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EFFECT OF FLOUR LIPIDS AND SOME LIPID DERIVATIVES ON COOKIE-BAKING CHARACTERISTICS OF LIPID-FREE FLOURS[1]

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ABSTRACT

Cookies baked from flours which had been extracted with water-saturated *n*-butyl alcohol were generally darker brown in color and had diameters appreciably smaller than those baked from untreated flours. The restoration of lipids to the extracted flours resulted in an increase in cookie diameter and an apparently complete recovery of the characteristics of cookies baked from the original flours. Approximately three-fourths of the total lipid must be removed from a flour before definite changes in cookie-baking behavior (top grain and color) are noted. When the lipids of two flours (which differed in cookie-spread properties) were interchanged, the diameter of the cookies was the same as that of cookies from untreated flours; that is, the diameter appears to depend on constituents other than flour lipids. When lipid-free flours were supplemented with the phosphorus-free fraction from flour oil, no improvement occurred in the cookie-baking quality of these flours; the phosphorus-containing fraction, however, produced complete recovery of cookie characteristics. Soybean or corn phosphatide preparations and a purified soybean lecithin also brought about a similar improvement.

Investigations concerned with the relationship of wheat flour lipids to the baking quality of flours have been carried on by several workers, among whom Sullivan *et al.* (13,14) and Cookson *et al.* (3,4) may be mentioned. Their work, however, has been confined chiefly to a study of the role of flour lipids in the breadmaking behavior of hard wheat

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flours, and little attention has been given to soft wheat flour varieties, which are the types best suited for pastries and cookies. The present study was undertaken to determine the extent to which cookie-baking properties of soft wheat flours are affected by flour lipids.

One difficulty encountered in the evaluation of flour lipids was that of the preparation of suitable flour samples which were free of lipids. In general, those procedures used to remove all of the lipids from flours also produced deleterious effects on gluten and other constituents of flour. Mecham and Mohammad (8) obtained an essentially complete removal of lipids from flour through use of water-saturated *n*-butyl alcohol as the extracting solvent. The few observations which these workers made on bread-baking behavior of flours indicated that extraction with this solvent resulted in a pronounced loss of loaf volume, but as much as two-thirds of the loss could be regained by replacement of the extracted material. These encouraging results prompted the use of water-saturated *n*-butyl alcohol as the extraction solvent for preparation of lipid-free flour samples used in the present study. Such solvents as carbon tetrachloride, petroleum ether, and chloroform were incapable of extracting all of the lipid from the flour. They were used in this series of experiments, however, to provide additional comparative information. The results of the cookie-baking tests which were made in this study and the effect of various lipids on the cookie-baking characteristics of lipid-free flours are described in this paper.

Materials and Methods

One hard and two soft wheat flours, all of which were commercially milled, and one hard wheat flour, which was experimentally milled from a pure variety (Rio), were used in this work. All were unbleached.

Lipid Extraction Procedures. Lipids were extracted from the flours with water-saturated *n*-butyl alcohol by the procedure of Mecham and Mohammad (8) with some modifications. A 500-g. sample of flour was placed in a beaker and covered with 1200 ml. of water-saturated *n*-butyl alcohol. This mixture was stirred to disperse all of the flour and allowed to stand for approximately 30 minutes. Then the clear supernatant was decanted and filtered through S&S No. 588 filter paper. The flour residue was extracted five more times in the manner just described, with 400-ml. portions of the solvent each time. Finally, the residue was placed on the filter paper and allowed to drain free of excess solvent. The final filtrate was tested for esters qualitatively with the hydroxamic acid method of Hestrin (6). Two milliliters of the filtrate from the extracted flour were added to 3 ml. of methanol. The hydroxamic acid test was made on this solution. The extraction of lipid was presumed complete when a negative hydroxamic acid test for fatty acid esters was obtained on the filtrate.

