Evaluation of Factors Affecting Lipid Binding in Wheat Flours

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The effects of lipid fractions, mixing time, and dough ingredients on lipid binding were studied. Up to 1.5% unsaturated corn oils were bound in dough mixed from petroleum ether–extracted flour; less saturated corn oils and fats were bound. Much more unsaturated oils than saturated fats were bound in bread crumb. Increasing length of dough mixing increased binding of free flour lipids; binding decreased slightly during prolonged overmixing. Adding 2 or 4% sodium chloride to the dough had little effect on the binding of polar wheat flour lipids (added to an extracted flour), but significantly reduced binding of nonpolar lipids. The results suggest that the action of salt was on the gluten proteins and not on salt-like linkages. Presence of nonpolar lipids reduced binding of polar lipids. More bound flour lipids were released by remixing a dough with an NaCl solution than by remixing with water. Practically no added nonpolar lipids were bound by dry-mixing with flour, but substantial amounts of free polar lipids were bound during dry-mixing with a flour containing 4.4% moisture. The binding increased with increases in moisture contents and was highest in dough.

Earlier studies on the interaction between lipids and other wheat flour components (Mecham and Weinstein, 1952; Olcott and Mecham, 1947) were extended recently by researchers from several laboratories (Daniels *et al.*, 1967; Ponte *et al.*, 1964; Wootton, 1966).

Patent wheat flour contains about 0.8% free lipids that are extractable with petroleum ether (Pomeranz, 1967). The free lipids can be separated into two fractions according to their elution from a silicic acid column. One fraction of about 0.6% lipids can be eluted with chloroform, and is arbitrarily called the nonpolar fraction. This fraction contains mainly triglycerides and smaller amounts of hydrocarbons, sterols, steryl esters, monoglycerides, diglycerides, and free fatty acids. The second fraction (0.2% free lipids) can be eluted from the column with a more polar solvent, like methanol, and is a mixture of free polar lipids. Free polar lipids are rich in glycolipids, and contain relatively small amounts of phospholipids.

In addition to 0.8% free lipids, patent wheat flour also contains 1.0% total bound lipids. About 0.6% bound lipids (mainly polar) can be extracted from flour with water-saturated butanol, following the petroleum ether extraction. However, during gluten washing or breadmaking, part of the nonpolar components becomes bound (unextractable with petroleum ether). Extractability of lipids from whole wheat flour or milled wheat products depends also on particle size of the material. Thus, more lipids will remain unextracted with petroleum ether from wheat ground to pass a 20-mesh sieve than from wheat ground to pass a 60-mesh sieve. In flour, which is milled to pass a 100-mesh sieve, particle size is less important. The bound polar lipids contain a mixture of glycolipids and phospholipids. While free and bound polar lipids contain the same kinds of components, the latter contain relatively more phospholipids.

Wheat flour also contains about 0.4% strongly bound lipids that cannot be extracted with any of the above solvents. Fatty acids in the strongly bound lipids can be isolated from the flour lipids after acid hydrolysis. Little is known about the nature of the strongly bound lipids in the intact flour.

Previous studies (Chiu and Pomeranz, 1966) showed that two thirds of the original free flour lipids were bound during dough-mixing and fermentation; after baking, five sixths were bound. Only small amounts of hydrogenated vegetable shortening were bound during dough-mixing, but one third to half of the added shortening lipids became bound during baking. Higher percentages of polar than nonpolar wheat flour lipids were bound during dough-mixing. More recently (Chiu *et al.*, 1968), gluten was found to contain five times as much proteins and lipids as the original flour. A mixture of starch and water-solubles contained a low concentration of lipids; the distribution of nonpolar and polar lipids in the mixture varied from that of lipids in gluten. No measurable amounts of shortening lipids were bound during mixing of doughs from untreated flours, but about 0.2% was bound in doughs from pe-

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troleum ether–extracted flours. Binding of flour lipids during dough mixing increased with protein content and mixing time, and decreased with decreasing protein quality. Daniels *et al.* (1967) found that binding of shortening fat in premixed doughs decreased during prolonged mixing at relatively high speeds (up to 378 r.p.m.).

