

# Functionality of Specific Flour Lipids in Cookies<sup>1</sup>

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## ABSTRACT

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Cookies baked from hexane-extracted flour exhibited limited spread and top grain as a result of breakdown of internal structure. Functionality was restored when total free lipids were added back to the extracted flour. To determine the source of functionality, free flour lipids were separated quantitatively by preparative thin-layer chromatography. Zones were removed, eluted, and added back to cookie mixes containing hexane-extracted flours. Two fractions (about 13% of free lipids) corresponding to digalactosyldiglyceride (plus phosphatidylcholine) and monogalactosyl-

diglyceride exhibited high degrees of restoration. Pure commercial digalactosyldiglyceride added alone at 0.1% of flour weight (dry basis) or pure phosphatidylcholine from egg yolk added at 0.5% resulted in essentially complete restoration. Monogalactosyldiglyceride added at levels up to 0.15% gave little response. The data suggests that digalactosyldiglyceride and/or phosphatidylcholine are the primary contributors to functionality. An unidentified glycolipid with chromatographic mobility similar to that of monogalactosyldiglyceride also appears to contribute to functionality.

Sugar-snap cookies exhibit characteristic deleterious effects when the free flour lipids are extracted before baking. These effects have been the subject of several reports (Cole et al 1960, Kissell et al 1971, Yamazaki and Donelson 1976). The most obvious effects are reductions in spread and top grain, but the changes in internal structure are of more significance (Clements 1980, Yamazaki and Donelson 1976). The reduced spread and consequent loss of top grain result from the breakdown of gas cells during expansion and their coalescence into larger cells. Cookies baked from a defatted (ie, hexane-extracted) flour from a typical soft wheat thus exhibit large voids enclosed within relatively thin shells. Cookies baked from nondefatted flours typically show a uniform fine cellular structure (Clements 1980). When extracted lipids are added back to defatted flours, this fine structure is restored.

Free lipids constitute about 1% (db) of a straight grade flour. Both polar and nonpolar free lipids (about 20 and 80%, respectively, of total free lipids) appear to be essential for complete restoration of functionality when added back to defatted flours (Kissell et al 1971). In this study, free flour lipids were separated by preparative thin-layer chromatography (TLC) into individual fractions. Each fraction was returned to an aliquot of defatted flour for cookie baking to determine which lipids are effective in restoring cookie structure.

## MATERIALS AND METHODS

### Flours

The flour employed in this study was a composite of straight grade flours from several soft red and soft white winter wheats milled in the Wooster laboratory. Its protein content was 12.4% (db, N × 5.7), and its ash content was 0.47 (db). Free lipids were extracted with hexane in a large Soxhlet extractor (Clements 1977), yielding 1.11% lipids (db).

### TLC

Preparative TLC was performed on precoated silica gel plates, 20 × 20 cm, 1,000 μm thick, with a diatomaceous earth application strip (Quantum PLQF, now Whatman PLKF, from Whatman, Inc., Clifton, NJ 07014). Plates were washed with chloroform/methanol/water (65:25:4, v/v/v) and air-dried before use.

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Generally, lipid equivalent to 40 g of flour (ie, one cookie bake, giving two cookies), or about 0.4 g of lipid, in 2-3 ml of chloroform/methanol (2:1), was placed in an aluminum foil trough. The application end of the plate was placed in the trough, and the sample was allowed to migrate about 2 cm into the application zone. The plate was then air-dried, and the process was repeated until the sample was taken up. Small amounts of chloroform/methanol were added to the trough in increments and allowed to migrate into the application zone (with drying between applications) until the sample was uniformly distributed in a band 1-2 cm deep along the leading edge of the application zone. The solvent system used for development was prepared by combining equal volumes of polar system (chloroform/methanol/water, 65:25:4, v/v/v) and a nonpolar system (hexane/diethyl ether/acetic acid, 70:40:3, v/v/v). The mixture was shaken vigorously and allowed to settle to give a heavy phase covered by a very shallow floating phase. The lower phase was withdrawn with a syringe as needed and added directly to the tank. Plates were equilibrated against the solvent for 1 hr, developed to 12 cm, and air-dried. Spraying with water revealed 10 hydrophobic zones, which were cut out with a scalpel while the plate was still moist, transferred to beakers, and air-dried.

