

Lipid Binding and Fatty Acid Distribution in Flour, Dough, and Baked and Steamed Bread

K. ADDO^{1,2} and Y. POMERANZ¹

ABSTRACT

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Lipid binding and fatty acid distribution were determined in flour, mixed dough, and crumb of baked or steamed bread. Dough mixing, baking, and steaming of the original flours reduced extractability of free (petroleum-ether extractable) flour lipids. No significant difference was found in the extent of lipid binding in native flour as a result of baking and steaming. In experiments with untreated flour, shortening lipids were bound during baking or steaming but not during dough mixing. In experiments with petroleum-ether defatted flour, shortening lipids were also bound in the dough. Flour lipids were bound in preference to short-

ening lipids. Protein content was apparently not the only factor associated with degree of lipid binding. More unsaturated oils than saturated fats became bound during dough mixing and baking. In untreated flour supplemented with 2% shortening, baking and steaming resulted in more binding of saturated fats and unsaturated oils than dough mixing did. In defatted flour, the binding of unsaturated oils increased in order of dough mixing, baking, and steaming. In general, steaming resulted in more shortening binding than baking did.

The effects of native wheat flour lipids and of added lipids on dough-handling properties and the quality of baked products have been well documented (Tao and Pomeranz 1968, DeStefanis and Ponte 1976, Morrison 1976). Reduction in the amounts of lipids extracted after hydration, dough development, and baking have been reported (Olcott and Mecham 1947, Chiu and Pomeranz 1966, Fisher et al 1973, Chung and Tsen 1975, Carr et al 1989). Such a reduction in freely extractable lipids has been attributed to an interaction between the lipids and other flour components. In dough mixing, lipid interaction is mainly with the gluten proteins, resulting in the structural modification and support of the latter. This is significant, as gluten is the skeleton or framework of wheat flour dough and is responsible for gas retention that is required in the production of light, yeast-leavened products. In baked bread, much of the interaction is with the starch. This is significant, as the starch governs, to a large extent, freshness retention of the baked bread (Pomeranz and Chung 1978).

Olcott and Mecham (1947) found that the amount of free flour lipids (ether-extractable) was reduced to one third as a result of dough mixing. Later, information on binding of specific lipids during breadmaking was obtained by Chiu and Pomeranz (1966). Flour lipids soluble in petroleum ether (PE) were reduced to one third during dough mixing or fermentation; subsequent baking lowered the residual free flour lipids by half. Small amounts of hydrogenated vegetable shortening added to the formulation became bound during dough mixing, but about one third to one half became bound during baking. Many more polar wheat flour lipids (phospholipids and glycolipids) than nonpolar components were bound during dough mixing. Pomeranz et al (1968) studied

binding of various lipids in doughs varying in composition and mixed for various times. Up to 1.5% (flour weight basis) of unsaturated corn oils were bound in dough mixed from PE-extracted flours; saturated corn oils and fats were bound to a lesser extent. In bread crumb, much more unsaturated oil than saturated fat was bound.

The present study was undertaken to compare free lipids in baked bread with those in steamed bread, as rate and extent of heating in the crumb of the two bread types may affect lipid binding, and to examine the distribution of fatty acids in PE-extractable lipids in the breadmaking steps.

MATERIALS AND METHODS

Materials

A hard red winter (HRW) wheat (cv. Montana) from the 1988 crop year was grown in Richland, Washington. The wheat was milled on a Miag Multomat Mill into a patent (straight-grade) flour. The flour had a protein content of 13.2% (N \times 5.7, 14% mb), ash 0.42% (14% mb), an optimum mixograph water absorption of 70.2% (as determined by an experienced experimental baker aided by results of a series of mixograph curves), and a mixograph dough development time of 3.3 min. A commercial soft white flour marketed under the name White Spear was obtained from Fisher Flour Mills, Seattle. This commercial unbleached soft wheat flour had a protein content of 8.9% (N \times 5.7, 14% mb) and ash 0.41% (14% mb). The flour was milled from a soft white winter (SWW) mix and had an optimum mixograph water absorption of 54.5%, and a mixograph dough development time of 2.7 min.

Shortening was a partially hydrogenated commercial vegetable product (Crisco) with a melting point of 41°C. Organic solvents were analytical reagent grade, and solutions were prepared from analytical reagent-grade compounds.

Analytical Methods

Flour moisture, protein, ash, mixograph water absorption, and dough mixing properties (mixograph) were determined by AACC

¹Graduate research assistant and research professor, respectively, Department of Food Science and Human Nutrition, Washington State University, Pullman, WA 99164-6376.

