Solvent Solubility Parameter and Flour Moisture Effects on Lipid Extractability

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ABSTRACT

Lipids were extracted with a series of solvent systems whose solubility parameter (δ) values ranged from 7.27 to 12.92 (hexane and its aqueous azeotrope, benzene, acetone and its azeotrope and 2-propanol and its azeotrope) from a flour with 1.2%, 7.2% or 13.8% moisture content (MC). The extracted total lipids (TL) were fractionated into nonpolar lipids (NL) and polar lipids (PL) by silicic acid column chromatography. NL and PL were further subfractionated by thin layer chromatography (TLC). PL were separated into glycolipids (GL) and phospholipids (PhL). TL, NL and PL extractabilities were affected by both flour MC and by the nature and composition of extractants: the overall solvent effects were substantially more pronounced than the flour MC effects. Free fatty acid and digalactosyldiglyceride (DGDG) extractabilities increased for all extractants, in general, as flour MC increased. Significant linear relations between the δ values of extractants and the average amounts of TL, PL, GL and PhL from flours with 3 MC were found. The high correlation (r = 0.981) between δ and extractability of TL was primarily caused by PL; extractabilities of monoglycerides (among NL) and all classes of GL and PhL were significantly (at the 0.01 level) linearly related to the δ values at all 3 flour MC levels. The present study indicated no selective binding between flour proteins and a specific PL class. Among PL, DGDG showed a clear-cut breaking point in irreversible restoration of breadmaking characteristics. Thus, the present study confirms the significant role of PL to breadmaking, resulting primarily from the contribution of DGDG.

INTRODUCTION

Flour lipids bound to proteins require the addition of water to an extracting organic solvent to disrupt lipoprotein complexes and increase lipid extractability (1-3). On the other hand, flour lipids bound to starch require an elevated temperature of extraction (90-100 C) besides the addition of water to an organic solvent, because starch lipids (mainly lysophosphatidyl cholines, PC) are tightly bound inside starch granules and can be extracted only when starch granules are gelatinized (4,5). Water-saturated n-butanol (WSB) is considered the most efficient extractant for nonstarch flour lipids at room temperature (5,6) and for starch lipids at elevated temperatures (4,5,7). WSB is inadequate, however, for studies on the role of lipids in breadmaking with the reconstituted flour (8). During lipid extraction, butanol forms a complex with starch and inhibits gas production during fermentation. At the present time, demonstrating the role of starch lipids in breadmaking by a reconstitution technique is almost impossible because starch granules of defatted flour are mostly gelatinized after starch lipids are removed.

Consequently, establishing conditions that maximize the extraction of lipids yet minimize damage to functional properties of wheat flour constituents is necessary in order to demonstrate the role of nonstarch flour lipids in breadmaking. Schmid and Hunter (9) reported that the relationship between polarity of lipids and solvent might have a significant effect on the solubility of lipid. Schmid (10) extended the solubility parameter concept of Hildebrand (11) to several binary solvent systems to interpret extractions of lipids from biological tissues. Chung et al. (12) found that total lipid extractability increased when the water content of wheat flour increased from 1.2% to 13.8% or when aqueous binary azeotropes were used as extractants. Rheological dough properties and baking characteristics of defatted and reconstituted flours were affected by moisture content (MC) of either flour samples or the solvent systems.

In this study, we determined the effects of flour MC and solubility parameter (δ) values of extractants on lipid composition. We used low levels of flour MC, because simply raising the MC of a flour-accelerated lipid binding to gluten proteins in work-free systems where no mixing was applied (13,14). When flour MC was between 20-45%, petroleum ether-extractable free lipids decreased, polar solventextractable bound lipids increased and the sum of extractable free and bound lipids decreased (15). Therefore, true extractabilities of lipids by solvents cannot be studied at higher ranges of flour MC. The information on the composition of extracted lipids as affected by low flour MC and δ values of extractants can be valuable. It can be used to relate adverse effects of irreversible binding of lipids on breadmaking properties and to demonstrate the role of specific lipid classes in native complex-formation in flour.

MATERIALS AND METHODS

Materials

An untreated, straight-grade flour (RBS-74) was experimentally milled (Allis) from a composite grist of many varieties of hard red winter wheat grown at locations throughout the Great Plains. The flour contained 12.4% protein (N \times 5.7) and 0.41% ash (both on 14% moisture basis) and had a good loaf volume (LV) potential and medium mixing and oxidation requirements.