Lipids were recovered by extracting the silicic acid with chloroform/methanol/water (65:25:4, v/v/v). The suspensions were heated to boiling in a water bath, with stirring, and allowed to settle, and the supernatants were decanted into columns containing glass wool plugs. The process was repeated twice, and suspensions were then transferred to the columns and allowed to drain. The columns were washed three times with hot chloroform/methanol/water (15-20 ml), and any remaining solvent was forced from the columns by pressure. A small sample (1-2 ml) was taken from each extract for qualitative TLC. Two grams of shortening (Creamtex, Durkee Famous Foods, SCM Corp., Cleveland, OH 44115) was added to each extract to serve as a vehicle for lipid residues, and the samples were evaporated under vacuum on a rotary evaporator at 60°C. Fractionations were replicated several times.

For quantitative determinations, weighed samples of flour lipids (0.4-0.6 g) were applied to preparative plates. Plates were developed and lipid bands recovered according to the above procedure (without addition of shortening to extracts). Extracts of TLC bands were filtered and evaporated to dryness, and residues were weighed after 1 hr at 100°C. Qualitative TLC verified that the fractions were essentially identical to those added to the cookie bakes. Duplicate determinations were carried out and averaged.

Qualitative TLC was performed on plates coated with silica gel in layers 250 μm thick (Quantum Q6, now Whatman K6, Whatman, Inc., Clifton, NJ 07014). Samples of extracted lipids and known lipids (Supelco, Inc., Bellefonte, PA 16823) were applied at concentrations adjusted to give spots of sufficient density, and the plates were developed in the solvent system used for preparative TLC after equilibration for 10 min. Lipids were detected by dipping the plates in 3% cupric acetate in 8% phosphoric acid and heating

them for 30 min at 145°C (Anonymous 1975). Identities of glycolipids were confirmed by spraying with Bial's orcinol reagent (Applied Science Laboratories, State College, PA 16801).

### Baking Procedures

Cookies were baked according to micro method III of Finney et al (1950). Composition of the creamed mass was adjusted to allow for the shortening used in lipid recovery. Lipids were added to individual batches by transferring the isolated lipids (dispersed in the 2 g of shortening added before solvent removal) to the appropriate creamed mass. Total free lipids were added back in the same manner. Lipids, shortening, and creamed mass were blended during mixing after the creamed mass was added to the mixing bowl. Monogalactosyldiglyceride (MGDG) and digalactosyldiglyceride (DGDG) from wheat flour (P. L. Biochemicals, Milwaukee, WI 53205) and phosphatidylcholine (PC), a purified L- $\alpha$ -lecithin from egg yolk (U.S. Biochemicals Corp., Cleveland, OH 44128) were dissolved in appropriate solvents; 2 g of shortening was added; the solvent was evaporated; and the shortening was added to the creamed mass as described above. However, bakes involving additions of pure lipids were scaled down to 20 g of flour (one cookie) to conserve lipids.

## RESULTS AND DISCUSSION

The separation procedure yielded 10 fractions that, although somewhat arbitrary, were qualitatively and quantitatively reproducible. Cookies resulting from additions of these fractions are shown in Fig. 1. Fraction 2 resulted in almost complete restoration of spread and top grain. Fractions 3 and 5 resulted in moderate response, and fractions 1, 4, and 7 showed slight restoration. The remaining four fractions produced cookies that did not vary noticeably from cookies baked from the defatted control flour.

Figure 2 shows the TLC patterns of the fractions that produced the baking responses illustrated in Fig. 1. Although the same solvent system was used for both preparative and qualitative TLC,  $R_f$  values varied slightly because of differences in the plates. However, preparative and qualitative patterns were essentially the same. Most eluted fractions exhibited a predominant component and several secondary constituents. Table I shows yields of lipids eluted from individual bands from preparative plates run specifically for qualitative determinations. Average recovery from duplicate plates was 97%, indicating negligible loss. Fraction 2 (about 7% of total free lipids), which gave maximum restoration, contained two major components corresponding to PC and DGDG. All material on the preparative plate was mobile, and

therefore material remaining at the origin presumably was an artifact of the isolation procedure. Because PC characteristically gives a weak spot when detected with cupric acetate/phosphoric acid, the density of the spot may not be a true indication of concentration relative to that of DGDG.