This study was conducted to determine the effects of lipid fractions, mixing time, and dough ingredients on lipid binding.

EXPERIMENTAL

Flours. Four experimentally milled, hard red winter wheat flours were used. Regional baking standard (RBS) was milled from a composite of wheat varieties. It had a good loaf volume potential and a medium mixing time. Qvivira-Tenmarq × Marquillo-Oro (C.I. 12995) had good loaf volume potential and a relatively long mixing time. Comanche had good loaf volume potential and medium mixing time. Chiefkan × Tenmarq (K501099) had a poor loaf volume potential and a short mixing time. The four flours were milled from composites of wheat harvested at many locations throughout the Southern and Central Great Plains, U.S.A.

Table I summarizes some of the chemical and breadmaking properties of the flours. The flours were untreated or were exhaustively extracted (twice for 8 to 10 hours) with petroleum ether (b.p. 35° to 60° C.) in a large Soxhlet extractor. Residual petroleum ether in the flours (after each extraction) was allowed to evaporate at room temperature.

Analytical Determinations. Moisture, ash, and Kjeldahl protein were determined by the AACC method (American Association of Cereal Chemists, 1962). All analytical results are expressed on a 14% moisture basis. Free lipids were extracted with petroleum ether in a Goldfish extractor from flour or from lyophilized and ground (40-mesh sieve on a micro-Wiley mill) doughs. To determine bound lipids, petroleum ether–extracted flours were extracted with water-saturated butanol. After the butanol extract was centrifuged, the supernatant was evaporated under reduced pressure and redissolved in petroleum ether prior to final evaporation of solvent. Total lipids were extracted directly from flour with water-saturated butanol. The lipids were characterized by thin-layer chromatography (Daftary and Pomeranz, 1965). The pure compounds used to identify lipid components included trilinolein, phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine, monogalactosyl diglyceride, and digalactosyl diglyceride. The solvents used for one-dimensional ascending development of 100-μg. lipids were: chloroform for nonpolar lipids and chloroform-methanol-water (65:25:4) for polar lipids. The spots were visualized by spraying with a saturated solution of $K_2Cr_2O_7$ in 70% (by volume) aqueous sulfuric acid, and charring at 180° C. for 30 minutes. Pictures were obtained under ultraviolet light. More specific spraying methods included ninhydrin (for amino acid–containing lipids), modified Dragendorff reagent and 1-naphthol (for glycolipids), and molybdenum spray (for phosphorus).

Lipids. For preparative purposes, free lipids were extracted from flour with petroleum ether in a large Soxhlet extractor. Total flour lipids were isolated from the composite flour with water-saturated butanol. The flour lipids were fractionated into nonpolar and polar components by silicic acid column chromatography (Daftary and Pomeranz, 1965). Oils and fats varying in iodine value and melting points were prepared on a laboratory scale by hydrogenation of commercial refined corn oil (iodine value 127.5) with 0.1% fresh nickel catalyst at atmospheric pressure. Samples with iodine values of 40.6 and above were hydrogenated at 165° C.; with iodine values of 22.5 and 2.3 at 190° C. Flour-lipid mixtures were prepared by premixing the lipids with part of the flour in a mortar, and remixing with the rest of the flour in a Stein mill for 45 seconds.

Breadmaking. Baking tests were made on a laboratory scale from 100 grams of flour (14% moisture basis). The formula included (on flour basis), in per cent: sucrose, 6; salt, 1.5; yeast, 2; 60° L. malt sirup, 0.5; nonfat milk solids, 4; commercial vegetable shortening, 3; water as needed; and optimum potassium bromate. An optimum mixing time with the straight-dough procedure and a 3-hour fermentation time at 30° C., were employed. Punching and panning were performed mechanically. Baking time was 24 minutes at 218° C. Loaf volumes were measured by dwarf rape seed displacement. Differences of 25 cc. were significant at the 5% level. After the loaves had cooled, they were cut and their crumb grains evaluated. The following code was employed: S satisfactory; Q questionable; and U unsatisfactory.

