Determination of Flour Glycolipids as Their Benzoyl Derivatives by High-Performance Liquid Chromatography with Ultraviolet Detection

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ABSTRACT

Monogalactosyldiglyceride and digalactosyldiglyceride from wheat flour were determined as their benzoyl derivatives by normal phase high-performance liquid chromatography with detection at 254 nm. Approximately 1 mg of lipid was dissolved in pyridine and benzoyl chloride was added. After heating at 65° C for 30 min, excess benzoyl chloride was hydrolyzed and the glycolipid derivatives were extracted into heptane concentrated to dryness and redissolved in heptane. The derivatives were separated by isocratic elution on Partisil-10 with an elution solvent of heptane/2-propanol (200:1). Recoveries of 90–109% of added glycolipid were achieved, and the calibration was linear up to at least 250 μg of glycolipid per test. The coefficient of variation ($n = 16$) was 2.4% for both mono- and digalactosyldiglyceride.

Polar lipids, especially glycolipids, are important factors in determining the breadmaking quality of wheat flour (Bekes et al 1983a,b; Chung et al 1982, 1984; Lin et al 1974; Pomeranz 1971), although correlations between free glycolipid and baking quality of Canadian (Bekes et al 1986) and U.S. (Chung et al 1982) wheats do not necessarily apply to English (Bell et al 1987) and Australian (Marston 1985) wheats. Methods for the quantitative determination of glycolipids rely on the analysis of either carbohydrate (Galanos et al 1965) or fatty acids (Morrison et al 1980) in the hydrolyzed lipid extract after either column or thin-layer chromatography (TLC). TLC followed by charring and densitometry is also used successfully (Chung et al 1984). Because these procedures are time-consuming, and we needed to perform several hundred lipid analyses as part of a project to investigate the quality determinants of wheat, a simpler, faster method was sought. This paper presents a method for the determination of two of the principal glycolipids of wheat flour, monogalactosyl-diglyceride (MGDG) and digalactosyldiglyceride (DGDG), as their benzoyl derivatives, by normal phase high-performance liquid chromatography (HPLC) with ultraviolet detection.

MATERIALS AND METHODS

Solvents used throughout the procedure were chromatography grade (Mallinckrodt, South Oakleigh, Victoria) unless stated otherwise. A 2.5-g sample of flour was extracted by shaking for 5 min with 25 ml of hexane (ACS grade) in a 50-ml centrifuge tube. After centrifuging (2,000 rpm for 10 min), 1.0 ml of supernatant, or a standard solution containing up to 250 μg of mixed glycolipids, was transferred to a screw cap vial and taken to dryness at 65° C under a stream of nitrogen. The residue was redissolved in 0.2 ml of pyridine and 0.1 ml of benzoyl chloride was added. After blowing away the HCl vapors formed, the vial was capped and the benzoylation reaction carried out at 65° C in a dry block heater. After 30 min the vial was cooled under running tap water, and excess benzoyl chloride was hydrolyzed with 2.0 ml of methanol/water (80:20) saturated with sodium carbonate, and 2.0 ml of heptane was added. The benzoyl derivatives of glycolipids were extracted into heptane with shaking (3 min) and, after the phases had separated (30 min), the lower aqueous phase was removed with a Pasteur pipette. The heptane layer was washed once more with sodium carbonate saturated aqueous 80% methanol and once with aqueous 80% methanol. 1.5 ml of the heptane layer was transferred to a clean vial and evaporated to dryness at 65° C (3–4 min) under a stream of nitrogen. The residue was redissolved in 0.5 ml of heptane for chromatography.

The recovery of glycolipid was determined using purified samples of glycolipids that were prepared by preparative TLC using silica-gel-coated plates (Merck 5553) and chloroform/methanol/water (90:20:2) as the developing solvent. Appropriate areas were scraped from the plate (R_f 0.46 and 0.15 for MGDG and DGDG, respectively), and the glycolipids were desorbed with methanol, the solutions evaporated to dryness, and the residues redissolved in chloroform (AR)/methanol (AR) (2:1). Aliquots of each solution were assayed for galactose by a phenol/sulfuric acid method (Galanos et al 1965) and the result expressed as the glycolipid.