To remove residual *n*-butyl alcohol, the extracted flour was dried *in vacuo* at 40°C. for approximately 16 hours. The vacuum-dried material was humidified in a proofing cabinet to a moisture content of approximately 20% and again dried *in vacuo* to approximately 2% moisture. Finally, the dried sample was ground in a small Wiley Mill[3] to pass the No. 20 mesh screen. Lipid-free samples prepared in this way had no detectable odor of butyl alcohol when they were moistened or mixed into a cookie dough. The flour lipid was recovered from extracts by removing the butyl alcohol with a rotary evaporator at a temperature not exceeding 40°C.

When carbon tetrachloride or petroleum ether was used to extract flour lipids, 500 g. of the flour were extracted by percolation in a glass column, using about 1500 ml. of solvent.

Reconstitution Procedures. To reconstitute the flours, desired proportions of lipid were dispersed in water-saturated *n*-butyl alcohol and added to the extracted sample. The residual solvent was removed by drying as described previously.

To reconstitute the flours which had been extracted with carbon tetrachloride or petroleum ether, the lipid was dispersed in the original extracting solvent and added to the extracted flour sample. This reconstituted flour was then spread on trays, and residual solvent was allowed to evaporate at room temperature. All flour samples were humidified to a moisture content of about 12% before they were mixed into doughs.

Fractionation of Flour Lipids. The lipid material obtained from a commercially milled flour by extraction with water-saturated *n*-butyl alcohol was freed of all solvent and dried in a desiccator *in vacuo* over phosphorus pentoxide prior to fractionation. The dried lipid was then dispersed in petroleum ether (b.p. 30°–60°C.). An insoluble fraction precipitated as a light-tan powder and was removed by centrifugation. The lipid soluble in petroleum ether was freed of solvent by evaporation and dissolved in chloroform. The chloroform solution of the lipid was then separated on a silicic acid:celite column into two fractions, one containing phosphorus and the other phosphorus-free, according to the procedure of Borgstrom (2). The fraction free of phosphorus was obtained by passing chloroform through the column containing the absorbed lipid until no more lipid material was found in the eluate. The phosphorus-containing fraction remaining on the

[3] Mention of trade names or products does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

column was then eluted wth methanol.

The fraction which contained phosphorus was further separated into various components with the gradient elution technique described by Weiss (15). The glass column used in this procedure was packed with 120 g. of the silicic acid:celite absorbant having an absorption capacity of 0.6 mg. phosphorus per gram of absorbant. The mixing chamber, attached above the column and equipped with a magnetic stirrer, contained 850 ml. of chloroform. Methanol was added to the chamber dropwise under a nitrogen pressure of about 3 p.s.i. The rate of flow of solvent eluate from the column was approximately one drop per 8 seconds. The eluate was collected as 5-ml. portions in test tubes. The tubes were grouped in successive units of four and the contents of the four combined as one fraction for analysis.

The amounts of solids in the different fractions were obtained by weight.

Analytical Methods. Total phosphorus was determined by the method of Allen (1). Nitrogen was determined by a standard micro-Kjeldahl method. Sugars were quantitatively determined by the anthrone method of Radin *et al.* (12) using glucose as the standard; and qualitatively determined using the paper chromatographic procedure of Williams *et al.* (16).

Cookies were baked by the micro-baking procedure No. III of Finney *et al.* (5) using 40 g. of flour per bake. Cookie quality was expressed as the average diameter and diameter-to-thickness ratio. The reflectance of the cookies was determined using a Hunter Color and Color Difference Meter with standard filter No. SKC-SBC-31.