RESULTS AND DISCUSSION

Binding of hydrogenated corn oils and fats (added at the level of 3 grams per 100 grams of flour) is summarized in Table II. The doughs contained, in addition to water, flour and corn lipids only. Both untreated and petroleum ether–extracted flours were used. Small amounts (0.2 to 0.7%) of unsaturated corn oils were bound in doughs prepared from the untreated flour. In doughs from petroleum ether–extracted flours, up to 1.5% of unsaturated corn oils were bound. The binding increased with the degree of unsaturation.

Table I. Chemical Analyses and Certain Breadmaking Properties of Flours

Flours	Ash, %	Protein (N × 5.7), %	Water Absorption, %	Bromate Requirement, P.P.M.	Loaf Volume, Cc.	Crumb Grain
RBS	0.42	12.9	62.3	30	960	S
C. I. 12995	0.42	12.3	63.2	10	918	S
Comanche	0.42	12.9	64.2	20	919	S
K 501099	0.43	12.6	65.1	40	700	U

Table II. Binding of Hydrogenated Corn Oils and Fats (Added at Level of 3 Grams per 100 Grams of RBS Flour) in Flour-Water Doughs

Iodine Value of Corn Oil	Melting Point of Corn Oil	Free Lipids (%) in Dough From: Untreated flour	Free Lipids (%) in Dough From: Petroleum ether–extracted flour
None	...	0.23	0.09
127.5	Below 25	2.72	1.53
115.0	Below 25	2.57	1.60
97.1	Below 25	2.49	1.50
60.2	34	2.96	2.22
40.6	50	2.80	2.54
22.5	58	2.93	2.75
2.3	69	2.98	2.76

Slightly more lipids were bound in doughs containing all baking ingredients (Table III) than in water-flour doughs (Table II). Bread baked from untreated flour had consistently a larger loaf volume and generally better crumb grain than bread baked from petroleum ether–extracted flour. In untreated flour, any of the added oils and fats increased loaf volume above that of the control. Adding fat with an iodine value of 60.2 increased loaf volume most with both untreated and extracted flours. Much more unsaturated oils (iodine value greater than 60) than saturated fats (iodine value less than 60) were bound in bread crumb.

The untreated flour contained 0.8% free lipids that were extractable with petroleum ether. The free lipids consisted of about three fourths nonpolar and one fourth polar components. In petroleum ether–extracted flours, increasing the length of mixing, in general, increased the binding of free lipids (Table IV). However, mixing for 8 minutes (considerably beyond the point of optimum consistency) freed a small amount of the bound lipids. Similar changes were observed during excessive mixing of doughs containing all baking ingredients (no data given). There was, however, no consistent effect of flour quality on rate and amount of lipid binding, except where 3.2% of lipids were added.

Mecham and Weinstein (1952) studied the effects of bread dough ingredients on lipid binding during dough formation and gluten washing. Sodium chloride decreased binding of total lipids and of phospholipids in doughs. Similarly, the lipid content of glutens washed out in salt solutions was lower than in glutens washed out in water. They concluded that lipid binding was partly ionic in nature. Involvement of saltlike linkages in gluten formation was suggested by Grosskreutz (1961) in a paper on the lipoprotein model of wheat gluten structure. The existence of an ionic or saltlike linkage between phospholipids and proteins was also postulated on the basis of radio-active studies conducted by Lee and Wan (1963). The importance of saltlike linkages, in gluten structure, between phospholipids and proteins was stressed by Pence *et al.* (1964).

The effects on lipid binding (in water-flour doughs from unextracted and petroleum ether–extracted RBS flour) of increasing concentration of sodium chloride are summarized in Table V. More added lipids were bound in petroleum ether–extracted than in untreated flour.