High-Performance Liquid Chromatography

The analytical separations were carried out with Laboratory Data Control instrumentation (Milton Roy Co., Ivyland, PA) consisting of a Constametric III pump and a Spectromonitor III variable wavelength detector. Pump and integration were controlled with an IBM PC/XT based chromatography workstation (Maxima, Dynamic Solutions Corp.). An HPLC column (150 × 4 mm) was packed with Partisil-10 (Whatman), and the eluant consisted of heptane/2-propanol (200:1), with a flow rate of 1.5 ml/min and an injection volume of 20 μl. The column temperature was held at 30° C with a forced-air oven temperature

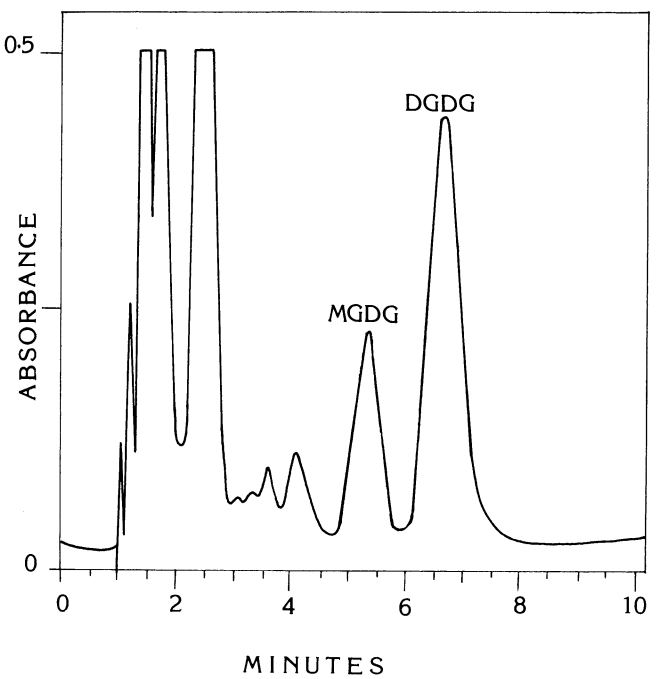

Fig. 1. High-performance liquid chromatographic separation of the benzoyl derivatives of mono- (**MGDG**) and digalactosyldiglyceride (**DGDG**) from wheat on Partisil-10 (150 × 4 mm) with heptane/2-propanol (200:1) as eluant at a flow rate of 1.5 ml/min and detection at 254 nm with sensitivity 0.5 AUFS. Column temperature: 30° C.

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controller (ICI Scientific Instruments, Dingley, Victoria, Australia). Detection was at 254 nm with a sensitivity of 0.5 absorbance units full scale.

RESULTS AND DISCUSSION

The procedure for benzoylation was originally developed for the analysis of mono- and disaccharides (Thompson 1978). In Thompson's procedure, the sugar oximes are formed in a preliminary derivatization with benzyloxylamine, so as to prevent the formation of multiple benzoylation products, which occur with many sugars due to the presence of alpha and beta anomers in equilibrium. As the configurations of the anomeric carbon atoms are defined for MGDG and DGDG (Kates 1982), the formation of multiple products upon benzoylation of the sugar residues in the lipids would not be expected, and the preliminary derivatization should not be necessary. Figure 1 shows the chromatogram of the derivatized free lipids extracted from flour. Peak identities were established by chromatography of glycolipids purified by TLC and derivatized as described. Figures 2 and 3 show the single peaks resulting from purified MGDG and DGDG, respectively.

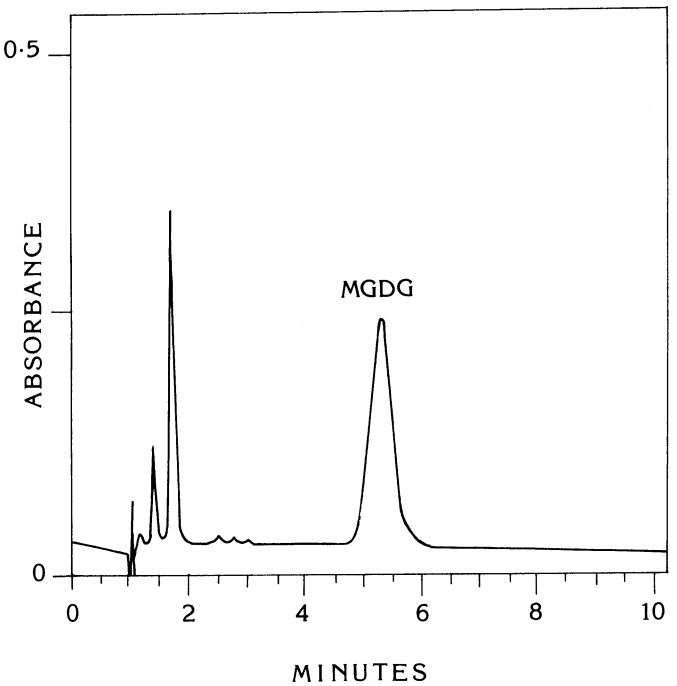

Fig. 2. Chromatogram of the benzoyl derivative of monogalactosyldiglyceride (**MGDG**) purified from wheat. Operating conditions as for Fig. 1.