Results and Discussion

Characteristics of Cookies Baked from Lipid-Free Flours. Cookies baked from flours whose lipids had been extracted with water-saturated *n*-butyl alcohol had consistently smaller diameters, exhibited more compact, hard-appearing top surfaces, and were darker in color than the cookies from the control flour. Restoration of lipids to the extracted flours resulted in an increase of the cookie diameter and a regain of the characteristics of cookies baked from the control. Figure 1 shows the cookies baked from a soft wheat commercial flour whose lipids were extracted with petroleum ether, carbon tetrachloride, and water-saturated *n*-butyl alcohol. The diameters and the diameter-to-thickness ratios of these cookies are shown in Table I. Carbon tetrachloride and petroleum ether extraction produced only minor changes in cookie-baking quality of the flour when compared with the effect produced by water-saturated *n*-butyl alcohol extraction. The amounts

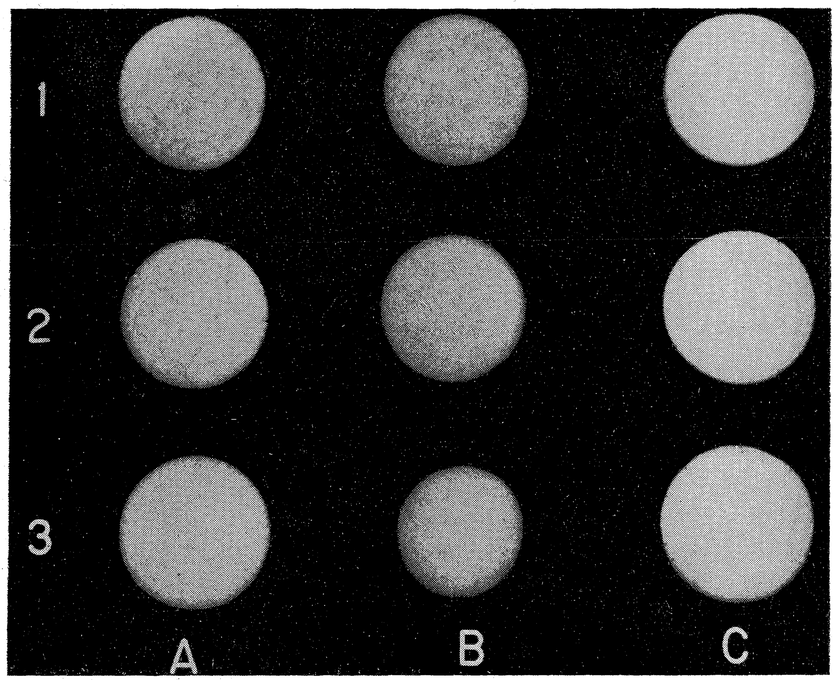

Fig. 1. Cookies baked from a soft wheat commercial flour extracted with different solvents. Top row: A, untreated; B, extracted with petroleum ether; C, reconstituted with petroleum ether extract. Middle row: A, untreated; B, extracted with carbon tetrachloride; C, reconstituted with carbon tetrachloride extract. Bottom row: A, untreated; B, extracted with water-saturated *n*-butyl alcohol; C, reconstituted with water-saturated *n*-butyl alcohol extract.

TABLE I

EFFECT OF SOLVENT EXTRACTION ON COOKIE SPREAD OF A SOFT WHEAT COMMERCIAL FLOUR

FLOUR SAMPLE	COOKIE DIAMETER[a]	DIAMETER/THICKNESS
	cm	*cm*
Untreated	8.79	12.0
Extracted with petroleum ether	8.49	11.0
Extracted with petroleum ether and reconstituted	8.61	12.0
Extracted with carbon tetrachloride	8.41	—
Extracted with carbon tetrachloride and reconstituted	8.52	12.0
Extracted with water-saturated *n*-butyl alcohol	7.49	6.8
Extracted with water-saturated *n*-butyl alcohol and reconstituted	8.94	13.6

[a] Average diameter in cm. of at least four cookies from two baking experiments.

of lipid removed from this flour (as-is basis) by these three solvents were as follows: petroleum ether, 0.88%; carbon tetrachloride, 0.97%; and water-saturated *n*-butyl alcohol, 2.0%.

