CHROM. 4539

Identification of wheat flour lipids by thin-layer chromatography

We are at present engaged in studies of the lipids in wheat flour, and have now isolated twenty-three lipid classes by combined column and thin-layer chromatographic (TLC) techniques[1]. McKILLICAN[2] previously separated twelve lipid classes (+ six unknowns) by two-dimensional TLC, and GRAVELAND[3] separated nineteen lipid classes (+ six unknowns) by unidimensional TLC with double development. Many other papers illustrate TLC separations of wheat flour lipids, but all contain incomplete or erroneous identifications of the lipid classes. We therefore consider it worth reporting our methods for the separation and identification of wheat flour lipids. These methods form the basis of quantitative and qualitative procedures which we are using to study changes in flour lipids during storage and dough mixing. Since most of the lipid classes in wheat are found in all plant lipids, the TLC methods described below may be usefully applied in the identification and analysis of plant lipids generally.

Lipids were extracted from untreated, unbleached spring wheat flour with water-saturated *n*-butanol[1]. The solvent was removed by rotary vacuum evaporation

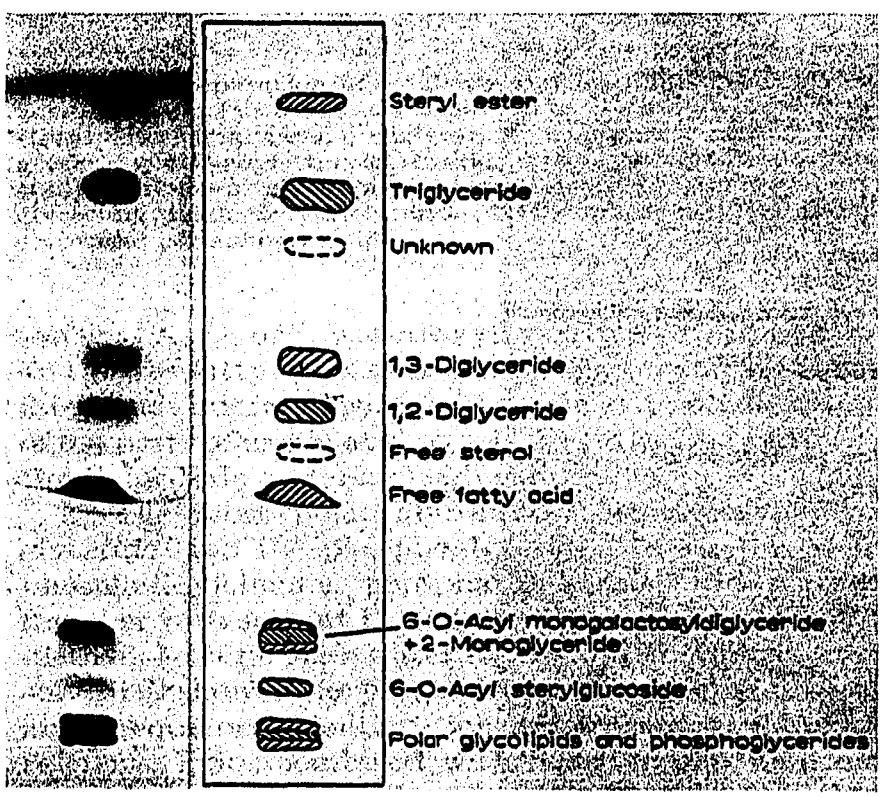

Fig. 1. TLC separation of total wheat flour lipids on Silica Gel G, developed first with diethyl ether–benzene–ethanol–acetic acid (40:50:2:0.2), air-dried, redeveloped in the same direction with diethyl ether–hexane (6:94), and visualised by charring with 50% sulphuric acid. From top to bottom: steryl ester; triglyceride; unknown; 1,3-diglyceride; 1,2-diglyceride; free sterol; free fatty acid; 6-O-acyl monogalactosyldiglyceride + 2-monoglyceride; 6-O-acyl sterylglucoside; polar glycolipids, phosphoglycerides.

and the lipids purified by partition in a biphasic chloroform–methanol–water system[4].

TLC plates made with a 250-μm layer of Silica Gel G (Merck, Darmstadt) were activated for 1 h at 120° immediately before use. The plates were developed in paper-lined tanks with the following solvents (given in parts by volume):

(A) First development with diethyl ether–benzene–ethanol–acetic acid (40:50:2:0.2), followed by air drying and a second development with diethyl ether–hexane (6:94)[5].

(B) Chloroform–acetone–methanol–acetic acid (73:25:1.5:0.5)[6].