²Present address: University of Kentucky, Department of Nutrition and Food Science, Lexington, KY 40506-0054.

TABLE I
Free Lipid Contents (% of Sample) of Hard Red Winter Flour, Dough, and Bread Samples^a

Wheat Sample	Flour	Dough	Baked Bread		Steamed Bread
			Crumb	Crust	
Original	0.98 a	0.36 b (63.2)	0.28 c (71.4)	0.13 d (86.7)	0.30 c (69.4)
Original + 2% shortening	3.08 a	2.37 b (23.1)	2.19 c (28.9)	1.39 e (54.9)	2.01 d (34.7)
Defatted + 2% shortening	2.03 a	1.20 b (40.9)	1.07 c (47.3)	0.54 e (73.4)	0.87 d (57.1)

^a Means of three determinations. Means followed by the same letter within a row are not significantly different at the $P = 0.05$ level. Values in parentheses are for percent of lipids made inextractable with petroleum ether as a result of making a dough or steamed bread.

TABLE II
Free Lipid Contents (% of Flour, Dough, and Steamed Bread Samples^a

Wheat Sample	Flour	Dough	Steamed Bread
Soft white winter			
Original	1.30 a	0.36 b (72.3)	0.21 c (83.9)
Original + 2% shortening	3.28 a	2.29 b (30.2)	2.08 c (36.6)
Defatted + 2% shortening	2.07 a	1.71 b (17.4)	1.19 c (42.5)
Hard red winter			
Original	0.98 a	0.36 b (63.3)	0.30 c (69.4)
Original + 2% shortening	3.08 a	2.37 b (23.1)	2.01 c (34.7)
Defatted + 2% shortening	2.03 a	1.20 b (40.9)	0.87 c (57.1)

^a Means of three determinations. Means followed by the same letter within a row are not significantly different at the $P = 0.05$ level. Values in parentheses are for percent of lipids made inextractable with petroleum ether as a result of making a dough or steamed bread.

methods (AACC 1983). Results were expressed on a 14% moisture basis, and protein was calculated as $N \times 5.7$, %.

Baking Procedures

Steamed bread. Ingredients for steamed bread consisted of flour, water, sugar, and shortening (when required in the formulation). All doughs were mixed to optimum consistency in a mixer (National Mfg., Div. of TMCO, Inc., Lincoln, NE) using 100 g of flour; rolled out, molded, and divided into seven pieces with equipment described by Rubenthaler et al (1990); placed on a steam tray; and steamed for 10 min in a steamer (Model 3005 Ultra Steamer, Market Forge Co., Everett, MA).

Pan bread. Ingredients for pan bread consisted of flour, water, sugar, and shortening (when required in the formulation). All doughs were mixed (using optimum water absorption from mixograph data) and baked in an Auto Bakery (Funai Electric Co. Ltd., Osaka, Japan) according to equipment instructions.

Sample Preparation

Dough, crumb, crust, and steamed bread samples were frozen and lyophilized in a freeze-drier (Freezemobile 12, Virtis Co. Inc., Gardiner, NY). Samples were then finely ground to pass through a 0.5-mm screen.

Lipid Extraction

Free lipids were extracted for 24 hr with PE (bp 35–60°C) in a Soxhlet from 5 g (water-free basis) of sample (flour, lyophilized doughs, crumbs, crusts, and steamed bread). The solvent in the PE extract was removed under vacuum in a rotary evaporator and the percent free lipid determined. Lipid extracts for gas chromatographic analysis were stored at –80°C under nitrogen until ready to use.

Gas Chromatography

Lipid samples for fatty acid profile analysis by gas chromatography (GC) were prepared as follows: Approximately 100 mg of lipid was butylated by heating the sample in a 0.5N butanolic potassium hydroxide solution on a hot plate for 2–3 min to boiling. After cooling to room temperature, 3 ml of 12.5% boron trifluoride-butanol reagent (Supelco, Inc., Bellefonte, PA) was added, and the resulting mixture was heated again for 3 min to boiling. Upon cooling, fatty acid butylesters (FABEs) were extracted from the reaction mixture with hexane. The FABEs were analyzed directly (Iverson and Sheppard 1977) using a Varian

TABLE III
Fatty Acid Composition (% of Sample) of Petroleum-Ether Extracts from Hard Red Winter Flour^a

Fatty Acids ^b	Flour	Dough	Baked Bread		Steamed Bread
			Crumb	Crust	
Saturated	0.13 a	0.05 b	0.04 b	0.02 b	0.05 b
Unsaturated	0.56 a	0.15 b	0.12 bc	0.05 d	0.10 c
Total	0.69 a	0.20 b	0.16 c	0.07 d	0.15 c

^a Means of two determinations. Means followed by the same letter within a row are not significantly different at the $P = 0.05$ level.