Similar changes occurred in the cookie-baking performance of hard wheat flours as well as the soft wheat flours examined when their lipids were removed with water-saturated *n*-butyl alcohol:

Flour sample	*Cookie Diameter*	*Diameter/Thickness*
	cm	
Commercial spring wheat flour		
Untreated	8.01	8.9
Extracted	7.46	7.4
Reconstituted	8.32	10.5

The changes, however, were more pronounced with the soft wheat flours. When lipids were removed from both types of flours, the decrease was greatest in the spread of cookies baked from the soft wheat flour.

Effect of Lipid Interchange between Flours. Although the soft wheat commercial flour and the Rio variety hard wheat flour contain approximately the same quantity of protein, they produced cookies with different diameters. Cookies were baked from lipid-free portions of these two flours after reconstitution in which their total lipids were interchanged. The diameters obtained for these cookies were as follows:

Flour sample	*Cookie Diameter*
	cm
Commercial soft wheat flour (6.7% protein)	8.79
Extracted	7.53
Reconstituted with own lipids	8.85
Reconstituted with Rio lipids	8.90
Rio variety (6.9% protein)	8.23
Extracted	7.46
Reconstituted with own lipids	8.54
Reconstituted with commercial soft wheat flour lipids	8.52

According to these results, when the flour lipids were interchanged, the average diameter of the cookies from the flour containing interchanged lipid was the same as the diameter observed in cookies from the reconstituted flour. Since the soft wheat flour used in these experiments was not from a pure-variety lot of wheat, it is not possible to determine whether flour lipids affect cookie spread strictly from a varietal standpoint. The results above, however, suggest that properties of flour lipids from soft and hard wheat flours are similar in improving the behavior of extracted flours.

Effect of Flour Lipid Concentration on Extracted Flours. The amount of flour lipid which was added to lipid-free flour in order to produce a cookie with top grain and color closely resembling the control cookie was found to be less than the total amount of lipid originally extracted from the flour. Flour lipid in amounts varying from 0 to 100% of the total extractable lipid was added back to portions of the lipid-free commercial soft wheat flour. Table II shows the effect produced on the color and diameters of cookies from the

TABLE II

EFFECT OF FLOUR LIPID CONCENTRATION ON CHARACTERISTICS OF COOKIES BAKED FROM A SOFT WHEAT COMMERCIAL FLOUR EXTRACTED WITH WATER-SATURATED *n*-BUTYL ALCOHOL

Total Flour Lipids Added to Extracted Flour	Cookie Diameter[a]	Reflectance of Cookies
%	*cm*	%
100	8.52	47.4
50	8.24	46.5
20	7.99	47.6
5	8.02	42.9
1	7.82	35.7
0	7.46	37.8

[a] Average diameter in cm. of at least four cookies from two baking experiments. The average diameter of cookies baked from the untreated flour was 8.50 cm., and the reflectance was 47.2%.

series in which the lipid concentration was varied. Cookies containing less than one-fifth of the total lipids showed a distinct difference in color and loss of top grain, whereas cookies with higher lipid concentrations closely resembled the control. In general, with each decrease in lipid concentration, there was a decrease in cookie diameter. When a similar concentration study was made with the commercial hard wheat flour of 13% protein, the amount of lipid needed to restore the optimum characteristics (top grain and color) to the cookie was found to be approximately one-fourth of the total extractable lipid. Since about three-fourths of the total lipid had to be removed from a flour before definite changes in the cookie-baking behavior were noted, a possible explanation is furnished as to why extraction of flours with carbon tetrachloride or petroleum ether has no effect on cookie-baking quality of flours. As was indicated previously, these solvents remove only about one-half of the total extractable lipid from flours. Moreover, only small fractions of the total extractable lipid phosphorus are removed by these solvents.

As was indicated earlier in this paper, the cookies baked from lipid-free flours were consistently darker in color than the control. Table II shows that cookies containing less than one-fifth of the total extractable lipids exhibited a corresponding increase in brown color compared with the control. Cookies from extracted flours with flour lipids or phosphatides added back in normal amounts possessed the original light-yellow color of the control. These lipids thus seem to interfere somehow in the browning reactions that normally occur during baking.

Effect of Flour Lipid Fractions on Baking Quality of Extracted Flours. All of the lipid fractions obtained from the commercially milled flour are listed in Table III along with the analytical data for sugar, nitrogen, and phosphorus content.

TABLE III

ANALYSIS OF FLOUR LIPID FRACTIONS

Fraction	Approximate Total Lipid	Nitrogen	Phosphorus	Approximate Glucose	Nitrogen/ Phosphorus Ratio
	%	%	%	%	
Petroleum ether, insoluble fraction	9.6	1.43	0.11	80	33.0
Petroleum ether, soluble fraction	90.5	—	0.75	18	
Tube group 7–12	34.0	0.46	0.69	23	1.5
Tube group 13–16	3.8	3.8	1.18	92	7.1
Tube group 17–20	3.6	1.4	0.23	64	13.5
Phosphorus-free fraction	49.0	0.0	0.0	0	. . .

The fraction which precipitated as the result of dispersing the total flour lipid in petroleum ether was light tan in color, contained chiefly sugars, and was partially soluble in water. Acid hydrolysis of this solid followed by paper chromatographic analysis for sugars only showed the presence of glucose and fructose. When added to lipid-free flours, this fraction produced no improvement in the cookie spread (Table IV). The first fraction eluted from the silicic acid:celite column with

TABLE IV

EFFECT OF FLOUR LIPID FRACTIONS ON DIAMETER OF COOKIES BAKED FROM LIPID-FREE FLOURS

Lipid Fraction [a]	Cookie Diameter [b]
	cm
Petroleum ether, insoluble fraction	7.31
Phosphorus-free fraction	7.74
Tube groups 7–12	8.33
Tube groups 13–16	7.41
Tube groups 17–20	7.51

[a] Lipid fractions were added to flour extracted with water-saturated *n*-butyl alcohol in the same proportionate amounts as were isolated from total flour lipid.

[b] Average diameter in cm. of at least four cookies from two baking experiments, each with 40 g. of extracted flour supplemented with the above lipid fractions.

chloroform contained no phosphorus, sugars, or nitrogen. This fraction probably contained mostly neutral fats and esters and did not appreciably improve the quality and spread of cookies baked from lipid-free flours to which the fraction had been added back. These cookies had a greasy appearance and were dark brown in color.

The second fraction eluted from the column with methanol was further separated by gradient elution into one major and two minor subfractions. Figure 2 shows the distribution curve for the solids eluted from the column. The contents of the fractions in tubes 7–12 inclusive, 13–16 inclusive, and 17–20 inclusive (Fig. 2 and Table III) were

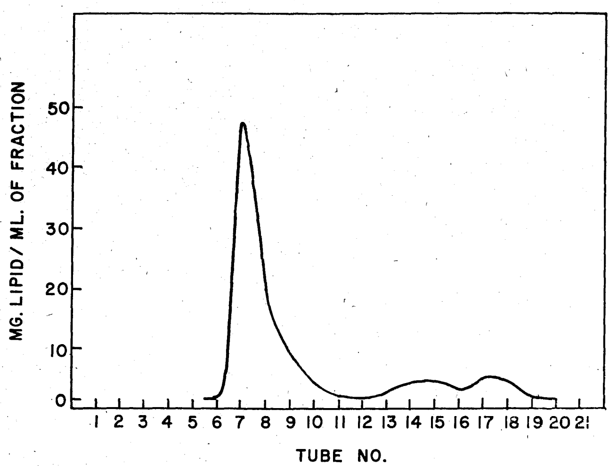

Fig. 2. A weight distribution curve of the phosphorus-containing fractions of flour lipids obtained by elution with chloroform-methanol.