The response from fraction 3 may have resulted from the small amounts of DGDG and PC detected in this fraction. Fraction 5 (almost 6% of total free lipids), which gave moderate restoration, contained a glycolipid corresponding to MGDG, as well as a second glycolipid (possibly a steryl glycoside). Overall, fractions 2, 3, and 5 (about 16% of total free lipids) gave substantial restoration, whereas the remaining seven fractions (about 84% of total free lipids) gave very little response. The three slow-moving bands in fraction 7 (with mobilities comparable to that of monoolein) were identified as glycolipids and may have contributed to the slight

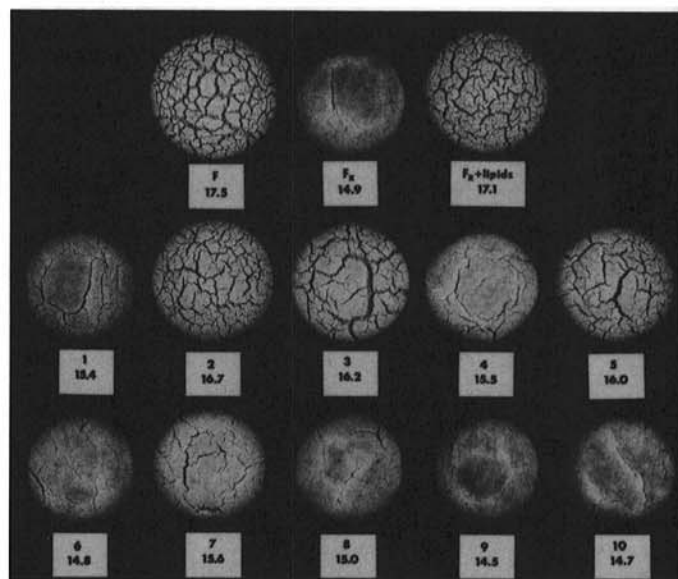


Fig. 1. Sugar-snap cookies baked from whole flour (F), hexane-extracted flour (F<sub>X</sub>), hexane-extracted flour with lipid added back (F<sub>X</sub> + lipids), and from hexane-extracted flour with lipids from individual thin-layer chromatography bands added back (1-10). The combined diameter (in centimeters) of two cookies is given below each cookie.

TABLE I

Quantitative Separation of Free Flour Lipids by Preparative Thin-Layer Chromatography and Efficacy of Individual Fractions in Restoring Lipid Functionality in Cookies

Lipid Fraction	$R_f$	Percent of Total Recovered Lipids <sup>a</sup>	Percent of Flour Weight	Baking Response <sup>b</sup>
Nonextracted control flour		100.00	1.11	+++++
Defatted flour + total free lipid		100.00	1.11	+++++
1	0.00-0.03	2.14	0.02	+
2	0.03-0.11	7.03	0.08	++++
3	0.11-0.16	3.22	0.04	+++
4	0.16-0.21	2.05	0.02	+
5	0.21-0.32	5.82	0.06	+++
6	0.32-0.37	2.53	0.03	0
7	0.37-0.58	16.82	0.19	+
8	0.58-0.74	54.31	0.60	0
9	0.74-0.79	4.88	0.05	-
10	0.79-1.00	1.21	0.01	0

<sup>a</sup>Averages of duplicates. Free lipids = 1.11% of flour (db).

<sup>b</sup>Degree of restoration of lipid functionality in cookies: no response, 0; negative response, -; positive response, +.

