AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

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1. SUBJECT CLASSIFICATION

A. PRIMARY: Public Health

B. SECONDARY: Nutrition

2. TITLE AND SUBTITLE

Improving breadmaking properties with glycolipids: improving soy products with sucroesters (and) improving various protein-enriched products

3. AUTHOR(S)

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4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1969	17 p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS

Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66502

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*

(In Cereal chemistry, v. 46, no. 5, pp. 503-518)

9. ABSTRACT

This paper summarizes studies of effects of synthetic sucroglycerides on quality of soy-enriched breads. The improving effects of synthetic glycolipids are compared with the effects of commercial shortening and of polar wheat flour lipids rich in galactolipids. The conclusions presented in Part I of this paper are that sucroesters seem to improve bread quality by rendering the soy proteins functional in breadmaking. Sucroesters counteracted the loaf volume-depressing effects of the soy products. Mixing synthetic glycolipids with soy flours or adding glycolipids at the dough-mixing stage therefore appears useful in producing nutritionally improved bread acceptable to consumers. The sucroesters appear useful in manufacturing bread low in the saturated fatty acids in shortening, or of protein-rich dietetic breads, which have been recommended as effective supplements to diets to treat obesity. The effects seem to indicate that the synthetic glycolipids make the noncereal proteins functional in breadmaking. The effects of sucroesters on breadmaking potentialities of proteins from other sources are reported in Part II of this series. The reasons for differences in mechanism and performance in breadmaking of synthetic and natural wheat flour glycolipids are being investigated.

10. CONTROL NUMBER

PN-AAB-877

11. PRICE OF DOCUMENT

12. DESCRIPTORS

Flours
Glycolipids
Soy supplements

13. PROJECT NUMBER

14. CONTRACT NUMBER

CSD-1586 Res

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

CSD-1586 Res.
PN-AAB-877
664.06.P785

Improving Breadmaking Properties with Glycolipids. I. Improving Soy Products with Sucroesters[1]

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ABSTRACT

Adding 0.5 g. sucroglycerides per 100 g. flour increased water absorption and maximum hot-paste viscosity; it had no significant effects on mixing time, mixing tolerance, or extensibility characteristics. The sucroesters counteracted the deleterious effects of up to 16% soy products on loaf volume. The improving effect of 0.5% sucroglycerides was equal to, or better than, the effect of 3.0% commercial vegetable shortening. The effect of sucroesters increased with increase in hydrophilic-lipophilic balance (i.e., with decrease in number and chain lengths of fatty acids attached to the sucrose molecule). In addition to increasing loaf volume, sucroesters substantially improved crumb grain and softness. Largest improvement was obtained by adding 0.5% free polar flour lipids, rich in glycolipids. Improvement of free polar flour lipids was observed both in the control and in soy-containing breads; the sucroesters rendered soy proteins functional in breadmaking.

Previous studies have shown that small amounts of polar wheat flour lipids substantially improve loaf volume, crumb grain, and freshness retention of bread (2). Preliminary investigations indicated that the improvement was due mainly to glycolipids (3). Synthetic glycolipids are available commercially. Sucrose esters or sucroglycerides are synthesized by esterification of fatty acids or natural glycerides with sucrose. They were originally designed to provide nonionic surfactants that can be easily broken down, and to eliminate problems of extremely low biodegradation in the disposal of sewage containing commonly used detergents. The procedure for preparing sucrose monoesters of fatty acids was described by Osipow and Rosenblatt (4) and in a patent by Haas et al. (5).

Audidier and LaPape (6) studied the effects of several sucroglycerides on rheological properties of dough and starch pastes. Gluten hydration was slightly reduced when 5.0% of sucroglycerides of palm oil was added to a dough. Gelatinization of starch was slowed when dough containing sucroglycerides was heated. Including sucroglycerides in dough improved handling properties, volume, and over-all quality of baked products (7).

