THE DETECTION OF POLYOXYETHYLENE GLYCOL IN BAKERY PRODUCTS[1]

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ABSTRACT

Polyoxyethylene (8) monostearate (POEMS) was separated from bread by chloroform extraction of acid-treated bread crumbs. The chloroform was passed through an alumina column which retained the POEMS, lipids, and other related substances. The polyoxethylene glycol portion of POEMS was eluted from the column with ethyl alcohol and determined semiquantitatively by standard ascending paper chromatography. This method overcame the false positive results obtained on bread containing a compound emulsifier when analyzed by the colorimetric method (Garrison *et al.* J. Assoc. Offic. Agr. Chemists **40:** 1085; 1957).

Polyoxyethylene (8) monostearate (POEMS) has been recommended for use in bakery products to increase softness and enhance palatability. A quantitative colorimetric procedure for the determination of POEMS in baked goods has been developed by Garrison *et al.* (3). However, this method has been found to give a false positive for products containing a compound emulsifier with a high percentage of lecithin. Therefore an investigation was initiated to develop a satisfactory procedure which would be applicable to all bakery products.

Polyoxyethylene (8) monostearate was shown by Birkmeier and Brandner (1) to be a mixture of polyoxyethylene glycol monostearate, polyoxyethylene distearate, and "free" polyoxyethylene glycol. This latter accounts for 15.8% by weight of POEMS on the dry, ash-free, free fatty-acid-free basis.

A method was developed for the extraction of the "free" polyoxyethylene glycol portion of POEMS in bakery products. The polyoxyethylene glycol was detected chromatographically by a modification of the method of Kliffmüller (4).

Materials

All chemicals were reagent grade unless noted.

1. Hydrochloric acid, 6*N*.
2. Alumina: Adsorption 80- to 200-mesh, from Brickman, Montreal, Canada.

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3. Development solvent: 200 ml. of butanol, 100 ml. of ammonium hydroxide (1 + 10), and 48 ml. of absolute alcohol.
4. Chromogenic agent: Modified Dragendorf Reagent: 850 mg. of bismuth subnitrate dissolved in 50 ml. of acetic acid (20%), mixed with 8 g. of potassium iodide in 20 ml. of water, filtered, and kept in the dark. Diluted 1:12 with acetic acid (20%) before use.
5. Standard ascending chromatographic equipment with Whatman 3MM paper.
6. Polyoxyethylene (8) monostearate: Commercial grade. A portion was separated into its polyoxyethylene glycol fraction and its ester fraction by the method of Birkmeier and Brandner (1).

Three samples of bread were baked in this laboratory: one did not contain POEMS and served as a blank; the other two were baked with 0.09% and 0.26% POEMS respectively on a dry basis. Buns from bakery X were baked with 0.38% POEMS on a dry basis. Bread from bakery Y was baked with a commercial emulsifier composed of flour, lecithin emulsifier, and a monoglyceride: glycerol mono-oleo-stearate, 40 – 45% in an animal oil base. Bread from bakery Z was baked with an unknown amount of POEMS. A commercial mixture of mono- and diglycerides was added at the 0.6% level to a portion of the blank sample of laboratory-baked bread.

Method

Extraction. Fresh samples of bread were air-dried and then crushed to pass through an 80-mesh sieve. A sample of 10 g. was heated with 25 ml. of hydrochloric acid (6*N*) on a water bath for 45 minutes, with occasional swirling. After cooling, 25 ml. of water were added and the mixture was filtered. The filtrate was shaken twice with 25 ml. of chloroform; if an emulsion formed it was broken by centrifuging.

Purification of the Extract. An alumina adsorption column was prepared by filling with chloroform a glass column, about 32 mm. in diameter, fitted with a sintered glass disk, and sifting in the adsorbant to a height of 40 mm. A disk of Whatman 41H filter paper was placed on top of the alumina.

The chloroform extract of the hydrolyzed crumbs was passed through the alumina, followed by 100 ml. of ethyl ether. The polyoxyethylene glycol was eluted with 25 ml. of 95% ethyl alcohol. This extract was taken to dryness on a steam bath, and the residue dissolved in 1 ml. of ethyl alcohol.

A strip of Whatman 3MM paper measuring 9 by 8 in. was prepared by drawing a starting line 1 in. from one end. The paper was

dipped in the development solvent, and after air-drying, 100-λ aliquots of the ethyl alcohol solution of the extracts were spotted at 2-in. intervals along the starting line. When the spots had dried, the two edges of the paper were stapled together.

Chromatographic Development. A tank, 10 in. high by 9½ in. in diameter, was equilibrated with development solvent for 18 hours and then the chromatogram developed for 5 hours by standard ascending chromatography. After drying in air, the chromatogram was sprayed with the chromogenic agent.

Results and Discussion

Previously it was found that a hot chloroform extraction of the untreated crumbs did not remove the polyoxyethylene glycol. With a preliminary acid treatment, extraction of the "free" polyoxyethylene glycol was readily accomplished. Comparison of the relative size and intensity of the spots given by known amounts of polyoxyethylene glycol with those given by the bread samples showed that this acid treatment did not cause hydrolysis of the polyoxyethylene glycol esters.