(C) Chloroform–acetone–water (30:60:2)[1].

(D) Chloroform–methanol–ammonia (30% w/v)–water (65:30:5:2.5)[6].

All lipids were detected by charring with 50% sulphuric acid. Glycolipids and sterol lipids were selectively detected with α-naphthol reagent[7], phospholipids with modified Zinzadze reagent[8], and amine groups with ninhydrin reagent[9].

The separation of neutral lipids with solvent A is shown in Fig. 1. The 2-monoglyceride was always inseparable from the 6-acyl monogalactosyldiglyceride with solvent A, or with any solvent system based on diethyl ether–hexane–acetic acid, but was well separated with solvent B (Fig. 2). The polar glycolipids were separated with solvent C (Fig. 3) and N-acyl phosphatidylethanolamine was the only phosphoglyceride to migrate from the origin in this solvent.

The mobility of free fatty acids relative to other lipids on plates developed with

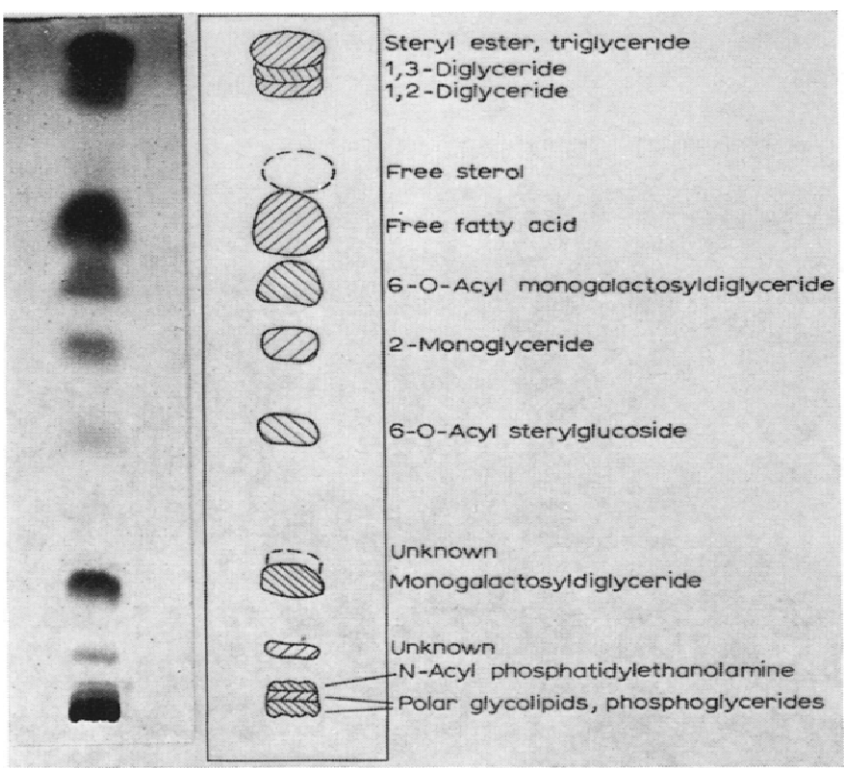

Fig. 2. TLC separation of total wheat flour lipids on Silica Gel G, developed with chloroform–acetone–methanol–acetic acid (73:25:1.5:0.5) and visualised by charring with 50% sulphuric acid. From top to bottom: steryl ester, triglyceride; 1,3-diglyceride; 1,2-diglyceride; free sterol; free fatty acid; 6-O-acyl monogalactosyldiglyceride; 2-monoglyceride; 6-O-acyl sterylglucoside; unknown; monogalactosyldiglyceride; unknown; N-acyl phosphatidylethanolamine; polar glycolipids, phosphoglycerides.