The use of soy flour and soy meal for edible purposes in the United States is estimated at 200 million lb. per year. The largest use of soy flour in the United States is about 50 million lb. for baked products (8,9). Despite increased interest in, and use of, soybean products in breadmaking, the volume of soy protein-enriched bread is relatively small. Acceptance of soy products as a bread ingredient has been relatively limited, because of functional disadvantages and because soy flours were not uniform in early stages of development (10). High consumption, acceptability, and low price make bread an ideal vehicle for protein supplementation. For that potential to be realized, however, the supplement must be economical and uniform.

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[1]Co-operative investigations between the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, and the Kansas Agricultural Experiment Station, Manhattan, Kansas. Approved March 25, 1968, for publication by the Director as Station Manual No. 236. Mention of a trademark name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may also be available.

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It must not change the breadmaking properties of the dough. It must require little adjustment in formula. It should not alter or impair the quality of bread (11).

This paper summarizes studies of effects of synthetic sucroglycerides on quality of soy-enriched breads. The improving effects of synthetic glycolipids are compared with the effects of commercial shortening and of polar wheat flour lipids, rich in galactolipids.

MATERIALS AND METHODS

Flour Samples

Most of the experiments were made with an untreated flour, experimentally milled on an Allis mill from a composite grist of several wheat varieties grown at several locations throughout the Great Plains. This flour is designated RBS (Regional Baking Standard). In addition, three wheat samples grown in 1965 were milled at an extraction of about 70% on a Miag Multomat. The wheat samples were composited by variety from equal portions of wheat, as described previously for samples from the 1963 crop (12). Some chemical and breadmaking characteristics of the flours (baked by the basic complete formula) are summarized in Table I.

Lipids

The shortening used was a commercial product of vegetable origin, partly hydrogenated, m.p. 41°C. Free polar flour lipids were obtained by fractionating free lipids (petroleum ether-extracted) by silicic acid column chromatography (13). Natural lecithin and nine commercially available sucroglycerides were studied. Palmitic acid, glycerol monopalmitate, glycerol dipalmitate, and glycerol tripalmitate were of analytical grade.

Soy Flours

Seven samples of soy flour from three manufacturers in the United States were selected to represent variations in chemical composition, particle size, and heat-treatment—variations believed to be typical of products marketed for use in the bread-baking industry. The composition and characteristics of the soy samples are given in Table II.

Analytical Determinations

Moisture, ash, and Kjeldahl protein were determined by the AACC method (14). Percent nitrogen was converted to percent protein with the factor 5.7 in wheat flour, and with the factor 6.25 in soy samples.

TABLE I. SOME CHEMICAL AND BREADMAKING CHARACTERISTICS OF FLOURS (14% Moisture Basis)

Flour	Protein	Ash	Baking Absorption	Mixing Time	Bromate Requirement	Loaf Volume	Crumb Grade
	N x 5.7%	%	%	min.	p.p.m.	cc.	
RBS	12.9	0.40	60.7	3-1/2	30	953	5
Comanche	12.7	0.42	64.2	3-5/8	15	919	5
Thatcher	13.0	0.54	64.3	3-1/4	20	922	5
Seneca	8.5	0.39	54.2	3	10	740	0.5

TABLE II. CHEMICAL AND PHYSICAL PROPERTIES OF SOY SAMPLES[a]

Soy Sample and Heat Treatment	Particle Size b	Protein N x 6.25 %	Water-Dispersible Protein %	Fat %	Remarks
A Slightly toasted	thru 100	50	70	2	1.75% Lecithin, chemically treated
B Toasted	thru 14	50	20	<1	
C Toasted	thru 100	50	20	<1	
D Toasted	thru 100	50	20	<1	
E ...	thru 100	70	...	...	Deflavored, 70% protein
F ...	thru 100	90	...	<1	Isolated soy protein
G ...	thru 100	85	80	<1	Sodium soy proteinate

[a]Manufacturer's data; expressed on "as-received" moisture basis, which varied from 6 to 10%.
[b]Mesh per in.

Rheological Properties

Mixograms were determined with 35.0 g. flour and optimum water absorption (15). Farinograms and extensigrams were obtained by the procedure described by Villegas et al. (16). The amylograph was used to record changes in hot-paste viscosity (14).