Silicic acid for column chromatography of lipids was 100-mesh size from Mallinckrodt, Paris, KY. Organic solvents were analytical reagent grade, and solutions were prepared from analytical reagent-grade compounds. Reference lipids were from Supelco, Inc., Bellefonte, PA and Applied Science Laboratories, Inc., State College, PA.

Analytical Procedures

Protein, ash and moisture contents were determined by Methods 46-11, 08-01 and 44-15A of the AACC Approved Methods (16).

Lyophilization of Flours

Flours were lyophilized to 7.2% and 1.2% MC. The MC of ca. 150 g of flour was reduced from 13.8% to 7.2% in ca. 30 hr and to 1.2% in ca. 72 hr.

Extraction and Fractionation of Flour Lipids

Lipids were extracted (4 replicates each) from 10 g (on dry basis) flour containing 1.2%, 7.2% or 13.8% MC with hexane, benzene, acetone, 2-propanol, or the aqueous binary azeotropes of hexane, acetone or 2-propanol in a Soxhlet apparatus, as reported previously (12). The dried extracts, except for the hexane extract, were purified by being redissolved in petroleum ether. Flour lipids were

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fractionated (at least 2 replicates) by silicic acid column chromatography (17) into nonpolar and polar lipids with chloroform and methanol, respectively, as eluting solvents. Total column recovery ranged from 94.1% to 101.8% (average, 98.7%).

Thin Layer Chromatography (TLC) and Quantitative TLC

The method is similar to that described previously (18). The chromatographic solvents used for one-dimensional ascending development were: a mixture of hexane/diethyl ether/methanol (80:20:1, by volume) for steryl esters (SE) and triglycerides (TG) of nonpolar fractions; a mixture of hexane/diethyl ether/ethanol/methanol (80:10:10:1, by volume) for diglycerides (DG), monoglycerides (MG), and free fatty acids (FFA) of nonpolar fractions; and a mixture of chloroform/methanol/water (65:25:4, by volume) for glycolipids (GL) and phospholipids (PhL) of polar fractions obtained by column chromatography.

A densitometer (Photovolt Multiplier Photometer Model 530) with a scanning stage, Model 52-C, and Varicord Variable Response Recorder, Model 43 (Photovolt Corporation, New York, NY), was used to quantitatively assay 50 μ g lipids separated by TLC after they were sprayed with a $K_2 Cr_2 O_7$ solution in 55% $H_2 SO_4$ and heated for 25 min at 180 C. Each peak area on the recording chart was integrated twice using a Gelman planimeter. The average peak area of each component obtained from quadruplicated chromatograms (duplicated chromatograms of lipids from 2 extractions) was converted to weight by using the least square regression equation of standard lipids (18). Because some lipid classes, e.g., esterified steryl glucosides (ESG), N-acylated phosphatidyl ethanolamines (APE) and its lyso-compound (ALPE) were not available commercially, the calibration curve of steryl glucosides (SG) was used to convert area to weight of ESG, and calibration curves of phosphatidyl ethanolamines (PE) and its lyso-compound (LPE) were used for APE and ALPE, respectively.

Two minor components, whose R_f values were close to that of LPC, were not quantitated because no related reference lipids were available. Those components were sugarcontaining lipids: sugars were identified as sucrose and raffinose by paper chromatography (19). Those were not extracted by hexane, hexane azeotrope and benzene but were extracted by acetone, 2-propanol or their aqueous azeotropes.

RESULTS AND DISCUSSIONS

Effects of Flour Moisture on Lipid Extractability

Analysis of variance and Fisher's least significant difference were used to determine significance of factors (20). The overall extractabilities of total lipids (TL), nonpolar lipids (NL) and polar lipids (PL) were significantly (at 0.01 level) affected by flour MC and the type of extractant: the overall solvent effects were substantially more pronounced than flour MC effects.

Although lipid extractability generally increased slightly as flour MC increased, the effects of flour MC differed, depending on the type of solvents. Extractability of TL or PL with hydrocarbons (hexane, its azcotrope and benzene) increased more when flour MC increased from 7.2% to 13.8% than when flour MC increased from 1.2% to 7.2%. Ketone (acetone and its azcotrope) and alcohol (2-propanol and its azeotrope) extractabilities increased more with an increase of MC from 1.2% to 7.2% than from 7.2% to 13.8%. Increases in amounts of extracted lipids were statistically significant; as MC increased from 1.2% to 13.8%, depending on extractant, TL increased by 6-12 mg, NL by 0.3-7.4 mg and PL by 3.6-6.8 mg/10 g flour (data not shown).