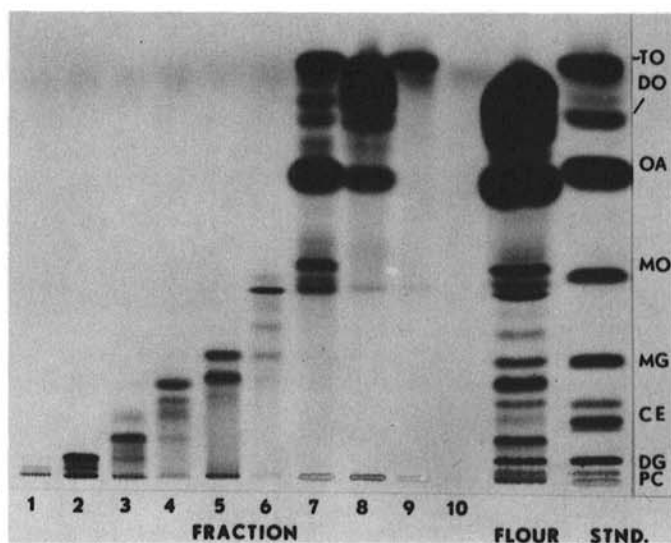
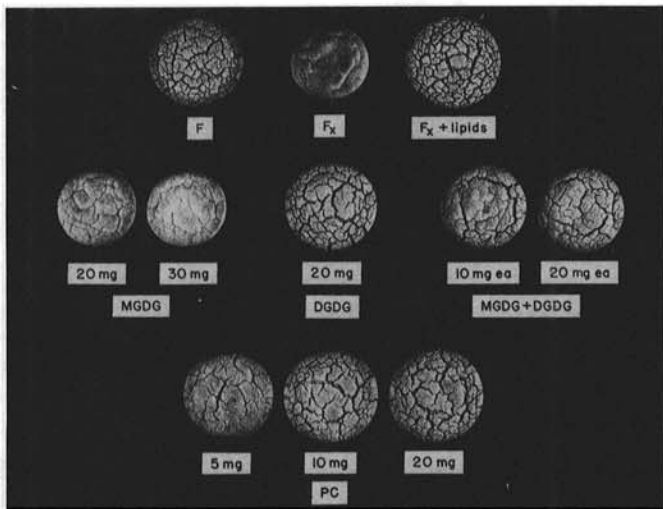


Fig. 2. Thin-layer chromatography patterns of lipids from preparative thin-layer chromatography of free flour lipids (1-10), total free flour lipids (FLOUR), and a standard lipid mixture (STND.). PC = phosphatidylcholine, DG = digalactosyldiglyceride, CE = cerebrosides, MG = monogalactosyldiglyceride, MO = monoolein, OA = oleic acid, DO = diolein, TO = triolein.



**Fig. 3.** Sugar-snap cookies baked from nonextracted control flour (F), hexane-extracted flour (Fx), hexane-extracted flour with total free lipids added back (Fx + lipids), and hexane-extracted flour with different levels and combinations of commercially available monogalactosyldiglyceride (MGDG), digalactosyldiglyceride (DGDG), and phosphatidylcholine (PC) added. Weights represent amounts added per 20 g of flour.

response shown by fraction 7. Thus, in this study, functionality appeared to reside almost exclusively in the polar lipids.

The preceding separation procedures were applied to lipids from other flours milled from both hard and soft wheats. Results were similar, with fraction 2 consistently giving a very high degree of restoration.

On the basis of these results, limited experiments were performed to determine the restorative properties of commercial MGDG and DGDG and of a commercial PC that gave a single TLC spot. MGDG added at levels of 0.10 and 0.15% of flour weight resulted in negligible response (Fig. 3). DGDG added at 0.1% gave essentially complete restoration. Surprisingly when MGDG and DGDG were both added in equal amounts at two levels (0.05 and 0.10%), restoration was less than from addition of DGDG alone (at 0.10%). PC added at 0.05% of flour weight resulted in total restoration. At 0.025%, the response was less, and at 0.10% was the same as at 0.05%.

The results suggest that DGDG and PC are the major contributors to functionality of free flour lipids in cookies. MGDG

does not appear to contribute significantly, but an unidentified glycolipid with TLC mobility similar to that of MGDG appears to be functional. Although both polar and nonpolar lipids have been reported to be necessary for full restoration of cookie quality (Kissell et al 1971), contribution by the nonpolar lipid fraction was negligible in this study. However, single fractions only were added back to defatted flours. Perhaps addition of combinations would give enhanced effects. Further experiments will be necessary to determine possible interactions and synergistic effects. Deletion (rather than addition) of fractions may be a useful approach, because some lipid classes may have negative effects on cookie quality.

Preparative TLC offers several advantages over more conventional column methods. It is convenient, offers high resolution, and can be closely correlated with qualitative TLC. Its limited capacity, however, restricts application to studies involving relatively small amounts of lipids (about 500 mg per plate). Application of lipids representing a single batch to a plate avoids the need for precise volumetric or gravimetric prating of individual fractions for adding back to batches because each isolated fraction represents the original native level of each lipid fraction. The technique of adding shortening to fractions to serve as a vehicle for residues facilitates transfer of lipids to the cookie formulation and circumvents the need for polar solvents (required to dissolve isolated polar lipids) that might interact with flour components.

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