^b Fatty acids detected: palmitic and stearic (saturated); oleic, linoleic, and linolenic (unsaturated).

2700 GC equipped with a flame ionizing detector on a stainless steel column (1.8 m × 2 mm i.d.) containing 10% SP2330 impregnated on 100–120-mesh Chromosorb W-AW (Supelco). Carrier gas (nitrogen) flow rate was set at 20 ml/min, and the injector and detector temperatures were 250°C. Initial column temperature was set at 150°C for 1 min and then increased to 200°C at a rate of 20°C/min. Column temperature was maintained at 200°C for the complete elution of all FABEs. Identification of individual fatty acids was based on the elution profiles of standards (Sigma Chemical Co., St. Louis, MO), and quantitation was based on detector response relative to known concentrations of standards (AOCS 1989).

Statistical Analysis

All determinations were made at least in duplicate. Data were analyzed using the statistical analysis system of the SAS Institute (SAS 1985).

RESULTS AND DISCUSSION

Dough mixing, baking, and steaming of the original flour decreased amounts of flour lipids extractable with PE, indicating interaction of the lipids with other flour components (Table I). No significant difference was seen in the amounts of PE-extractable lipids in the baked bread crumb or steamed bread.

When added to the untreated flour, no measurable amounts of shortening lipids were bound during dough mixing, confirming earlier results reported by Pomeranz et al (1968). Small amounts of shortening, however, became bound during baking or steaming. When added to PE-defatted flour, significant amounts of shortening became bound during all stages of breadmaking. Results seem to indicate a competition between native flour lipids and shortening lipids for binding sites. Flour lipids appear to be bound in preference to shortening lipids. Steaming resulted in more shortening binding than baking in the untreated or PE-defatted flours.

Mecham and Weinstein (1952) reported higher lipid binding in flours with higher protein content than in those with lower protein content during dough mixing. The difference was attributed to more binding sites in the former flour. Lipid binding in dough and steamed bread in the HRW (13.2% protein) and the SWW (8.3% protein) flour samples are summarized in Table II. In this study, more lipids became bound during dough mixing in the original SWW flour than in the original HRW flour. Steaming also resulted in more lipid binding in the SWW flour than in the HRW flour.

TABLE IV
Fatty Acid Composition (% of Sample) of Petroleum-Ether Extracts from Hard Red Winter Flour + 2% Shortening^a

Fatty Acids ^b	Flour	Dough	Baked Bread		Steamed Bread
			Crumb	Crust	
Saturated	0.54 a	0.47 b	0.41 c	0.26 d	0.42 c
Unsaturated	1.72 a	1.51 b	1.15 c	0.83 d	1.16 c
Total	2.26 a	1.98 b	1.56 c	1.09 d	1.58 c

^a Means of two determinations. Means followed by the same letter within a row are not significantly different at the $P = 0.05$ level.

^b Fatty acids detected: palmitic and stearic (saturated); oleic, linoleic, and linolenic (unsaturated).

Slightly more shortening became bound during both dough mixing and steaming in the HRW flour than in the SWW flour (Table II). In the absence of native flour lipids, however, much more shortening became bound during both dough mixing and steaming in the HRW flour than in the SWW flour. Results suggest that factors other than protein content may account for the degree of lipid binding. For instance, the SWW wheat flour was milled commercially, whereas the HRW wheat flour was milled on a laboratory mill. The two flours differed widely in their water-binding capacity, and, while the two flours had comparable ash contents, their free lipid contents differed considerably.

Dough mixing, baking, and steaming all resulted in reduced fatty acid contents in the PE-extracts from the HRW flour samples (Table III). The reduction was, however, much more pronounced in the unsaturated fatty acids than in the saturated fatty acids. Results confirm the work of Pomeranz et al (1968), who showed that much more unsaturated oils than saturated fats became bound during dough mixing and baking.

There was no significant difference in binding of saturated fats as a result of dough mixing, baking (crumb), or steaming (Table III). However, whereas steaming resulted in more binding of unsaturated oils (indicated by a significantly lower unsaturated fatty acid content in PE-extracts) than in dough mixing, no significant difference was seen between dough mixing and baking (crumb). When shortening was added to the untreated HRW flour (Table IV), both baking and steaming resulted in more binding of saturated fats and unsaturated oils than did dough mixing. When shortening was added to the defatted flour (Table V), dough mixing, baking, and steaming showed no significant differences in binding of saturated fats, much like in the untreated flour with no added shortening. In contrast, the binding of unsaturated oils increased in order of dough mixing, baking, and steaming.

