

Effects of Various Lipid Fractions of Wheat Flour on Expansion of Sponge Cake

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ABSTRACT

Cereal Chem. 71(1):6-9

To elucidate the role of lipids in wheat flour during cake baking, five different fractions were separated from free lipids of wheat flour. The fractions were added to defatted flours at a 0.5% level by flour weight. In addition to these five fractions, one extra sample was prepared by adding the same amount of commercial pure linoleic acid to the defatted flours. Fraction 1 (76.5% steryl ester) and fraction 2 (81.1% triglyceride) had no effect on gelatinization properties of the defatted flours or on the expansion of the cakes made from the defatted flours, whereas fraction

4 (58.9% monogalactosyl diglyceride and 28.8% digalactosyl diglyceride) and fraction 5 (74.7% digalactosyl diglyceride) improved cake expansion. Fraction 3 (64.4% diglyceride and 22.0% free fatty acids) and the 99% pure linoleic acid decreased gel hardness of the defatted flours, indicating the suppression of retrogradation of wheat starch by free fatty acids. They also increased cake volume, but this effect was smaller than that of the glycolipids.

Lipids, minor constituents of wheat, play a major role in bread baking. The functionality of wheat flour lipids in bread baking was reviewed by several researchers (Chung et al 1978, MacRichie 1984, Chung 1989). Polar lipids of wheat flour were effective in improving bread baking, whereas nonpolar lipids had no such improving effects (Daftary et al 1968, MacRichie 1977). De Stefanis and Ponte (1976) reported that the free fatty acids of wheat flour have detrimental effects, and glycolipids, especially digalactosyl diglyceride, have improving effects.

Different results were obtained in studies of cookie baking. Extraction of the flour lipids greatly reduced cookie spread. The original diameter was restored fully with the free lipids and polar lipids; it was only partially restored with the nonpolar lipids (Clements 1980). Among the polar lipids, digalactosyl diglyceride plus phosphatidyl choline and mono galactosyl diglyceride gave essentially complete recovery of the original diameter of the cookie (Clements and Donelson 1981).

The role of wheat flour lipids in cake baking has not been reported. The effects of flour lipid fractions on pan-cake quality were studied by Seguchi and Matsuki (1977). Previously, Takeda (1990) reported that the polar lipids of wheat flour had larger effects than those of the nonpolar lipids on restoring the volume of sponge cakes made from defatted flours. This result was in agreement with that for cookie baking as mentioned above.

The purpose of this study was to determine the source of the functionality of the wheat flour lipids in sponge-cake baking by defatting and constituting procedures.

MATERIALS AND METHODS

Materials

Violet brand wheat flour (Nissin Milling Co., Tokyo, Japan) was used in this study. The protein and ash content was 7.5 and 0.37%, respectively (14.0% mb). Wheat starch was obtained from Chiba Milling Co., Chiba, Japan. Protein content and hydrolysate lipid content were 0.23 and 0.83%, respectively (14.0% mb). The purity of all reagents used was >99%, except *n*-hexane (95%). Linoleic acid and trilinolein were obtained from Sigma Chemical Co., St. Louis, MO.

Extraction and Fractionation of Wheat Flour Lipids

Free lipids of wheat flour were extracted with diethyl ether using a Soxhlet apparatus. Wheat flour (30 g) was extracted in a cylindrical paper filter (28 mm diameter \times 100 mm) using 150 ml of diethyl ether for 16 hr. A 6-kg portion of the wheat flour was defatted by repeating this procedure. The extracted lipid solutions were collected and dried under reduced pressure. Batch fractionation (Ponte and De Stefanis 1969) of the free lipids extracted (45 g) produced nonpolar and polar fractions.

