to yield predominantly mixtures of the corresponding glucosides and galactosides, as shown below: