Note on the Separation and Baking Properties of Polar and Nonpolar Wheat Flour Lipids

J. G. PONTE, Jr., and V. A. De STEFANIS, Research Laboratories,
ITT Continental Baking Co., Rye, N. Y.

Separation of polar and nonpolar fractions of lipid mixtures is usually achieved by such methods as thin-layer chromatography (TLC), column chromatography, and countercurrent extraction. While these procedures afford powerful means of separating lipid mixtures for characterization studies, they suffer from at least several disadvantages. In some cases, fairly elaborate equipment is required; long separation times (except for TLC) are often involved, with the attendant hazard of degradation of labile lipids; and only small amounts of lipids may normally be processed at one time. The last-mentioned disadvantage is particularly troublesome when relatively large quantities of separated lipids are desired for studies of functionality, for example in baking experiments. We have employed a procedure for fractionating lipid mixtures into polar and nonpolar components that largely overcomes the disadvantages cited above. This method, a batch technique based on adsorption of lipid mixtures onto activated silica gel, followed by selective elution with appropriate solvents, is rapid, can handle large amounts of material, and demands no special equipment. Hornstein and co-workers recently utilized a similar method for the separation of muscle lipids into various classes (1). Morrison (2), in a study on the role of free fatty acids in flour-water mixtures, employed activated silica gel to adsorb phospholipids from wheat flour lipids. Activated silica gel has also been used to remove phospholipids from vegetable oils (3) and pork muscle lipids (4). The present paper describes the application of the technique to the fractionation of wheat flour lipids, and shows that polar lipids thus separated increase loaf volume, whereas nonpolar lipids have the opposite effect.

MATERIALS AND METHODS

Lipid Extraction

Lipids were removed from a commercial baker's patent flour (12.2% protein, 0.43% ash on 14% m.b.) utilizing a solvent system of ethanol-benzene (1:1, v./v.). Three successive extractions were conducted per lot of flour by mechanically stirring, over a 15-min. period, flour and solvent in the ratio of 1:2 (w./v.), followed by Buechner filtration through Whatman No. 1 filter paper. The combined extracts were dried under vacuum employing a flash evaporator and a bath temperature of $< 45°C$. The crude lipid material was extracted with n-hexane, centrifuged to remove insoluble material, and again taken down to dryness under vacuum. Under these conditions, 1.4% hexane-soluble lipids were extracted from the flour (solids basis).

Activation of Silica Gel

Kieselgel D-O (Camag, Muttenz, Switzerland) was activated by washing three times with acetone (5); residual acetone was eluted by four washings with anhydrous ether. Lipids were adsorbed onto the activated silica gel by slurrying lipids (dissolved in ethyl ether) and gel in the ratio of at least 1:3

for 15 min. Ninety-five g. of flour lipids was processed for the baking studies described below, while 5-g. portions of lipids were used to provide quantitative data on the separations, as well as material for TLC studies.

Separation of Nonpolar Lipids

Diethyl ether-petroleum ether (90:10, v./v.) was stirred into portions of the above silica gel slurry, such that the ratio of diethyl ether-petroleum ether to lipids was roughly 12:1 (v.:w.). A 5-min. contact period was allowed, then the mixture was centrifuged at 2,000 r.p.m. for 2 min. The supernatant was filtered through Whatman No. 1. Three additional elutions were conducted. The extracts were combined and the solvent was removed by heating on a steam bath under a stream of nitrogen. To remove small quantities of silica gel evidently soluble in diethyl ether, the nonpolar lipids were taken up with petroleum ether, then dried under vacuum.

Separation of Polar Lipids

The remaining lipids were eluted with methanol (95:5,v./v.), following the procedure described for the nonpolar lipids. The eluted polar lipids were also taken up with petroleum ether before a final concentration under vacuum.

Baking Studies

A conventional laboratory sponge-dough procedure was used (two 1-lb. loaves per dough, duplicate doughs on different days), essentially as described elsewhere (6), except that no surfactants were employed in the formulation. Loaf compressions were determined with a Baker compressimeter (7), on 4-day-old bread that had been stored in plastic bags at room temperature.

TLC Analyses

Plates were prepared as previously described (8). Five-microliter aliquots of 10% lipid solutions in chloroform were applied to the plates. Developments were conducted with solvent systems of hexane:ethyl ether:acetic acid (65:35:2, by volume) (9), and chloroform:methanol:water (90:20:2, by volume) (10). The developed plates were exposed to iodine vapors for visualization of spots. Clean glass plates were then placed directly on top of the silica gel layers, forming "sandwiches" of the iodine-exposed layers between glass plates. This prevents evaporation of the iodine from the lipid spots, preserving the TLC pattern for at least several days and allowing photography of the plates when convenient.

Spots were identified with reference to standards. Digalactosyl diglyceride standard was a gift of D. H. Hughes, Procter & Gamble Co., Cincinnati, Ohio; monogalactosyl diglyceride was obtained from Applied Science Labs., State College, Pa. Phosphatidyl choline was obtained from K&K Laboratories, Plainview, N. Y.

RESULTS AND DISCUSSION

Lipid Fractionation

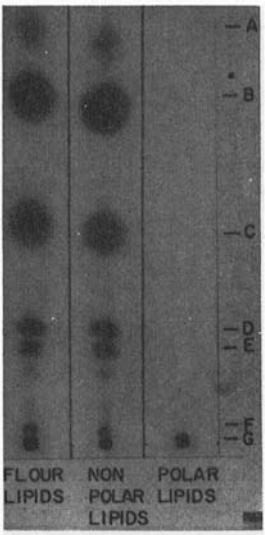
Utilizing the batch technique outlined above, triplicate determinations on

the ethanol-benzene-extracted flour lipids yielded the following proportions of nonpolar and polar fractions.

Trial	*Nonpolar Lipids*	*Polar Lipids*
1	62.5	37.7
2	63.1	37.2
3	62.5	37.5
Mean	62.7	37.5

These values were based on recovered material; average recovery of lipids from the silica gel was 100.1%. Pomeranz and co-workers (11) reported somewhat lower ratios of nonpolar:polar lipids, employing water-saturated butanol to extract lipids (from experimentally milled flour) and a column chromatographic procedure to effect separation.

A qualitative comparison of the lipid fractions as indicated by TLC is shown in Fig. 1.



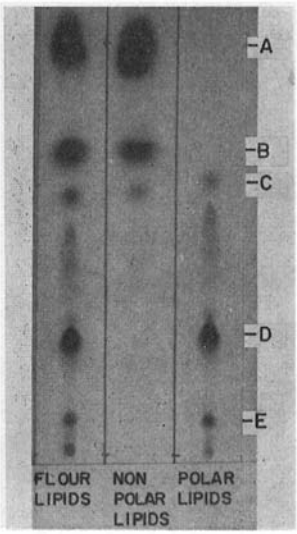


Fig. 1 (left). Thin-layer chromatogram of flour, nonpolar and polar lipids; solvent system of n-hexane:ethyl ether:acetic acid (65:35:2, by volume). A, neutral components; B, triglycerides; C, free fatty acids; D, 1,3-diglycerides; E, 1,2-diglycerides; F, monoglycerides; G, material at origin.

Fig. 2 (right). Thin-layer chromatogram of flour, nonpolar, and polar lipids; a solvent system of chloroform:methanol:water (90:20:2, by volume). A, neutral components; B, monoglycerides; C, monogalactosyl diglyceride; D, digalactosyl diglyceride; E, phosphatidyl choline.

The nonpolar lipid fraction comprised components ranging in polarity (i.e. mobility) from the neutral components (hydrocarbons and sterol esters) to monoglycerides, in roughly the same proportion as the starting flour lipids; in addition, the presence of a small amount of material at the origin was indicated. The polar lipid fraction appeared to be virtually free of monoglycerides and faster-moving (less mobile) components.

A TLC comparison of the same lipid fractions, utilizing a more polar solvent system to induce migration of the polar components, is shown in Fig. 2. This illustration again indicates that the polar lipid fraction was free of monoglycerides and less polar components. The nonpolar lipid fraction contained some monogalactosyl diglyceride, but virtually nothing else of greater polarity.

Baking Studies

Table I summarizes the baking data obtained in this study. In general, the bread made with added fat (lard) was of better over-all quality than the bread made without added fat, in agreement with general experience.

TABLE I. EFFECT OF LIPID FRACTIONS ON BREAD QUALITY

	Loaf volume cc.	Grain score (max. 100)	Loaf compressibility compr. units
With 3% lard: Control	2,626	78	15.3
1% Total flour lipids	2,557	82	15.4
1% Nonpolar fraction	2,364	85	17.9
1% Polar fraction	2,704	80	15.0
Without lard: Control	2,528	73	16.3
1% Total flour lipids	2,561	80	16.3
1% Nonpolar fraction	2,262	87	20.5
1% Polar fraction	2,667	78	16.8

The addition of 1% total flour lipids had a slightly negative influence on volume in the dough systems containing added fat, but had little effect in the doughs without fat; in both systems the total flour lipids caused a small improvement in grain score.