Separation of Five Lipid Fractions

Nonpolar and polar fractions were each absorbed onto activated silica gel and selectively eluted with appropriate solvents. To separate steryl ester, hexane was stirred into portions of the silica gel slurry of the nonpolar fraction, such that the ratio of hexane to the lipids was roughly 12:1 (v/w). After a 5-min contact period, the mixture was centrifuged at $715 \times g$ for 5 min. Four additional elutions were conducted with the same amount of hexane. For fraction 1, extracts were combined and solvent was removed at 40°C under reduced pressure. For fraction 2, triglyceride in the remaining lipids was eluted 10 times, each with 300 ml of hexane-diethyl ether (90:10, v/v), following the procedure for fraction 1. All of the remaining nonpolar lipids were eluted with 300 ml of diethyl ether, followed by the absorption onto Florisil (magnesium silicate) pretreated with 10% boric acid. For fraction 3, 1,3- and 1,2-diglycerides were eluted 10 times, each with 300 ml of hexane-diethyl ether (90:10, v/v). For fraction 4, monogalactosyl diglyceride in the silica gel slurry of the polar fraction was eluted four times, each with 200 ml of benzene-acetone (30:70, v/v) (De Stefanis and Ponte 1968). For fraction 5, the remaining glycolipids were eluted three times, each with 200 ml of acetone-methanol (97:3, v/v).

Extracts of the five fractions were dried at 40°C under reduced pressure and resolved in diethyl ether for the constitution. The lipids in the diethyl ether solutions were determined gravimetrically from an aliquot dried for 1 hr at 105°C.

To measure foam stability and qualities of the cake made from the wheat starch, crude glycolipids were prepared by mixing equal amounts of fractions 4 and 5.

Analysis of Flour Lipid Fractions

The five fractions of the flour lipids were analyzed by thin-layer chromatography and flame ionization detection (Iatron Lab, Inc., Tokyo, Japan) as described previously (Takeda 1992a).

Preparation of Defatted Flours and Flours Constituted with Lipids

The defatted flours were air-dried and sifted through a 100-mesh screen. The five separated flour lipid fractions were added to the defatted flours by pipetting 150 ml of diethyl ether solutions from 1.5 g (solid weight) of lipids to 300 g of defatted flours (0.5% level). After the air-drying process, the treated flour was hand-mixed and sifted through a 100-mesh screen. Because free fatty acids could not be clearly separated from the slurry of the nonpolar fraction in this experiment, 99% pure commercial linoleic acid, which was the major free fatty acid in the flour lipids (Clayton and Morrison 1972), was added to the defatted flour (14.6% mb) at the 0.5% level by flour weight.

Amylograms

The pasting characteristics of the flour samples were determined with a Brabender amylograph using 64.4 g of the flour sample (14.0% mb) and 450.6 g of deionized water. Flour-water sus-

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pensions were cooked in the amylograph with a temperature controller (Sasaki Co., Kawasaki, Japan) at 1.5°C per minute from 25 to 95°C. The gelatinization temperature and the maximum viscosity were measured.

Gel Texture

Gels with 10.8% (db) wheat flour were prepared by the amylograph. Hot paste (95°C) was poured into shallow dishes (7 cm diameter × 2.3 cm). The depth of each dish was increased 5 mm by attaching adhesive tape around the rim. The dishes were covered, and the gels were stored for 2 hr at 20°C. After the tape had been removed, the excess gel above the rim was cut by a cotton thread. The gels were removed from the dishes and cut into a cylindrical shape with a round cutter (2 cm diameter). The texture of the wheat flour gels was measured by a texturometer (Zenken Co., Tokyo, Japan) (Takeda 1992a).

Swelling Power and Solubility

The swelling power and solubility were determined by the method of Nishida et al (1980). Mixtures of flour (1 g) and water (50 ml) were heated in glass centrifuge tubes at 65, 75, 85, and 92°C for 30 min. During heating, the suspension was stirred by a stainless bar at 114 rpm. After the suspension was centrifuged at 2,960 × *g* for 30 min, weights of the swelled flour sample and the dried soluble matter in the supernatant were measured.