Table IV. Effects of Mixing Time on Binding Free Flour Lipids in Water Doughs from Four Petroleum Ether–Extracted Flours

Dough Mixing Time, Min.	Free Lipids in Doughs Containing 0 to 3.2 Grams of Added Lipids/100 Grams of Four, %: 0	0.8	1.6	3.2
	K501099			
3/4	0.10	0.25	0.55	1.56
Optimum[a]	0.09 (1 1/4)	0.18 (1 3/8)	0.41 (1 1/2)	0.91 (1 1/2)
4	0.06	0.18	0.33	0.86
8	0.07	0.18	0.33	0.89
	RBS			
3/4	0.06	0.35	0.63	1.58
Optimum[a]	0.03 (2 3/8)	0.27 (2 3/8)	0.43 (2 1/2)	1.08 (2 5/8)
4	0.04	0.25	0.37	0.81
8	0.04	0.28	0.48	1.03
	Comanche			
1	0.04	0.25	0.58	1.56
Optimum[a]	0.06 (1 3/4)	0.22 (2 1/8)	0.35 (2 7/8)	0.87 (2 7/8)
4	0.05	0.24	0.36	0.74
8	0.05	0.24	0.42	0.88
	C.I. 12995			
3/4	0.07	0.24	0.61	1.74
2	0.04	0.15	0.31	0.90
Optimum[a]	0.04 (3)	0.13 (4 1/8)	0.20 (4 3/8)	0.63 (4 5/8)
8	0.05	0.16	0.27	0.67

[a] Optimum mixing time for each lipid level in parenthesis.

Table III. Binding of Hydrogenated Corn Oils and Fats (Added at Level of 3 Grams per 100 Grams of RBS Flour) in Dough and Crumb of Bread Baked by Complete Formula

Iodine Value of Corn Oil	Untreated Flour: Loaf volume, cc.	Crumb grain	Free Lipids, %: In dough	In bread	Petroleum Ether–Extracted Flour: Loaf volume, cc.	Crumb grain	Free Lipids,[a] %: In dough	In bread
None	850	Q-S	0.26	0.13	793	S	0.11	0.04
127.5	905	Q-S	2.69	1.53	748	Q-S	1.58	1.02
97.1	903	Q-S	2.54	1.48	768	Q-S	1.50	1.01
78.0	895	S	2.63	1.51	753	Q-S	1.60	1.02
60.2	923	S	2.63	2.30	828	Q-S	1.97	1.40
22.5	898	S	2.73	2.69	773	Q-S	2.19	2.39

[a] Expressed on 14% moisture basis.

Increasing the level of sodium chloride decreased lipid binding. In extracted flour, the effect is much more pronounced with nonpolar and total free than with polar lipids. Actually, with petroleum ether–extracted flours binding of polar lipids (in the absence of nonpolar lipids) was virtually unaffected by adding sodium chloride (Table V). Thus, it appears unlikely that adding NaCl disrupts saltlike linkages between proteins and lipids. That binding of nonpolar lipids is substantially reduced by the presence and level of added sodium chloride points to the possibility of lipid binding's being affected by sodium chloride effects on the gluten proteins, and not on ionic bonds between lipids and proteins.

Table V. Effects of NaCl Concentration on Binding of Lipids in Doughs from Untreated and Petroleum Ether–Extracted RBS Flour

NaCl Concn., %	Lipids Added, %	Free Lipids[a] (%) in Doughs Mixed with: Total free lipids	Polar lipids	Nonpolar lipids
		Untreated		
0	0	0.20	0.20	0.20
	0.75	0.42	0.44	...
	1.50	0.83	0.64	0.61
	3.00	1.87	2.25	...
	6.00	4.51	3.75	2.39
2	0	0.27	0.27	0.27
	0.75	0.71	1.08	...
	1.50	1.47	1.62	1.17
	3.00	2.77	2.75	...
	6.00	5.24	4.95	3.55
4	0	0.33	0.33	0.33
	0.75	1.06	1.25	...
	1.50	1.43	1.76	1.65
	3.00	2.68	3.10	...
	6.00	5.38	5.49	5.83
		Petroleum Ether–Extracted		
0	0	0.06	0.06	0.06
	1.50	0.72	0.17	0.30
	6.00	3.52	3.17	1.70
2	0	0.08	0.08	0.08
	1.50	0.90	0.36	0.42
	6.00	4.61	3.23	2.54
4	0	0.06	0.06	0.06
	1.50	0.98	0.35	0.65
	6.00	4.37	3.30	2.80

[a] Expressed on a 14% moisture basis.