Breadmaking

The breadmaking formula included 100 g. flour, 1.5 g. salt, 2 g. yeast, 4 g. NFDM, 6 g. sucrose, 0.50 g. 60°L. malt syrup, 3 g. shortening, water as needed, and optimum potassium bromate (17,18), except for these changes: In all experimental baking with soy products, NFDM was replaced by 4 to 32 g. soy products. Similarly, shortening was replaced by, or supplemented with, 0.25 g. to 1.00 g. polar lipids in some experimental baking tests. An optimum mixing time with the straight-dough procedure and a 3-hr. fermentation time at 30°C. were employed. Punching and panning were performed mechanically. Baking time was 24 min. at 218°C. Baking tests were replicated at least twice. A third replicate was made when loaf volumes differed by more than 25 cc. Average loaf-volume differences of 20 cc. were significant at the 0.05 level. Loaf volumes were determined by dwarf rapeseed displacement immediately after the bread was taken from the oven. After cooling, the loaves were cut and their crumb grain and texture evaluated. This code was employed: S, satisfactory; Q, questionable; and U, unsatisfactory. Crumb compressibility was measured with a compressimeter. The plunger, 25 mm. in diameter, was depressed 4 mm. into the bread crumb after the crust was removed. The weight in g. required to depress the plunger was taken as compressibility parameter. Compressibility was measured on bread crumb from wrapped and sealed loaves, stored at room temperature (about 25°C.), for 24 or 48

RESULTS AND DISCUSSION

Effects of Lipids on Rheological Dough Properties

Water-absorption of dough containing 3% shortening was lower than of dough

TABLE III. LOAF VOLUME AND CRUMB GRAIN OF BREAD BAKED FROM RBS FLOUR WITH SOY FLOUR D

Soy Flour Level g.	Shortening g.	Sucrose Tallowate g.	Potassium Bromate p.p.m.	Loaf Volume cc.	Crumb Grain
			15	860	Q-S
	1.0		15	890	S
	2.0		15	900	S
	3.0		15	893	S
		0.25	15	893	S
		0.50	15	900	S
4			35	820	U
8			50	717	U
4	1.0		35	883	Q-S
4	3.0		35	898	Q-S
8	1.0		50	878	Q-U
8	3.0		50	918	Q-S
4		0.25	35	915	Q-S
4		0.50	35	918	Q-S
8		0.25	50	878	Q-U
8		0.50	50	945	Q-S

containing no added lipids. Adding 0.5% polar lipids increased water-absorption of the dough about 1.0%, and adding 0.5% sucroesters increased absorption up to 1.5%. A similar increase was observed in farinograph water-absorption. Adding 0.5 g. (per 100 g. flour) sucroesters had no significant effect on mixing time or mixing tolerance. Similarly, extensimeter parameters (extensibility and resistance to stretching) were affected very little. Sucroesters had little effect on temperature of hot paste peak viscosity; the peak height was, however, increased substantially. Maximum amylograph viscosity of 590 B.U. in the control was increased to 750, 780, and 860 B.U. by adding, respectively, 0.5 g. of sucrose monopalmitate, sucrose monostearate, or sucrose monolaurate.

Improving Soy Flours with Sucroesters

Adding, per 100 g. flour, 1.0 g. shortening or 0.25 g. sucrose tallowate increased loaf volume and improved crumb grain of bread; higher levels of lipids had no additional improving effect in bread baked without soy flour (Table III). Adding soy flour decreased loaf volume; the decrease was greater when 8 g. soy flour was added than when 4 g. was added. To counteract the deleterious effect of 4 g. soy flour, 1 g. shortening or 0.25 g. sucroglyceride was added. At the level of 8 g. soy flour, 3 g. shortening or 0.50 g. sucroglyceride increased loaf volume of soy-enriched bread above that of the control containing no soy flour; the increase from adding 0.5 g. sucroglyceride was greater than from adding 3.0 g. shortening.