Generally, extractabilities of FFA and digalactosyldiglyceride (DGDG) increased for all extractants as flour MC increased. Effects on extractabilities of FFA and DGDG contents only are shown in Table I to simplify presentation. The official AACC methods (02-01 and 02-02) for the determination of fat acidity (16) specify that for most accurate results, MC of grain should not exceed 11.0%. MC above 11.0% at time of extraction raises fat acidity significantly (16). Our results confirm the importance of flour moisture on the extractability of FFA, which is used as an index of changes in grain or flour during storage.

The water molecules are associated with specific chemical groups in starch, proteins, gums and other flour constituents (21). Especially important is the bonding between proteins and lipids (22). The adsorption isotherm of flour exhibits a sigmoid relationship between water activity (Aw) and MC (23). Water is bound at the low moisture region (MC < 14%, Aw < 0.7); not bound and free to condense as a liquid phase at moisture levels between 14% and 23%; and free in the true liquid phase and separated from the flour particles only in the high moisture region (MC > 23%, Aw < 0.95). In flour, water is bound tightly as a monomolecular layer between 0.0% and 6.5% MC and bound

TABLE I

FFA and DGDG (mg per 10 g flour, db) Extracted from Flours with 3 Moisture Contents with 4 Solvents and 3 Aqueous Binary Azeotropes

Lipids (mg)	Flour Mc (%)	Extractant (solvent/water, % composition) ^a							
		Hexane (100) bp=69.0 $\delta=7.27$	Hexane/water (94.4:5.6) bp=61.6 δ=8.18	Benzene (100) bp=80.1 $\delta=9.16$	Acetone (100) bp=56.5 δ=9.62	Acetone/water (88.5:11.5) bp=56.1 $\delta=11.22$	2-Propanol (100) bp=82.3 $\delta=11.44$	2-Propanol/water (87.8:12.2) bp=80.4 δ=12.92	
FFA					·				
	1.2	4.9	4.9	4.5	3.9	4.0	4.7	5.1	
	7.2	5.4	5.5	5.0	4.8	4.5	4.7	5.7	
	13.8	6.6	6.8	7.1	6.7	6.9	6.5	8.2	
DGDG									
	1.2	8.5	8.7	17.0	20.0	20.4	25.0	29.6	
	7.2	8.5	8.5	16.8	20.4	22.8	28.1	30.6	
	13.8	11.0	11.0	17.2	24.0	26.5	30.1	31.1	

 a_{bp} = boiling point (C). δ = Solubility parameter values from Hoy (24). The values for binary azeotropes were calculated from % composition of solvent and water.

fairly strongly as a second monolayer between 6.5% and 14% MC (23).

The packing of flour components is loosened to some extent by filling the first monolayer space with water as a result of increasing the MC from 1.2% to 7.2% and, to a larger extent, through formation of the second water monolayer by an increase in MC from 7.2% to 13.8%. Therefore, the increase in lipid extractability might be caused, at least in part, by the ease with which the solvent penetrates into flour components that pack in a less compact system as a result of the presence of bound water layers. Other factors that may be involved include degree of solvent accessibility, interaction-effects of solvents and the ability of water in extractant to break bonds between lipids and proteins.

Effects of Solubility Parameter Values of Extractants on Lipid Extractability

Extractants were pure solvents or aqueous binary azeotropes varying in solubility parameter (δ) values shown in Table I. Solubility parameter value is defined as the square root of cohesive energy density: $\delta = (\Delta E/V)^{1/2} = [(\Delta H - P\Delta V)/V]^{1/2}$, where δ is the solubility parameter, E the internal energy, V the molar volume, ΔH the heat of vaporization, and P the pressure (11). Extensive data on δ values of numerous solvents were reported by Hoy (24) by calculating from vapor pressure data of solvents. According to the solubility parameter theory (11), for a binary solvent $\delta = V_1\delta_1 + V_2\delta_2$, where V_1 and V_2 are volume fractions of solvents 1 and 2 for the binary solvents and δ_1 and δ_2 are corresponding δ values for the pure solvents (11). Adding water to the organic solvent increases the δ value of the binary azeotropes (Table I), because the δ value of pure water (23.53) is higher than δ of organic solvents. Lipids could not be extracted from flour with the benzene azeotrope. Benzene and water form an azeotrope, but are immiscible and form 2 layers. During refluxing, water formed a dough layer around the flour sample in the flour sample in the extractor. Benzene could not penetrate that layer; in addition, some free lipids were bound to gluten and made practically inextractable. The hexane azeotrope also formed 2 layers; we accomplished the extractions as reported previously (12), presumably because less water was in the hexane azeotrope than in the benzene azeotrope.

The average data (to simplify the presentation of results) of lipids extracted from flours with 3 MC (1.2%, 7.2% and 13.8%) are given in Table II. Extractabilities of TL and PL were significantly affected by extractants. Extractability of NL by 2-propanol azcotrope was significantly different from those by the other extractants.

Significant linear relations were found between δ values of extractants and the average amounts of TL, PL, GL and PhL but not with the average NL (Table III). Extractabilities of TL, PL, GL and PhL were linearly related to the extractant δ at the 3 flour MC levels; extractability of NL was linearly related to δ values at the 1.2% MC level only (data not shown).

The significant linear relation between TL and δ value was mainly from PL, because the rate of extraction (B values in Table III) for PL was ca. 8.5-fold greater than that for NL. Extractability of flour lipids was linearly related to δ values in the range of 7.27 to 12.92, even though Schmid and coworkers postulated that solvents ranging in δ values between 10.5 and 11 would maximize extraction of lipid mixtures from biological samples (10,25).

Among NL, extractabilities of MG and DG were linearly

TABLE II

Average Flour Lipids (mg per 10 g flour, db) Extracted from Flours with 3 Moisture Contents (1.2%, 7.2% and 13.8%) with 4 Solvents and 3 Aqueous Binary Azeotropes

	Extractant (solvent/water, % composition) ^a								
Lipids ^b (mg)	Hexane (100)	Hexane/water (94.4:5.6)	Benzene (100)	Acetone (100)	Acetone/water (88.5:11.5)	2-Propanol (100)	2-Propanol/water (87.8:12.2)		
Total	92.38	96.3h	118.7 ⁱ	126.3 ^j	134.3 ^k	147.3 ¹	170.7 ^m		
Nonpolar	66.9g	72.1g	69,3g	68.0g	69.0 g	70.0g	80.0 ^h		
SE	5.3	5.6	5.3	4.6	4.8	5.0	5.6		
TG	44.6	48.8	46.4	45.4	46.0	45.2	51.7		
DG	6.4	7.0	6.9	6.9	6.6	7.2	8.0		
MG	4.9	5.0	5.1	6.0	6.5	7.4	8.5		
FFA	5.6	5.7	5.5	5.1	5.1	5.3	6.3		
Polar	25 5g	24.3g	49.4h	58.3 ⁱ	65.4j	77.3k	90.6 ¹		
ESG	0.4	0.4	0.5	0.6	0.6	0.7	0.8		
SG	0.6	0.5	1.1	1.1	1.4	1.4	1.8		
MGDG	6.9	7.0	11.4	10.4	11.9	12.1	17.6		
DGDG	9.3	9.4	17.0	21.5	23.2	27.7	30.4		
Sum of GL	17.2	17.3	30.0	33.6	37.1	41.9	50.6		
PE + APE	3.5	2.9	7.3	5.8	8.0	8.2	10.1		
PC	2.5	2.4	7.5	6.2	6.4	8.5	9.9		
LPE + ALPE	0.5 c	0.5 c	1.0 ^c	3.2	3.8	5.2	5.8		
LPC		_	1.6 ^c	7.6	7.7	10.1	10. 6		
Sum of PhL	6.5	5.8	17.4	22.8	25.9	32.0	36.4		

^aSee Table I for boiling points and solubility parameter values of solvents.

bWithin total, nonpolar and polar lipids, values with different letters differ significantly at the 0.01 level. SE = steryl esters, TG = triglycerides, DG = diglycerides, MG = monoglycerides, FFA = free fatty acids, SG = steryl glucosides, ESG = esterified SG, MGDG = monogalactosyldiglycerides, DGDG = digalactosyldiglycerides, GL = glycolipids, PE = phosphatidyl ethanolamines, APE = N-acylated PE, PC = phosphatidyl choline, LPE = lyso-PE, ALPE = N-acylated LPE, LPC = lyso-PC, PhL = Phospholipids.