The results of this study also make it possible to make certain deductions regarding the possible mode of lipid binding in dough. Grosskreutz (1961) proposed a lipoprotein model of wheat gluten structure favoring the existence of bimolecular leaflets of the type found in myelin structures. Benson (1966) pointed to limitations and inadequacies of the bimolecular lipid leaflet model in organization of chloroplast and cell membranes. To control lipid orientation most directly, the proteins involved would have to be essentially basic. Wheat proteins, however, are not basic and contain large amounts of acidic amino acids. Benson (1966) proposed a corpuscular model of protein, the interior of which is largely hydrophobic, with hydrophilic and charged amino acids predominating on the molecule's exterior.

Adding sodium chloride decreases the solubility of gluten proteins and makes them more compact. This, in turn, decreases binding of nonpolar lipids by the hydrophobic regions in the interior of the protein. Certain sites on the surface of the protein molecule seem to bind polar lipids preferentially. The binding of polar lipids is, however, limited, and in doughs from petroleum ether–extracted flours (Table V) to which 6.0% lipids were added more nonpolar than polar lipids were bound. It seems also that binding of large amounts of nonpolar lipids reduces binding of polar lipids. In doughs to which 6% lipids were added, less total free lipids (containing a mixture of 75% nonpolar and 25% polar components) were bound than calculated from the amounts bound when each of the lipids was added alone. Similarly, much less nonpolar or polar lipids were bound in untreated than in petroleum ether–extracted flours (Table V).

To study the possible effects of polar lipids on binding of nonpolar lipids, lipids were added and doughs were mixed from petroleum ether–extracted flours in two stages. The amount of lipids added in each stage was 1.5%. The doughs were remixed after stage II (without adding lipids) with either water or 4% sodium chloride. After each mixing stage, the doughs were frozen, lyophilized, and pulverized prior to lipid extraction and moisture determination. The doughs were mixed in stage I for 2 minutes, and remixed in stages II and III to optimum (Table VI). The presence of polar lipids reduced the

Table VI. Effects on Lipid Binding of Mixing Doughs from Petroleum Ether–Extracted RBS Flour in Three Stages

Stage I[a]		Stage II[b]		Stage III[c] Free Lipids[f] in Doughs Remixed with	
Description of added lipid[d,e]	Free lipids,[f] %	Description of added lipid[d]	Free lipids,[f] %	Water, %	4% NaCl, %
O	0.06	O	0.05	0.12	0.08
NP	0.32	NP	0.65	0.37	1.06
P	0.22	P	0.33	0.34	0.74
NP	0.33	P	1.03	0.62	0.90
P	0.24	NP	0.93	0.73	1.28
NP	0.32	...	...	0.33	0.46
P	0.22	...	...	0.17	0.19

[a] Mixed for 2 minutes, frozen, lyophilized, and ground for use in stage II.
[b] Remixed to optimum consistency following stage I, frozen, lyophilized, and ground for use in stage III.
[c] Remixed (or mixed) to optimum consistency following stage II (or I).
[d] At level of 1.5 grams per 100 grams of pulverized material on 14% moisture basis.
[e] O = control, NP = nonpolar, P = polar.
[f] Expressed on 14% moisture basis.

binding of nonpolar lipids (and vice versa) as less lipids were bound in stage II if 1.5% nonpolar and 1.5% polar lipids were added, than if 3.0% of either alone was added. The order in which the polar and nonpolar lipids were added seems, however, to have little effect on the total amount of bound lipids (stage II). Similarly, fractionation by silicic acid column and thin-layer chromatography (Figure 1) showed that the order of lipid addition had no effect on binding nonpolar or polar components. The weak forces involved in binding of lipids to flour components are indicated by partial release of bound lipids on repeated mixing (stage III). The release was higher in doughs mixed in stage III with a 4% NaCl solution than in doughs mixed with water alone.