Sponge-Cake Baking

The sponge cake formula was: egg white (130 g), egg yolk (70 g), refined sugar (186 g), flour (140.7 g, 14.0% mb), and deionized water (29.3 g). The egg white, egg yolk, and sugar were put into a bowl and warmed in a 90°C water bath to 30°C. This mixture was whipped for 6 min in a KENMIX CHEF mixer (Kenwood Co., Havant Hants, UK) at 455 rpm (speed 8). After the water was added to the foam and whipped for 30 sec at 378 rpm (speed 5), the flour was added and mixed for 30 sec at 245 rpm (speed 2). The batter (80 g) was weighed into a stainless cake pan (9.0 cm diameter × 6.0 cm deep). A thin paper scale was inserted to measure the maximum height of the batter during baking. The cake was baked in a gas oven at 180°C for 20 min. After 5 min, the cake was removed from the pan and cooled for 24 hr at 20°C. The cake volume was measured by the rapeseed displacement method. Specific volume was defined by: (cake volume/cake weight) × 100.

In the baking test with wheat starch, three kinds of lipids (linoleic acid, trilinolein, and crude glycolipids) were added to the cake batter at the end of the mixing process because these lipids, especially linoleic acid, acted as defoaming agents in the egg foam and in the batter with wheat starch.

Foam Stability

The egg foam was prepared by whipping a mixture of egg white (130 g), egg yolk (70 g), and refined sugar (186 g) at 30°C for 6 min, as already described. Each of the three kinds of the lipids (99% pure linoleic acid, 99% pure trilinolein, and crude

glycolipids [mixture of fractions 4 and 5]) was added to the egg foam at a 0.1% level by foam weight. To measure the foam stability after standing, the 10-g egg foam with each lipid fraction was weighed into a centrifuging tube with a scale and allowed to stand at 20°C for 30 min. After centrifugation at 185 × *g* for 5 min, the volume of the remaining foam was measured. To measure the foam stability upon heating, 5 g of egg foam with each lipid fraction was weighed into the centrifuging tube and heated in an electric oven at 150°C. The maximum volume of the foam during heating was measured.

All experiments were repeated twice and evaluated by analysis of variance. Least significant difference values at 0.05 level of significance were calculated from replication error mean squares.

RESULTS AND DISCUSSION

Yields and Compositions of Lipids

The yield of the free lipids from the flour was 0.79% (14.0% mb), which consisted of 62% nonpolar and 38% polar fractions. The recovery was about 95% for both the nonpolar and polar fractions.

The percent of total free lipids for fractions 1–5 were: 5.2, 37.5, 8.8, 12.3, and 21.6%, respectively.

The composition of the five fractions separated from the flour lipids are shown in Table I. The major components were steryl ester (76.5%) in fraction 1, triglyceride (81.1%) in fraction 2, diglycerides (64.4%) in fraction 3, monogalactosyl diglyceride (58.9%) in fraction 4, and digalactosyl diglyceride (74.7%) in fraction 5.

The prepared crude glycolipids were composed of monogalactosyl diglyceride (29.5%), digalactosyl diglyceride (51.8%), and other glycolipids (18.8%).

Gelatinization Properties

The maximum viscosity and gelatinization temperature measured by the amylograph are shown in Table II. When the defatted flour was enriched with linoleic acid, the maximum viscosity and gelatinization temperature greatly increased. Swelling power and solubility at 92°C decreased (Table II), indicating suppression of the gelatinization by linoleic acid (Nihara and Yonezawa 1981, Takeda 1992b). None of the five lipid fractions affected the maximum viscosity or swelling power of the enriched flours.