Different Soy Flours and Bread Quality

Table IV gives a comparison of the improving effects on loaf volume and crumb grain of 3 g. shortening and 0.5 g. sucrose tallowate in bread containing 8 g. (per 100 g. flour) of the seven soy products. The soy flours varied considerably in effects on loaf volume and crumb grain. All soy flours decreased loaf volume of bread baked without shortening or sucrose tallowate. Bread baked with chemically treated soy flour A, which contained 1.75% lecithin, had consistently the largest and generally the best loaves. Soy flour A was better than the toasted and chemically unmodified soy flour D from the same manufacturer. Soy flours B and C were from the same manufacturer and varied only in particle size. The coarse

TABLE IV. LOAF VOLUME AND CRUMB GRAIN OF BREAD BAKED AT OPTIMUM BROMATE LEVEL WITH 8 g. OF SEVEN SOY PRODUCTS PER 100 g. RBS FLOUR

Soy Product	Control		With 3% Shortening		With 0.5% Sucrose Tallowate	
	Loaf Volume cc	Crumb Grain	Loaf Volume cc.	Crumb Grain	Loaf Volume cc.	Crumb Grain
None	860	Q-S	893	S	893	S
A	766	Q	933	S	963	Q-S
B	723	Q	860	Q-S	858	Q
C	658	U	833	Q	850	Q
D	717	U	918	Q-S	945	Q-S
E	680	Q-U	735	Q-U	813	Q
F	673	Q-U	798	Q	885	Q-S
G	618	Q-U	805	Q-S	835	Q-S

product, B, was better than the finely pulverized soy flour, C, particularly for the controls. Loaf volumes of bread baked with the three soy protein concentrates and isolates were substantially improved by either shortening or sucrose tallowate. Yet the loaf volumes were substantially lower than those of bread containing 8% of soy flour. The results indicate that removing nonprotein soy flour components does not eliminate the loaf volume-depressing effect of soy flour. On the other hand, added at an 8% level, the soy isolates or concentrates increased protein contents up to twice as much as did adding regular soy flour. The improvement from adding the sucroglyceride was substantially greater than from adding shortening to doughs containing any of the six finely ground soy flours. The improvement in bread baked with the coarse soy product, B, was the same whether shortening or sucrose glyceride was added.

Lipid Composition and Soy Flour Improvement

Table V compares effects on loaf volume and crumb grain of adding 0.5 g. (per 100 g. flour) of various lipids to bread baked with 8 g. soy flour A. Free polar flour lipids improved bread most; the loaf volume of bread baked with 0.5 g. polar flour lipids and 8 g. soy flour was substantially higher (1,015 cc.) than that of bread baked with 3 g. shortening and 4 g. NFDM (953 cc., Table I) or with 3 g. shortening and 8 g. soy flour (933 cc., Table V). Lecithin had a significant, though relatively small, improving effect. The sucroglycerides investigated affected the results consistently and significantly. The improving effect was greater with fewer and shorter chain lengths of fatty acids attached to the sucrose molecule; i.e., with higher hydrophilic-lipophilic balance.

Palmitic acid, glycerol monopalmitate, glycerol dipalmitate, and glycerol tripalmitate had little or no improving effect, which indicates that a combination between the polar character of sugar polyols and the lipophilic contribution of the aliphatic chains of fatty acids is essential.

Whereas in soy-containing bread sucroglycerides with short-chain fatty acids increased loaf volume more than sucroglycerides with long-chain fatty acids (Table V), no such effect was noted in bread baked without added soy flour (Table VI). The sucroesters generally increased loaf volume of bread baked without soy products as much as vegetable shortening, which seems to point to the possibility of an improvement related to physical properties rather than to chemical characteristics of the added lipids. In soy-enriched bread, the sucroesters seem to

TABLE V. LOAF VOLUME AND CRUMB GRAIN OF BREAD BAKED AT OPTIMUM BROMATE LEVEL WITH 8 g. SOY FLOUR A PER 100 g. RBS FLOUR, VARIOUS LIPIDS, AND LIPID DERIVATIVES

Lipid Description	Lipid Level	Loaf Volume	Crumb Grain
	g.	cc.	
None	None	766	Q-U
Shortening	3.0	933	Q-S
Polar flour	0.5	1,015	S
Lecithin	0.5	873	Q
Sucroesters			
Monolaurate	0.5	997	Q-S
Sesquilaurate	0.5	970	Q-S
Dilaurate	0.5	955	Q-S
Monopalmitate	0.5	965	Q-S
Dipalmitate	0.5	840	Q
Monostearate	0.5	915	Q-S
Sesquistearate	0.5	853	Q
Tristearate	0.5	795	Q
Lipid derivatives			
Palmitic acid	0.5	778	Q-U
Glycerol monopalmitate	0.5	815	Q-U
Glycerol dipalmitate	0.5	805	Q-U
Glycerol tripalmitate	0.5	765	Q

interact with the soy proteins, so they are functional in breadmaking. That hypothesis is currently being investigated.