The polar lipid fraction brought about small grain score improvements and substantial loaf volume increases in both dough systems, particularly in the system without lard. This essentially confirms the recently reported researches of Pomeranz and co-workers (12, 13), who worked with straight-dough systems and with lipid fractions prepared by column chromatography. Cookson et al. (14), employing English breadmaking procedures and lipid fractions obtained by countercurrent distribution, observed that waxy lipid fractions improved the baking properties of defatted flour, while oily lipid fractions had a deteriorating effect; in the presence of lard, however, differing results were obtained, depending on the solvent system used to fractionate the lipids.

The present data show further that the nonpolar lipid fraction markedly decreased loaf volumes and also increased crumb firmness; the grain of this bread, on the other hand, appeared to be finer and more uniform.

The results of this study thus indicate that lipid mixtures can be fractionated quickly and efficiently into relatively polar and nonpolar components, by a simple batch technique that should be amenable to considerable scaling-up. Baking studies with the sponge-dough system confirmed that polar lipids had an improving effect on loaf volume and showed that nonpolar lipids had a deteriorating effect on this bread parameter.

Acknowledgments

The authors are grateful to A. Young, A. Juers, and E. Reed for their technical assistance.

Literature Cited

1. HORNSTEIN, I., CROW, P. F., and RUCK, J. B. Separation of muscle lipids into classes by nonchromatographic techniques. Anal. Chem. 39: 352-354 (1967).
2. MORRISON, W. R. The free fatty acids of wheat flour: their role in simple flour-water mixtures. J. Sci. Food Agr. 14: 245-251 (1963).
3. CHOUDHURY, R. B. R., and ARNOLD, L. K. The determination of the neutral oil content of crude vegetable oils. J. Am. Oil Chemists' Soc. 37:87-88 (1960).
4. KUCHMAK, M., and DUGAN, L. R., Jr. Phospholipids of pork muscle tissues. J. Am. Oil Chemists' Soc. 40: 734-736 (1963).
5. HAMILTON, J. Gelman Eastern Regional TLC, ITLC Workshop, New York City, April, 1967.
6. PONTE, J. G., JR., TITCOMB, S. T., ROSEN, JOCELYN, DRAKERT, W., and COTTON, R. H. The starch damage of white bread flours. Cereal Sci. Today 6: 108-110, 112, 121 (1961).
7. PLATT, W., and POWERS, R. Compressibility of bread crumbs. Cereal Chem. 17: 601-621 (1940).
8. PONTE, J. G., Jr., De STEFANIS, V. A., and COTTON, R. H. Studies of gluten lipids. I. Distribution of lipids in gluten fractions separated by solubility in 70% ethanol. Cereal Chem. 44: 427-435 (1967).
9. MANGOLD, H. K., and MALINS, D. C. Fractionation of fats, oils, and waxes on thin layers of silicic acid. J. Am. Oil Chemists' Soc. 37: 383-385 (1960).
10. WAGNER, H., HORHAMMER, L., and WOLFF, P. TLC of phosphatides and glycolipids. Biochem. Z. 334: 175-184 (1961).
11. POMERANZ, Y., CHUNG, O., and ROBINSON, R. J. The lipid composition of wheat flours varying widely in bread-making potentialities. J. Am. Oil Chemists' Soc. 43: 45-48 (1966).
12. POMERANZ, Y., RUBENTHALER, G., and FINNEY, K. Polar vs. nonpolar wheat flour lipids in bread-making. Food Technol. 19: 1724-1725 (1965).
13. DAFTARY, R. D., POMERANZ, Y., SHOGREN, M., and FINNEY, K. Functional bread-making properties of wheat flour lipids. 2. The role of flour lipid fractions in bread-making. Food Technol. 22: 327-330 (1968).
14. COOKSON, M. A., RITCHIE, M. L., and COPPOCK, J. B. M. The role of lipids in baking. IV. Some further properties of flour lipids and defatted flours. J. Sci. Food Agr. 8: 105-116 (1957).

[Received May 13, 1968. Accepted July 9, 1968.]