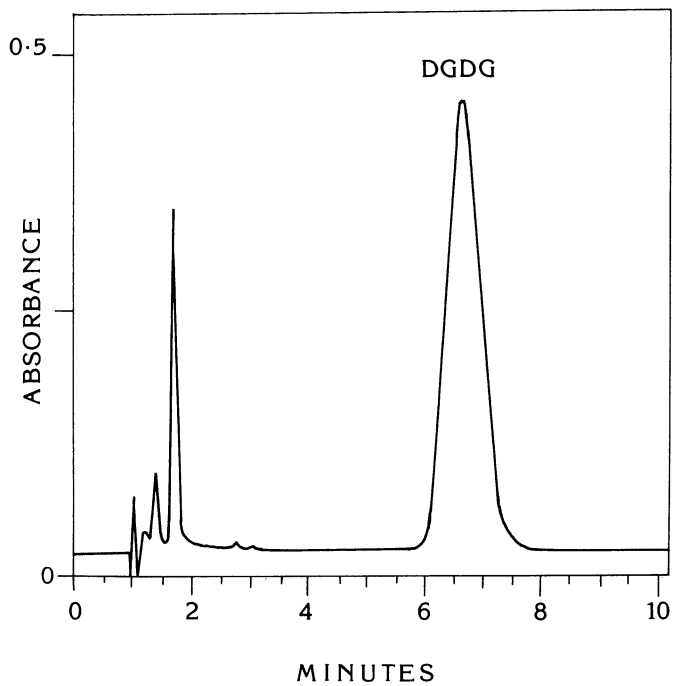

Fig. 3. Chromatogram of the benzoyl derivative of digalactosyldiglyceride (**DGDG**) purified from wheat. Operating conditions as for Fig. 1.

TABLE I
Recovery of Glycolipids Added to Flour Extracts

Glycolipid	Added (μg)	Found (μg)	Recovery (%)
Monogalactosyldiglyceride	8.6	8.0	93
	11.4	12.4	109
	22.9	23.9	102
Digalactosyldiglyceride	26.9	24.3	90
	35.9	37.2	104
	53.8	52.6	98

TABLE II
Mono- (MGDG) and Digalactosyldiglyceride (DGDG) for Pure Variety Wheat Flours

Variety	Glycolipids (mg/10g flour)		
	MGDG	DGDG	MGDG + DGDG
Banks	7.8	12.3	20.1
Condor	6.4	10.2	16.6
Cook	3.9	9.1	13.0
Halberd	4.6	7.0	11.6
Millewa	3.9	7.8	11.7
Matong	7.7	12.4	20.1
Osprey	6.1	9.6	15.7

The method was optimized by examining the time and temperature of derivatization, the quantity of benzoyl chloride added per test, and the number of heptane extractions necessary to achieve maximum formation and recovery of the derivatives. Maximum peak area response was achieved by reacting the lipid extracts with 100 μl of benzoyl chloride for either 45 min at 22° C or for 30 min at 65° C. A single heptane extraction was sufficient to remove the glycolipid derivatives from the aqueous methanol washings, as was shown by the absence of detectable peaks when these washings were reextracted with a second portion of heptane, which was then concentrated and chromatographed.

Recoveries of standard glycolipids added to sample extracts were 90 to 109% (Table I), and the calibration graphs were linear up to a total of at least 250 μg of glycolipid per test. Linear regression of the calibration data gave r^2 values of 0.999 for MGDG and DGDG and the precision of the method as measured by the coefficient of variation ($n = 16$) was 2.4% for both MGDG and DGDG. Samples of pure variety wheats were milled on a Buhler laboratory mill to an extraction of 78–80%. Typical glycolipid results obtained using the method for a selection of flours are shown in Table II. The sums of MGDG and DGDG are in agreement with the ranges of total glycolipids obtained by other workers (Bell et al 1987, Chung et al 1982).

This method should satisfy the call for a direct procedure for the determination of glycolipids in flour lipid extracts without the need for hydrolysis (Chung et al 1982).

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