^cValues for flour with 13.8% moisture were 1.5mg, 1.5mg, 3.1mg and 4.8 mg, respectively; none was extracted from flours with 1.2% or 7.2% moisture.

TABLE III

Intercept (A) and Slope (B) of Equation Y = A + BX and Simple Linear Correlation Coefficient (r) between Solubility Parameter Value (δ) of Extractant (X) and Average Amount (mg/10 g flour) of Lipids Extracted (Y) from Flours with 3 Moisture Contents (1.2%, 7.2% and 13.8%)

Lipids ^a	А	В	rb
Total	-9.6	13.65	0.981
Nonpolar	56.4	1.44	0.652 (NS)
ŜĒ	5.4	-0.02	-0.102 (NS)
TG	40.9	0.60	0.475 (NS)
DG	5.1	0.19	0.732
MG	-0.3	0.66	0.954
FFA	5.1	0.05	0.216 (NS)
Polar	-65.7	12.19	0.972
ESG	-0.2	0.07	0.962
SG	-1.1	0.22	0.965
MGDG	-5.9	1.70	0.934
DGDG	-20.5	4.04	0.966
Sum of GL	-27.7	6.04	0.975
PE + APE	-5.7	1.23	0.933
PC	-6.5	1.28	0.890
LPE + ALPE	-7.8	1.07	0.952
LPC	-16.4	2.18	0.924
Sum of PhL	-36.4	5.75	0.967

^aAbbreviations are given in Table II.

bFor 5 degrees of freedom, r values of 0.875, 0.755 and 0.669 were significant at the 0.01, 0.05 and 0.1 levels, respectively. NS = not significant at the 0.1 level.

related to δ values of extractants (0.01 and 0.1 levels of significance; respectively) (Table III); significant linear relations occurred between extractabilities and δ values for MG at all 3 MC levels and for DG at MC level 13.8% only (data not shown). No significant linear correlation was found between amounts of the other NL classes and δ values of extractant.

The effect of the extractant δ values on the rate of extraction (B values in Table III) for GL and PhL was similar. Among GL, δ values of extractants affected extractability of DGDG most, of monogalactosyldiglycerides (MGDG) less and of ESG least. The effects of extractant δ values seemed to be related to the polarities of GL lipid classes: the more polar the GL component, the more affected its extractability by the δ value of the extractant. Among PhL, extractability of LPC was affected most by the δ values of extractants (Tables II and III); hexane (δ 7.27) and hexane azeotrope (δ 8.18) extracted no measurable quantities of lysophosphatidyl cholines (LPC) at any MC (Table II). The flour contained comparable quantities of PC and PE + APE (Table II) and their extractability rates were almost equal (Tables II and III).

Effects of Restoring Flour Lipids on Breadmaking Properties

We reported previously that adverse effects of lipid extraction and reconstitution on rheological and breadmaking properties were smallest for hexane and largest for 2propanol and that the adverse effects increased with MC of the flour, especially from 7.2% to 13.8%, or from the addition of water to the solvent (12). Loaf volumes (LV) of breads baked from reconstituted flours (12) are plotted against the quantity of lipids in reconstitution studies (Fig. 1).

LV depended on the amount of lipids involved in recon-



FIG. 1. Loaf volumes of breads (data from ref. 12) baked from flour defatted and then reconstituted in relation to amounts of unfractionated total lipids (TL) and polar lipids (PL), extracted by pure solvents (hexane, benzene, acetone and 2-propanol) or aqueous binary azeotropes of hexane, acetone and 2-propanol and then added back to the defatted flour before baking.

stitution and on the extractant (pure solvents or aqueous binary azeotropes). With pure solvents, the more TL or PL were removed and then added back, the more adverse were the effects on LV. Sharp drops occurred in LV when 150 mg TL (79.8 mg PL) were reconstituted (Fig. 1). With aqueous azeotropes, LV from reconstituted flours were more or less constant up to 138 mg TL (67.7 mg PL) and then decreased sharply (Fig. 1). Similar trends were shown when LV were plotted against GL, PhL or individual lipid classes including MGDG, DGDG, PE + APE and PC (results not shown). Thus, the presence of water in extractants had adverse effects on breadmaking properties while enhancing extraction of lipids bound to flour proteins. An irreversible modification of protein structure is probably involved.