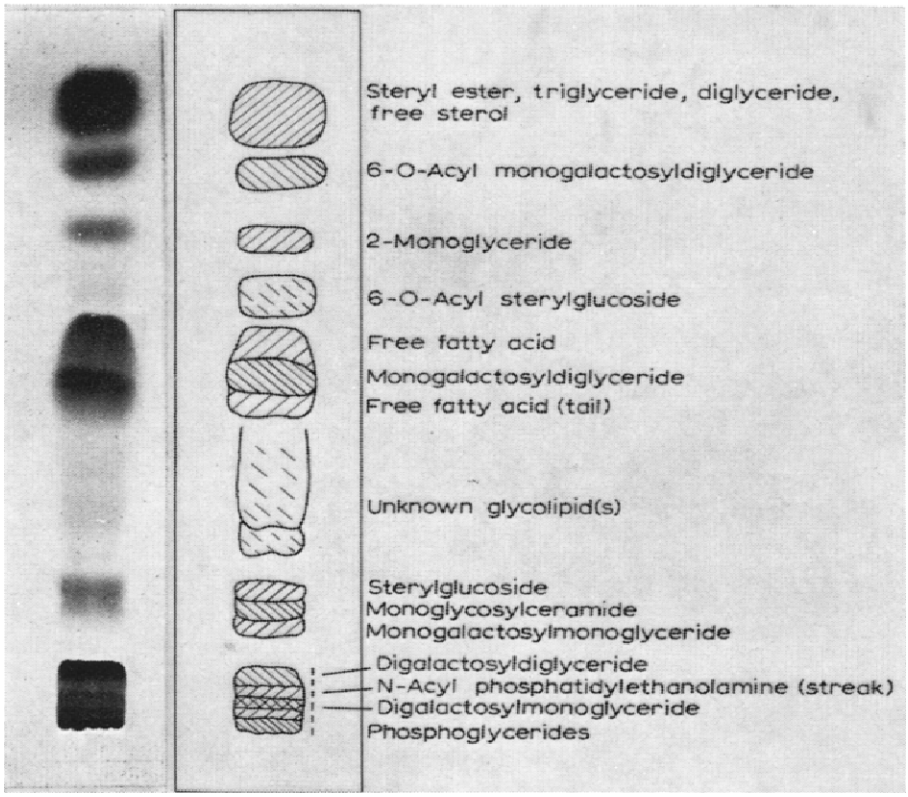

Fig. 3. TLC separation of total wheat flour lipids on Silica Gel G, developed with chloroform–acetone–water (30:60:2) and visualised by charring with 50% sulphuric acid. From top to bottom: steryl ester, triglyceride, diglyceride, free sterol; 6-O-acyl monogalactosyldiglyceride; 2-monoglyceride; 6-O-acyl sterylglucoside; free fatty acid; monogalactosyldiglyceride; free fatty acid (tail); unknown glycolipids; steryl glucoside; monoglycosylceramide; monogalactosylmonoglyceride; digalactosyldiglyceride; N-acyl phosphatidylethanolamine (streak); digalactosylmonoglyceride; phosphoglycerides.

solvents A, B, or C was always variable within narrow limits. Thus with solvents A and B free fatty acids sometimes overlapped the free sterols, and with solvent C free fatty acids migrated with, or just ahead of, monogalactosyldiglyceride. After addition of 0.5 parts of acetic acid to solvent C the free fatty acids migrated between 6-O-acyl sterylglucoside and 2-monoglyceride.

The most polar glycolipids, digalactosyldiglyceride and digalactosylmonoglyceride, and the phosphoglycerides were resolved with solvent D (Fig. 4). Lysophosphatidylcholine, phosphatidylserine, and phosphatidylinositol migrated close together with solvent D, and were better separated by development with chloroform–methanol–acetic acid–water (65:25:8:4)[10]. Detection of lipids on plates developed in solvent D showed the presence of polar glycolipids throughout the phosphoglycerides, and unequivocal identification of the lipids could only be achieved by careful use of reference lipids combined with selective detection methods. The characteristic red and blue colours of sterols and burgundy colours of glycolipids which appeared during the early stages of charring with 50% sulphuric acid were also invaluable for locating these lipids.

The TLC separations described above were adequate for most preparative purposes, except that many of the phosphoglycerides could not be isolated by a