In summary, dough mixing, baking, and steaming decreased amounts of PE-extractable lipids due to binding of the lipids to other flour components. A competition for binding sites between shortening lipids and native flour lipids seems to occur during all stages of breadmaking, with flour lipids bound in preference to shortening lipids. Finally, baking and steaming (depending on the presence or absence of shortening) resulted in different degrees of lipid binding to flour components.

TABLE V
Fatty Acid Composition (% of Sample) of Petroleum-Ether Extracts from Defatted Hard Red Winter Flour + 2 % Shortening^a

Fatty Acids ^b	Flour	Dough	Baked Bread		Steamed Bread
			Crumb	Crust	
Saturated	0.44 a	0.25 b	0.25 b	0.13 c	0.22 b
Unsaturated	1.25 a	0.79 b	0.67 c	0.33 e	0.51 d
Total	1.69 a	1.04 b	0.92 c	0.46 e	0.73 d

^a Means of two determinations. Means followed by the same letter within a row are not significantly different at the $P = 0.05$ level.

^b Fatty acids detected: palmitic and stearic (saturated); oleic, linoleic, and linolenic (unsaturated).

LITERATURE CITED

- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Method 08-01, approved April 1961, revised October 1981; 44-15A, approved October 1975, revised October 1981; 46-12, approved October 1976, revised October 1986; 54-30, approved October 1984. The Association: St. Paul, MN.
- AMERICAN OIL CHEMISTS SOCIETY. 1989. Method Ce 1-62. The Society: Champaign, IL.
- CARR, N. O., DANIELS, W. R. N., and FRAZIER, P. J. 1989. Lipid complex formation in wheat flour doughs. Pages 151-172 in: *Wheat End-Use Properties: Wheat and Flour Characterization for Specific End-Uses*. Proc. ICC 1989. H. Salovaara, ed. Yliopistopaino: Helsinki, Finland.
- CHIU, C.-M., and POMERANZ, Y. 1966. Changes in extractability of lipids during bread-making. *J. Food Sci.* 31:753.
- CHUNG, O. K., and TSEN, C. C. 1975. Changes in lipid binding and distribution during dough mixing. *Cereal Chem.* 52:533.
- DeSTEFANIS, V. A., and PONTE, J. G. Jr. 1976. Studies on the breadmaking properties of wheat-flour nonpolar lipids. *Cereal Chem.* 53:636.
- FISHER, N., BELL, B. M., and RAWLINGS, C. E. B. 1973. Lipid binding in flour, dough and bread. *J. Sci. Food Agric.* 24:147.
- IVERSON, J. L., and SHEPPARD, A. J. 1977. Butyl ester preparation for gas-liquid chromatography determination of fatty acids in butter. *J. Assoc. Off. Anal. Chem.* 60:284.
- MECHAM, D. K., and WEINSTEIN, N. E. 1952. Lipid binding in doughs. Effects of dough ingredients. *Cereal Chem.* 29:448.
- MORRISON, W. R. 1976. Lipids in flour, dough and bread. *Baker's Dig.* 50(4):29.
- OLCOTT, H. S., and MECHAM, D. K. 1947. Characterization of wheat gluten. I. Protein-lipid complex formation during doughing of flours. Lipoprotein nature of the glutenin fraction. *Cereal Chem.* 24:407.
- POMERANZ, Y., and CHUNG, O. K. 1978. Interaction of lipids with proteins and carbohydrates in breadmaking. *J. Am. Oil Chem. Soc.* 55:285.
- POMERANZ, Y., TAO, R. P.-C., HOSENEY, R. C., SHOGREN, M. D., and FINNEY, K. F. 1968. Evaluation of factors affecting lipid binding in wheat flours. *J. Agric. Food Chem.* 16:974.
- RUBENTHALER, G. L., HUANG, M. L., and POMERANZ, Y. 1990. Steamed bread. I. Chinese steamed bread formulation and interactions. *Cereal Chem.* 67:471.
- SAS INSTITUTE. 1985. SAS User's Guide: Statistics, Version 5 edition. The Institute: Cary, NC.
- TAO, R. P.-C., and POMERANZ, Y. 1968. Functional breadmaking properties of wheat flour lipids. III. Effects of lipids on rheological properties of wheat flour doughs. *Food Technol. Chicago* 22:1145.

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