The results (summarized in Tables V and VI) also point to a difference between the effects of free polar wheat flour lipids and synthetic sucroglycerides. Whereas certain synthetic sucroesters were comparable in their improving effects to the flour lipids in bread baked with soy flours (Table V), there was a large difference in bread baked without soy flour (Table VI). Bread containing no soy flour had substantially higher volume when baked with 0.5% polar flour lipids (922 cc.) than when baked with 0.5% sucroesters (865 to 905 cc.) or with 3.0% commercial vegetable shortening (893 cc.). It seems, therefore, that polar wheat flour lipids have an improving action in addition to, and above, that made by the synthetic sucroglycerides.

Table VII summarizes the effects of 8% soy flour C on loaf volume and crumb grain of bread baked from three wheat flours. Of the four soy flours (A through D) described in Table IV, the soy flour used in this series of baking experiments was the most harmful to breadmaking. Yet, in each case, after 0.5 g. of free polar wheat flour lipids was added, the loaf volume of bread baked with 8 g. soy flour was equal

TABLE VI. LOAF VOLUME AND CRUMB GRAIN OF BREAD BAKED FROM 100 g. RBS FLOUR AT OPTIMUM BROMATE LEVEL WITH VARIOUS LIPIDS

Lipid Description	Lipid Level g.	Loaf Volume cc.	Crumb Grain
		860	Q-S
Shortening	3.0	893	S
Polar flour	0.5	992	Slaty
Sucroesters			
Monolaurate	0.5	865	S
Monopalmitate	0.5	905	S
Monostearate	0.5	895	S

TABLE VII. LOAF VOLUME AND CRUMB GRAIN OF BREAD BAKED AT OPTIMUM BROMATE LEVEL FROM THREE WHEAT FLOURS WITH 8 g. OF SOY FLOUR C AND 3 g. SHORTENING OR 3 g. POLAR LIPIDS PER 100 g. WHEAT FLOUR

Lipid	Comanche		Thatcher		Seneca	
	Loaf Volume cc.	Crumb Grain	Loaf Volume cc.	Crumb Grain	Loaf Volume cc.	Crumb Grain
None	710	U	663	U	563	U
Shortening	848	Q-S	853	Q-S	695	Q
Polar flour	923	Q-S	918	Q-S	730	Q
Sucrose monolaurate	895	Q-S	860	Q-S	683	Q-U
Sucrose monopalmitate	860	Q-S	868	Q-S	713	Q-S
Sucrose monostearate	865	Q-S	805	Q-S	705	Q

that of bread baked with 4 g. NFDM (Table I). Improvement was greatest when the polar wheat flour lipids were added; 0.5 g. synthetic sucroglycerides were generally equal to 3.0 g. shortening.

Table VIII summarizes the effects of 6 g. shortening and 2.0 g. sucrose monolaurate on bread baked with 16 or 32 g. soy flour A per 100 g. RBS flour. At both soy flour levels, loaf volume was improved much more by sucrose monolaurate than by commercial vegetable shortening. Loaf volume of bread baked with 16% soy flour A and 2% sucrose monolaurate was essentially equal to that of bread baked by the complete formula without soy flour (953 cc., Table I). Adding g. shortening in addition to 2 g. sucrose monolaurate had no additional effect. Crumb grain of bread baked with 2 g. sucrose monolaurate was substantially more open than that of bread baked with 6 g. shortening.

Loaf volume of bread baked with 32% soy flour A was unaffected by addition of 6 g. shortening, and only partially restored (compared with the control) by addition of 2 g. sucrose monolaurate. The results indicate that at very high levels of soy flour and monolaurate, factors other than glycolipids limit loaf-volume potential.