Gel Texture

The physical properties of the flour gels measured by the texturometer are shown in Table II. When the flour was defatted, gel hardness increased and adhesiveness greatly decreased, indicating a retrogradation during storage. In the nonpolar lipids, fraction 1 (76.5% steryl ester) and fraction 2 (81.1% triglyceride) had no effect on gel texture, whereas fraction 3 (64.4% diglyceride) and linoleic acid greatly decreased hardness and increased adhesiveness of the gels. In contrast, fractions 4 and 5 (crude glycolipids) had little effect on softening the gels, but they greatly increased adhesiveness. Free fatty acids and glycolipids in the flour lipids can prevent the retrogradation of wheat starch (Chung

TABLE I
Lipid Composition of the Fractions (%) Separated from Free Lipids of Wheat Flour^a

Lipid	Fraction				
	1	2	3	4	5
% Total free lipids	5.2	37.5	8.8	12.3	21.6
Steryl ester	76.5	0.7			
Triglyceride	18.1	81.1	12.2		
Free fatty acid	4.9	14.6	22.0		
1,3-Diglyceride		1.0	24.8		
1,2-Diglyceride		2.3	39.6		
Monoglyceride			1.1		
Unknown (nonpolar lipid)	0.5	0.3	0.3		
Monogalactosyl diglyceride				58.9	
Digalactosyl diglyceride				28.8	74.7
Unknown (polar lipids)				12.3	25.3

^a Average of two experiments.

TABLE II
Gelatinization Properties and Gel Texture of Defatted Flours Combined with Lipid Fractions^a

	Original Flour	Defatted Flour	Fraction					Linoleic Acid	LSD ^g
			1 ^b	2 ^c	3 ^d	4 ^e	5 ^f		
Maximum viscosity, BU ^h	720	680	660	660	700	600	620	1,060	91
Gelatinization temperature, °C ^h	74	70	67	70	70	71	72	80	...
Swelling power at 92°C ⁱ	11.2	12.2	11.9	11.9	11.5	11.1	11.2	10.2	1.2
Solubility at 92°C, % ^j	14.4	16.5	15.4	15.7	15.5	14.9	14.8	12.2	3.0
Hardness, kg/V ^k	0.4	0.46	0.45	0.39	0.26	0.44	0.42	0.25	0.05
Adhesiveness, cm ² /V ^k	0.28	0.01	0.01	0.02	0.39	0.28	0.20	0.35	0.05
Cohesiveness ^k	0.30	0.20	0.27	0.27	0.33	0.29	0.27	0.39	0.04
Gumminess ^l	0.12	0.09	0.12	0.11	0.09	0.13	0.11	0.10	0.02

^a 0.5% level by flour weight.

^b Steryl ester (76.5%).

^c Triglyceride (81.1%).

^d Diglyceride (64.4%).

^e Monogalactosyl diglyceride (58.9%).

^f Digalactosyl diglyceride (74.7%).

^g Least significant difference (0.05).

^h Measured with an amylograph.

ⁱ Swelling power defined by $B/(S-A)$, where A is weight of the dried soluble matter in supernatant after centrifugation. B is weight of the swelled flour sample after standing at 92°C for 30 min, and S is weight of the dried flour sample.

^j Solubility (%) defined by $(A/S) \times 100$.

^k Measured with a texturometer.

^l Gumminess = hardness \times cohesiveness.

TABLE III
Baking Characteristics of Flours Constituted with Lipid Fractions^a

	Original Flour	Defatted Flour	Fraction					Linoleic Acid	LSD ^g
			1 ^b	2 ^c	3 ^d	4 ^e	5 ^f		
Specific gravity of cake batter	0.43	0.42	0.41	0.39	0.42	0.41	0.41	0.41	0.02
Expansion during baking									
Degree of maximum expansion, cm ³ ^h	297.3	324.6	281.8	267.3	280.1	269.1	262.7	281.8	23.8
Degree of baking shrinkage, cm ³ ⁱ	215.2	291.4	239.3	233.0	213.8	187.6	191.2	222.9	13.3
Degree of true expansion, cm ³ ^j	82.1	33.2	42.5	34.3	66.3	81.5	71.5	58.9	26.4
Cake weight, g	62.5	62.7	61.7	61.8	61.8	63.3	61.5	61.0	1.7
Cake volume, cm ³	268.1	223.7	237.6	239.4	256.8	276.6	266.6	254.0	26.4
Specific volume	429	357	385	387	416	438	434	416	45.1

^a 0.5% level by flour weight.