combined into three groups: A, B, and C, respectively. Large values were found for the nitrogen-to-phosphorus ratios of the solids in groups B and C, which suggested that these groups were contaminated with nonphospholipid nitrogen. One group (A), however, which contained phosphorus to the extent of one-third of the phosphorus in the total lipid, had a nitrogen-phosphorus ratio of 1.5 and may have been enriched with phospholipids, such as lecithin or cephalin (a ratio of 1.0 indicated presence of phospholipid only). Group A contained the constituents responsible for cookie spread and quality as shown in Table IV and Fig. 3. The remaining two groups produced no appreciable improvement. Group C on standing at 4°C. for several days deposited a white crystalline material. Hydrolysis of the material followed by paper-chromatographic sugar analysis showed presence of glucose and fructose. No further attempts were made to determine the

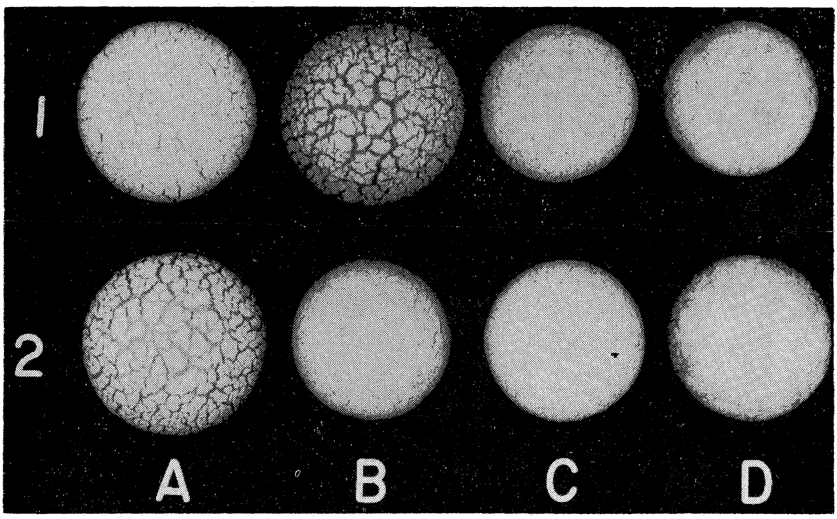

Fig. 3. Effect of flour lipids on the cookie-baking properties of a flour extracted with water-saturated *n*-butyl alcohol. Fractions added: 1A (untreated flour); 1B, total flour lipid; 1C, none; 1D, petroleum ether-insoluble solids; 2A, tube group 7–12; 2B, tube group 13–16; 2C, tube group 17–20; 2D, nonphosphorus fraction.

nature of the constituents in these groups.

Effect of Other Lipids on Baking Quality of Extracted Flours. To determine whether improvement in cookie-baking performance of extracted flours, brought about by flour lipid, was due to a property characteristic only of flour lipid or whether lipids foreign to flour could also bring about cookie-baking improvement, a variety of lipids, both natural and synthetic, were added individually to portions of lipid-free flours. Tables V and VI list some of the lipids used and the

TABLE V

Diameters of Cookies Baked from a Soft Wheat Commercial Flour Extracted with Water-Saturated *n*-Butyl Alcohol and Supplemented with Various Lipids

Lipid Added to Extracted Flour [a]	Cookie Diameter [b]
	cm
(Untreated)	8.46
Total flour lipid	8.53
None	7.42
Sucrose fatty acid ester	8.26
Vegetable tocopherols	7.76
Mono-olein	7.89
Wheat germ oil	8.66

[a] Lipid added to the extent of 2.3% to the lipid-free flour (dry-weight basis).
[b] Average diameter in cm. of at least four cookies from two baking experiments, each with 40 g. of extracted flour supplemented with the above lipids.