Olcott and Mecham (1947) postulated that about one third of the lipids present in flour are in a bound form. Additional lipid (about one third) forms complexes when flour is wetted and a major part of the residual free lipids is bound when a dough is mixed. To investigate the effects of flour moisture content on lipid binding, lipid fractions were added to flours varying in moisture contents (Table VII). Neither total free nor nonpolar lipids were bound by flours of 13.4% or less moisture when mixed in a Stein mill for 45 seconds. Small amounts—about 0.5%—of total polar lipids (extracted with water-saturated butanol) were bound by a flour with 8.5% moisture. About 2.2% polar free lipids were bound by flour that contained

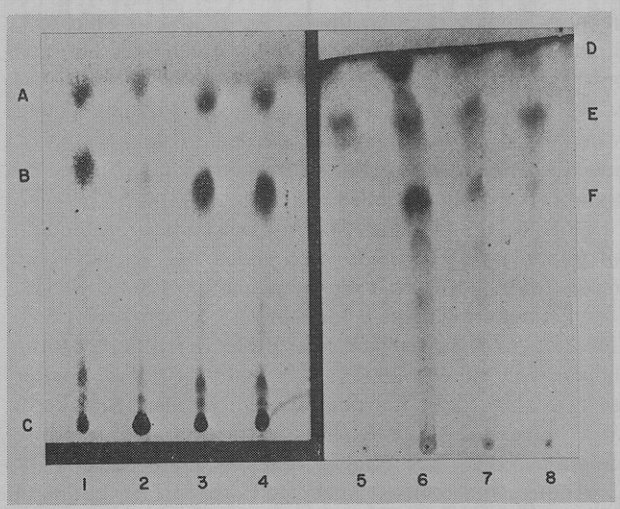

Figure 1. Thin-layer chromatography of free lipids from doughs

Mixed with:
1,5. 1.5 % nonpolar flour lipids
2,6. 1.5% polar flour lipids
3,7. 1.5% nonpolar followed by 1.5% polar lipids
4,8. 1.5% polar followed by 1.5% nonpolar lipids

Fractionation of 100-μg. lipids with chloroform (1 to 4) or chloroform-methanol-water (65:25:4) (5 to 8)
Tentative identification.
A. Hydrocarbons and sterol esters
B. Triglycerides
C. Unfractionated polar lipids
D. Unfractionated nonpolar lipids
E. Mixture of monogalactosyl diglyceride and phosphatidyl ethanolamine
F. Digalactosyl diglyceride

Charred with saturated $K_2Cr_2O_7$ in 70% H_2SO_4

Table VII. Binding of Added Flour Lipids (3 Grams per 100 Grams of Flour) by Petroleum Ether–Extracted RBS Flour Varying in Moisture Contents

Lipid Description	Water Contents, %	Free Lipids,[a] %
Total free	8.5	3.04
	13.4	3.03
Nonpolar	13.4	3.04
Polar total	8.5	2.53
Polar free	4.4	1.20
	13.4	0.80
	45.6	0.27

[a] Expressed on 14% moisture basis.

13.4% moisture. When flour moisture content was reduced to 4.4% (under vacuum at room temperature) 1.8% polar free lipids were bound. Increasing the moisture to 45.6%, in a dough mixed to optimum consistency, increased the amount of bound polar free lipids to about 2.7%. Fractionating free lipids by thin-layer chromatography showed no consistent or significant differences in composition of free polar lipids bound by flours varying in moisture content. The reasons for less binding of total polar lipids (extracted with water-saturated butanol) than of free polar lipids (extracted with petroleum ether) is unknown. Those differences suggest the need for further investigations.

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