^b Steryl ester (76.5%).

^c Triglyceride (81.1%).

^d Diglyceride (64.4%).

^e Monogalactosyl diglyceride (58.9%).

^f Digalactosyl diglyceride (74.7%).

^g Least significant difference (0.05).

^h Difference between the cake volume at the maximum expansion and the batter volume.

ⁱ Difference between the cake volume at the maximum expansion and the cake volume after baking.

^j Difference between the cake volume after baking and the batter volume before baking.

et al 1978, Takeda 1992a). For this reason, the gels of the flours enriched with linoleic acid and crude glycolipid fractions were stickier than those of the defatted flours. The softening effects of fraction 3 on the flour gels might be due to its high content of free fatty acids (22.0%).

Baking Test of Sponge Cakes

Baking characteristics of the sponge cakes made from defatted and constituted flours are shown in Table III. The sponge cake baked from defatted flour had the smallest volume and a largely collapsed contour; it had the largest expansion and shrinkage of the batter during baking.

Fractions 1 and 2 had no effect on restoring the volume of the cake baked from the defatted flour. However, adding 0.5% of glycolipid fractions 4 and 5 to the defatted flour restored the baking potential completely to that of the original flour. These results were in agreement with studies on bread baking (De Stefanis and Ponte 1976) and cookie baking (Clements 1980).

Linoleic acid and the fraction 3 (64.4% diglyceride, 22.0% free fatty acids) had improving effects on the cake baking, but these effects were smaller than those of glycolipids. Free fatty acids, especially linoleic acid, have detrimental effects in bread baking (De Stefanis and Ponte 1976) because they destabilize foam in the dough (MacRichie 1984). Table IV shows the effects of lipids

TABLE IV
Effects of Lipids on the Volume of Egg Foam (cm³)

	After Centrifugation ^a During Heating ^b	
No lipid	27	63
Trilinolein, 0.1%	27	58
Linoleic acid, 0.1%	19	44
Mixture of glycolipids, 0.1% ^c	24	55
LSD ^d	0.9	5.9

^a 10 g of egg foam with each lipid was allowed to stand at 20°C for 30 min, then it was centrifuged at $185 \times g$ 1,000 rpm for 5 min. The volume of remaining foam was measured.

^b 5 g of egg foam with each lipid was heated at 150°C; the volume of the foam at maximum expansion was measured.

^c Mixture of fractions 4 and 5 (29.5% monogalactosyl diglyceride, 51.8% digalactosyl diglyceride, and 18.8% other glycolipids).

^d Least significant difference (0.05).

on the stability of the egg foam. Linoleic acid had detrimental effects on the foam stability after centrifugation as well as during heating. Triglyceride and crude glycolipid fraction, however, showed little effect as defoaming agents.

Wheat starch is a major component supporting sponge structure in cake baking, thus gelatinization properties and gel texture of the wheat flour may be responsible for the expansion of the cakes.