TABLE VI

DIAMETERS OF COOKIES BAKED FROM A SOFT WHEAT COMMERCIAL FLOUR EXTRACTED WITH WATER-SATURATED *n*-BUTYL ALCOHOL AND SUPPLEMENTED WITH PHOSPHATIDES

LIPID ADDED TO EXTRACTED FLOUR	AMOUNT ADDED[a]	COOKIE DIAMETER[b]
		cm
(Untreated)		8.20
Total flour lipid	2.30	8.27
None	0.0	7.45
Soybean phosphatide	0.59	7.98
Corn phosphatide	0.75	8.02
Hydroxylated soybean lecithin	0.92	8.28
Alcohol-soluble soybean phosphatides	0.59	8.10
Alcohol-insoluble phosphatides	0.59	8.00
Purified soybean lecithin	0.88	8.15

[a] Percent lipid added to the lipid-free flour (dry wt. basis). The phosphatide preparations were added in quantities containing the same amount of phosphorus as was contained in the natural flour lipid.
[b] Average diameter, in cm. of at least four cookies baked from two baking experiments, each with 40 g. of extracted flour supplemented with the above lipids.

diameters of cookies baked from extracted flours supplemented with these lipids. Such constituents as wheat germ lipids, soy phosphatides, and a sucrose fatty acid ester improved the baking quality of the extracted flours (see Fig. 4 also). An extracted flour to which Tween 80 had been added produced a cookie dough that was sticky and unmanageable, and the baked cookies were very dark in color and irregular in shape.

The results given in Table VI show that phospholipids improved the baking characteristics of the extracted flour, because cookies baked from all doughs containing the crude phosphatide preparations showed an improvement in spread, color, and top grain, as compared with cookies from the extracted flour alone. To add support to the contention that phospholipids exert an improving action, a sample of lecithin prepared from a crude soybean preparation by the procedure of Pangborn (11) and Macpherson (7) was added to the extracted flour. Cookies baked from this flour (Fig. 4) closely resembled the control in all respects.

Whether the flour lipids exert an improving action by themselves or in combination with other constituents of lipid-free flour, such as starch or protein, is not known. There is evidence that a phospholipid-protein complex exists in flour dough, and suggestions have been made that this complex is necessary for optimum bread-baking behavior (9,10). The same type of complex may also play an important part in the cookie-baking behavior of flour.

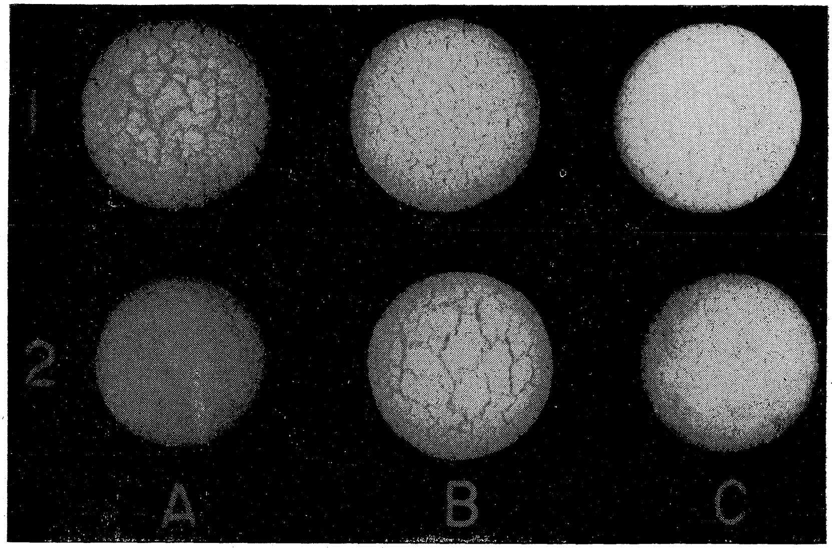

Fig. 4. Effect of various lipids on the cookie-baking properties of a flour extracted with water-saturated *n*-butyl alcohol. Lipids added: 1A, flour lipid; 1B, soybean lecithin, crude; 1C, soybean lecithin, purified; 2A, none; 2B, sucrose fatty acid ester; 2C, vegetable tocopherols.

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