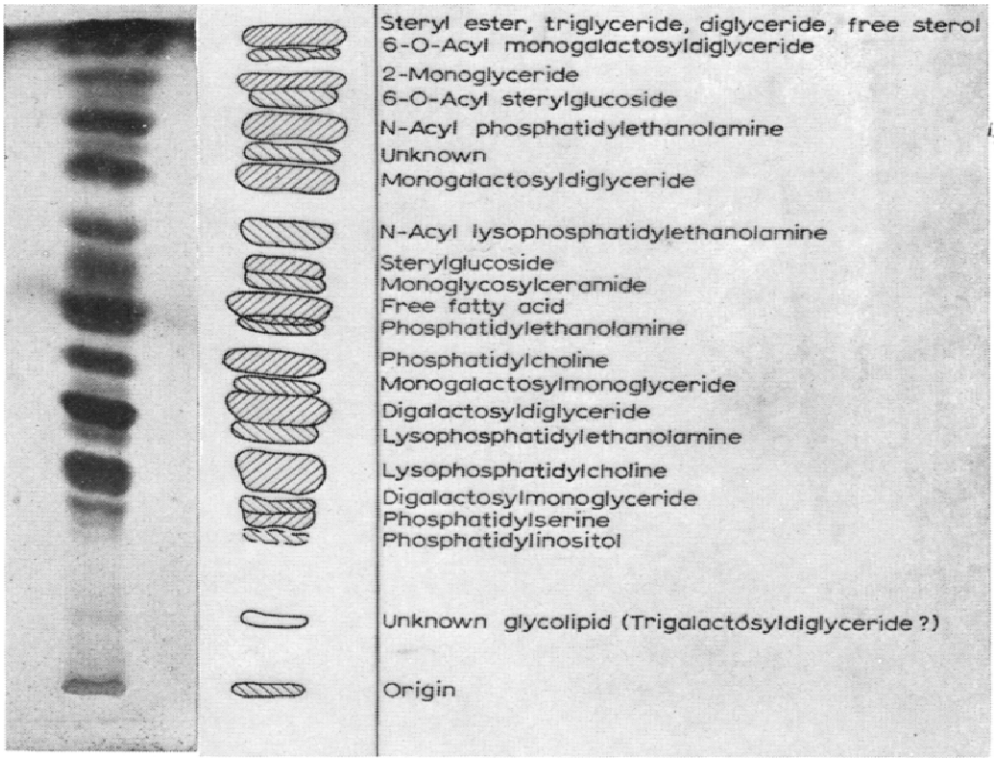

Fig. 4. TLC separation of total wheat flour lipids on Silica Gel G, developed with chloroform–methanol–ammonia (30% w/v)–water (60:35:5:2.5) and visualised by charring with 50% sulphuric acid. From top to bottom: steryl ester, triglyceride, diglyceride, free sterol; 6-O-acyl monogalactosyldiglyceride; 2-monoglyceride; 6-O-acyl sterylglucoside; N-acyl phosphatidylethanolamine; unknown; monogalactosyldiglyceride; N-acyl lysophosphatidylethanolamine; steryl glucoside; monoglycosylceramide; free fatty acid; phosphatidylethanolamine; phosphatidylcholine; monogalactosylmonoglyceride; digalactosyldiglyceride; lysophosphatidylethanolamine; digalactosylmonoglyceride; phosphatidylserine; phosphatidylinositol; unknown glycolipid (trigalactosyldiglyceride?); origin.

single TLC step. While an initial separation with solvent D followed by rechromatography with solvent C (or *vice versa*) could be used, we prefered to prefractionate the total wheat lipids on a silicic acid column[1] before preparative TLC. The neutral lipids were eluted with a gradient of diethyl ether in light petroleum (b.p. 40–60°), followed by chloroform; the glycolipids were eluted with acetone in chloroform, followed by acetone; and the phosphoglycerides were eluted with a gradient of methanol in chloroform.

Comparing the present results and previously published results some points of difficulty in lipid identification are apparent. 6-O-acyl monogalactosyldiglyceride[11] is present in larger amounts than monoglyceride in wheat lipids, and is sometimes wrongly identified as monoglyceride. Sterylglucoside and monoglycosylceramide are almost inseparable in all solvent systems we have studied. Hydrolysis of galactosyldiglycerides takes place during storage of flour, and galactosylmonoglycerides are produced in measurable amounts—these lipids have not been identified in wheat flour lipids before. The N-acyl phosphatidylethanolamines were originally identified by BOMSTEIN[12], but have been consistently overlooked since then, although they are present in appreciable amounts in flour[1] and other plant tissues[13–16]. Lysophosphati-

dylcholine is also a major phosphoglyceride in wheat flour, but in this case failure to find more than trace amounts can be attributed to the use of solvents which do not extract this lipid efficiently. In our experience water-saturated *n*-butanol is the best solvent for this purpose[1].

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Separation of isomers of malathion monocarboxylic acid by thin-layer chromatography

The insecticide malathion (O,O-dimethyl-S-[1,2-di(ethoxycarbonyl)ethyl] phosphorodithioate) has been shown to be degraded by carboxyesterases of mammals and insects to malathion monoacid. The latter compound exists in two isomeric forms, denoted as α- and β-monoacid (cf. p. 282).

We encountered this effect in a study of the malathion resistance mechanism of a certain strain of housefly. A simple chromatographic technique, which may be of wider application, was developed to separate the α- and β-monoacids.

Thin-layer chromatography was carried out on silica gel (DC-Fertigplatten, Kieselgel F_{254}, Merck A.G., Darmstadt, G.F.R.). Before use the plates (10 × 20 cm) were washed with acetone and activated for 15 min at 90°. They were developed in