TABLE V
Effects of Adding Various Lipids to Wheat Starch

	Gel Texture ^a		Sponge Cake Baking Qualities			
	Hardness (kg/v)	Adhesiveness (cm ² /v)	Volume of 80 g of Batter (cm ³)	Cake Volume (cm ³)	Specific Volume	Degree of True Expansion ^b (cm ³)
Wheat starch	0.46	0	205.0	259.2	409	54.2
Wheat starch + 0.075% Linoleic acid (LA)	0.41	0.01	188.7	286.8	431	98.1
+ 0.15% LA	0.34	0.05	187.6	290.5	433	102.9
+ 0.3% LA	0.25	0.10	190.8	300.4	454	109.6
Wheat starch + 0.15% Trilinolein (TL)	0.46	0	203.5	263.2	413	60.2
+ 0.3% TL	0.46	0	190.8	269.0	403	78.2
Wheat starch + 0.075% Glycolipids (GL) ^c	0.43	0.01	190.8	294.9	449	104.1
+ 0.15% GL	0.41	0.02	190.8	309.9	468	119.1
+ 0.3% GL	0.40	0.06	178.1	309.3	458	131.2
Diethyl ether-extracted flour	0.72	0	190.5	223.1	349	32.6
Diethyl ether-extracted flour + 0.25% LA	0.30	0.31	195.1	212.0	338	16.9
+ 0.5% LA	0.33	0.30	181.8	263.3	405	81.5
+ 0.75% LA	0.16	0.38	177.8	264.9	420	87.1
+ 1.0% LA	0.18	0.36	173.9	276.4	425	102.5
LSD ^d	0.06	0.05	7.0	18.0	25.9	13.2

^a Dry solids in the gels were 7.8% for wheat starch and 10.8% for defatted wheat flour. Gels were prepared by the amylograph and measured with the texturometer.

^b Difference between the cake volume after baking and the batter volume before baking.

^c Composition of the mixture of glycolipids is the same as described in Table IV.

^d Least significant difference (0.05).

Previously, a highly negative correlation was found between the adhesiveness of the gels and the volume of the baking shrinkage of the sponge cakes made from the flours constituted with the flour lipid fractions (Takeda 1992a).

In this report, no correlation was found between the adhesiveness of the gels and the baking shrinkage, but a highly positive correlation was found between the adhesiveness of the gels and the cake volume ($r = 0.7471$, $P < 0.05$). In this experiment, linoleic acid suppressed gelatinization and retrogradation of the wheat flour starch; the gels of the flours enriched with linoleic acid and fraction 3 (diglyceride plus free fatty acids) were softer and stickier than those from the defatted flours. Therefore, linoleic acid decreased baking shrinkage; the volume of the cake made from the flour enriched with linoleic acid was larger than that from defatted flour, in spite of its defoaming action.

Adding Linoleic Acid to Wheat Starch and Defatted Flour

To elucidate the effects of linoleic acid on cake baking with wheat starch containing no gluten protein, 99% pure linoleic acid was added to the wheat starch at various lipid levels (0.075, 0.15, and 0.3% by starch weight). The crude glycolipid mixtures described earlier and 99% pure trilinolein were also added to the wheat starch for comparison.

The gel texture and baking characteristics of these samples are compared in Table V. The wheat starch gels were prepared by cooking the suspension (7.8% dry solids) of wheat starch (46 g) and deionized water (469 g) in the amylograph. When trilinolein was added to the wheat starch, no change was observed in gel texture or cake volume. In contrast, adding linoleic acid to the wheat starch decreased gel hardness, increased adhesiveness, and had improving effects on the cake volume. These results were in agreement with those from cakes made from the defatted (diethyl ether-extracted) flour (Table V), which indicated that a strong interaction between wheat starch and linoleic acid during and after baking induced gel softening and increased cake volume. Crude glycolipids had little effect on gel texture, but they greatly increased the volume of cakes made from wheat starch.

Finally, it should be noted that the wheat flour used for the data in Table V belongs to a different lot than that used for the data in Tables II and III. Discrepancies between data in those tables may be attributed to the variation between wheat lots from the same brand.

In conclusion, because of their foam-stabilizing actions, glycolipids in the wheat flour had improving effects on the volume of the cakes made from wheat starch and from defatted flour.

However, linoleic acid had a softening effect on the starch gels, and it reduced the baking shrinkage of the cakes and increased the cake volume in spite of its defoaming action in the egg foam.

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