As DGDG is the major class among PL (Table II), and numerous studies have demonstrated that GL, especially DGDG, are more significant in breadmaking than PhL (26-30), characteristics of breads from reconstituted flours (12) were related to the amount of DGDG involved in reconstitution (Figs. 2 and 3). The breads in the top row were baked from flours reconstituted with lipids extracted by 2-propanol and the breads in the bottom row from flours reconstituted with lipids extracted by 2-propanol azeotrope (Fig. 2). Flour MC before lipid extraction were different: breads 1 and 4 were from flour with 1.2% MC, breads 2 and 5 were from flour with 7.2% MC and breads 3 and 6 were from flour with 13.8% MC before lipid extraction. The bar on the right side of each bread represents the amount of reconstituted DGDG. The amount (mg/10 g flour) of DGDG was 25.0 for bread 1 and 30.1 for bread 3. Thus, a relatively small increment of only 5 mg DGDG/10 g flour must have been structurally involved in lipid-protein interaction of flour. When the extractant contained water, an increase in flour moisture from 1.2% to 13.8%, which was bound water, did not affect breadmaking properties of flours defatted with the aqueous 2-propanol azeotrope because the extraction of DGDG already irreversibly destroyed breadmaking quality (Fig. 2).

Fig. 3 shows the effects of extractants with various δ values on breadmaking properties. All breads were baked from defatted flours of 7.2% MC that had been reconstituted. Breads in the top row were from flours defatted with pure solvents and breads in the bottom row from flours



FIG. 2. Bread (10 g reconstituted flours baked with 3% shortening) made from flours extracted with 2-propanol (top row) and with 2-propanol aqueous azeotrope (bottom row) and then reconstituted (picture of breads adapted from reference 12) in relation to the amounts of digalactosyldiglycerides (DGDG) involved in flour defension and memory flour with 1.2% defatting and reconstituting. Breads 1 and 4, from flour with 1.2% moisture; breads 2 and 5, flour with 7.2% moisture; and breads 3 and 6, flour with 13.8% moisture before lipid extraction.

defatted with aqueous azeotropes and then reconstituted. Breads 1 and 4 were from flours treated with hexane and its azeotrope, respectively; breads 2 and 5 from acetoneand its azeotrope-treated flours; breads 3 and 6 from 2-propanol- and its azeotrope-treated flours. The more DGDG that was taken out and then added back, the smaller the loaf of bread (Fig. 3). Extracted lipids, in each case, were added back to defatted flours and all breads were baked from flours that contained the same quantity of lipids as the original flours (12).

As more lipids were extracted, bonds between PL (GL + PhL) and proteins apparently broke and adding back the lipids did not restore the original lipid-protein interaction. The present study shows no selective binding between flour proteins and a specific PL class. DGDG was the main PL. In addition, extraction of DGDG showed (Table I, Figs. 2 and 3) the most clear-cut breaking point in irreversible restoration of breadmaking characteristics. Thus, the present study confirms that the significant contribution of PL to breadmaking results primarily from DGDG (26-30). Nevertheless, we cannot exclude significant effects of moisture in flour and especially in extractants on breadmaking characteristics of reconstituted flours. If one is interested only in maximizing lipid extraction, 2-propanol aqueous azeotrope extraction by a Soxhlet from flour with a normal MC may be the right choice. However, if one is interested in maximizing lipid extraction and, at the same time, minimizing irreversible damage to functional properties, extraction with 2-propanol by a Soxhlet from a flour with low MC may be a sensible thing to do.

Our results indicate that δ values of extractants, ranging from 7.27 to 12.92, are important, irrespective of solvent, for establishing a linear relation with lipids extracted by 3 classes of solvents (hydrocarbons, ketone and alcohol) and their aqueous azeotropes. The relation between the δ values of extractants and lipid extractability could have applications in maximizing lipid extraction from samples of biological origin.

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30 -(MG/IOG FLOUR) 20 10 ٥L 30 20 DG 10 ഗ õ 0

FIG. 3. Bread (10 g reconstituted flours baked with 3% shortening) made from flour (7.2% moisture) extracted with pure solvents (top row) and with their aqueous azeotropes (bottom row) and then reconstituted (picture of breads adapted from reference 12) in rela-tion to the amounts of digalactosyldiglycerides (DGDG) involved in flour defatting and reconstituting. Breads 1 and 4, treated with hexane; breads 2 and 5, acetone; and breads 3 and 6, 2-propanol.

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[Received July 18, 1983]