In passing the chloroform extract after acid hydrolysis through alumina, fatty acids and other interfering substances, as well as polyoxyethylene glycol, were adsorbed. The ether acted mainly as a drying agent. The volume of ethyl alcohol eluent (25 ml.) was the maximum amount which would allow for complete extraction of the polyoxyethylene glycol without removal of interfering substances. The resultant chromatograms were very clean.

Results obtained on polyoxyethylene (8) monostearate, the poly-

TABLE I

COMPARISON OF COLORIMETRIC AND CHROMATOGRAPHIC METHODS FOR THE DETECTION OF POLYOXYETHYLENE GLYCOL IN BAKERY PRODUCTS

SAMPLE	PERCENT POEMS ON DRY BASIS		CHROMATOGRAPHIC DETECTION: R_f VALUES
	Added	Colorimetric Method	
POEMS (not purified)			0.63–0.65 (red) 0.91–0.92 (orange)
Polyoxyethylene glycol fraction			0.62–0.65 (red)
Ester fraction			0.91–0.92 (orange)
Laboratory-baked bread	None	None	No spot
	0.26	0.25	0.63 (red)
	0.09	0.11	0.62 (red)
Laboratory-baked bread + 0.6% of mono- + diglycerides	None	None	No spot
Bakery X	0.38	0.37	0.62 (red)
Bakery Y	None	0.32	No spot
Bakery Z	Admitted	0.16	0.64 (red)

oxyethylene glycol and ester fraction, and a number of breads are given in Table I. These data show that a chloroform solution of POEMS, spotted directly on the paper, gave two spots which were in good agreement with those obtained with the polyoxyethylene glycol and ester fractions, separated from POEMS by the method of Birkmeier and Brandner (1). Samples of laboratory-baked bread and commercial bread from bakeries X and Z, all known to contain POEMS, gave good results by the colorimetric method (3), and when the samples were extracted and run by the chromatographic method the resultant spots agreed with that of polyoxyethylene glycol. However, all commercial samples from bakery Y, which gave very definite results by the colorimetric method (3), did not give a spot when run by the chromatographic method. It would appear that there is no interference in the chromatographic method from mono- and diglycerides or other ingredients normally in bread.

TABLE II

COMPARISON BETWEEN THE TWO METHODS OF ANALYSIS OF THE EMULSIFIER AND BREAD OF BAKERY Y

SAMPLE	AGE	PERCENT POEMS ON DRY BASIS, BY COLORIMETRIC METHOD	CHROMATOGRAPHIC METHOD: R_f VALUE
Emulsifier (a)	Unknown	5–12	No spot
Emulsifier (b)	Unknown	25–78	No spot
Emulsifier (c)	Unknown	37–72	No spot
Bread 1	1 week	0.32	No spot
Bread 1	6 weeks	....	No spot
Bread 1	1 year	....	0.63
Bread 2	1 week	....	No spot
Bread 2	4 months	....	0.64 (faint)
Bread 3	1 week	0.13	No spot
Bread 3	6 weeks	....	No spot

This method was developed following a bread survey of the local market to determine if POEMS was being used as the emulsifying agent, the analyses being done by the colorimetric method (3). Over a two-year period six samples of bread from bakery Y gave apparent positive results varying from a trace amount to 0.32% of POEMS, while its use was denied by the manufacturer. The bread from this bakery contained an emulsifier which was found to be a mixture of flour and two commercial emulsifiers: a lecithin emulsifier and 40 – 45% glycerol mono-oleo-stearate in an animal oil base. These emulsifiers are differentiated by the letters (a), (b), and (c), respectively, and the results of their analysis by the colorimetric method (3) are given in Table II.

When the very erratic results obtained with the colorimetric

method (3) on the emulsifiers were observed, as shown in Table II, it was decided to analyze these emulsifiers and the bread of bakery Y by the chromatographic method of Kliffmüller (4). However, the extraction procedure for bread samples outlined in this method was found to extract too many substances which interfered on the chromatogram. As the same was found with the method of Boari (2), it was necessary to develop a new method for the extraction and purification of the polyoxyethylene glycol.

It is seen in Table II that no spot indicative of polyoxyethylene glycol was given on the chromatogram by the three emulsifiers, spotted directly from chloroform. When three different samples of bread from bakery Y were aged, all containing emulsifier (a), a false positive result for polyoxyethylene glycol was given by the two samples analyzed by the chromatographic method 4 months after their manufacture. However, when these same samples were analyzed within 6 weeks of their manufacture, no spot was given.

With increasing concentrations of polyoxyethylene glycol it was possible to obtain a series of spots, all with the same R_f values but increasing in intensity and size. The difference in 0.1% additions of POEMS to bakery products was detected. The method can be made semiquantitative by spotting a series of polyoxyethylene glycol standards with the unknown on the same chromatogram.

This method for the detection and semiquantitative determination of polyoxyethylene glycol in bakery products provides faster and more reliable results than the colorimetric method (3), and is not affected by any of the normal ingredients present in bakery products.

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