Lipids and Bread Compressibility

Effects of lipids on compressibility in bread baked with 8% soy flour and stored for 24 hr. are summarized in Fig. 1. Loaf volume is negatively correlated with weight required to depress the plunger into bread crumb. Two regression lines can be drawn: one for bread baked from the HRW Comanche and HRS Thatcher, and one for the SRW Seneca. The flours from the hard wheats contained substantially more protein (12.7 and 13.0%, Table I) than that from SRW wheat (8.5%). Crumb

TABLE VIII. LOAF VOLUME AND CRUMB GRAIN OF BREAD BAKED FROM 100 g. RBS FLOUR WITH 16 AND 32 g. OF SOY FLOUR A AND SHORTENING OR SUCROSE MONOLAURATE

Flour level g.	Shortening g.	Sucrose Monolaurate g.	Potassium Bromate p.p.m.	Loaf Volume cc.	Crumb Grain
16	...	...	15	860	Q-S
16	...	...	70	700	U
16	6	...	70	883	Q-S
16	6	2	70	955	Q-S
32	...	...	100	505	U
32	6	...	100	505	U
32	...	2	100	607	U
32	6	2	100	580	U

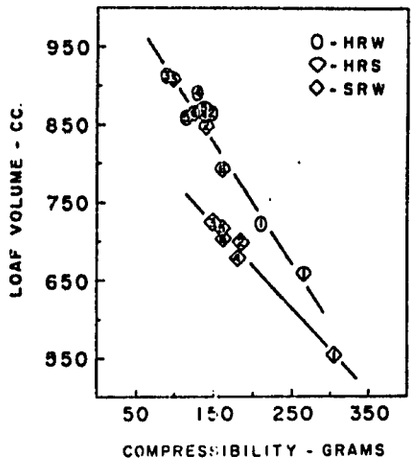

Fig. 1. Effects of 3.0 g. of shortening and 0.5 g. of certain polar lipids on loaf volume and compressibility (after 24 hr.) of bread baked from three wheat flours with 8 g. soy flour per 100 g. wheat flour: 1, control; 2, shortening; 3, wheat flour lipids; 4, sucrose monolaurate; 5, sucrose monopalmitate; 6, sucrose monostearate.

softness was improved most by adding 0.5% free polar flour lipids. Crumb of bread baked with 0.5% synthetic glycolipids was equal to or softer than bread baked with 3.0% commercial vegetable shortening. After 48 hr., softness decreased proportionately for all samples.

CONCLUSIONS

The sucroesters seem to improve bread quality by rendering the soy protein functional in breadmaking. Sucroesters counteracted the loaf volume-depressing effects of the soy products. Mixing synthetic glycolipids with soy flours or adding glycolipids at the dough-mixing stage, therefore, appears useful in producing nutritionally improved bread acceptable to consumers.

The sucroesters appear useful in manufactuirng bread low in the saturated fatty acids in shortening, or of protein-rich dietetic breads, which have been recommended as effective supplements to diets to treat obesity (19). The effects of sucroesters on quality of bread baked with soy products seem to indicate that the synthetic glycolipids make the noncereal proteins functional in breadmaking. The effects of sucroesters on breadmaking potentialities of proteins from other sources are reported in Part II of this series. The reasons for differences in mechanism and performance in breadmaking of synthetic and natural wheat flour glycolipids are being investigated.

Acknowledgment

Sucroglyceride samples were a gift of Colonial Sugars Company, Gramercy, Louisiana.

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[Received August 5, 1968. Accepted October 18, 1968]

Improving Breadmaking Properties with Glycolipids. II. Improving Various Protein-Enriched Products[1]

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ABSTRACT

Bread was baked with 8 or 16 g. soy flour, and 8 g. (per 100 g. wheat flour) defatted cottonseed flour, fish protein concentrate, nonfat dry milk, expeller-extracted sesame seed flour, wheat gluten, defatted wheat germ, air-fractionated protein-rich wheat flour, or food-grade Torula yeast. All protein-rich additives substantially lowered loaf volume and impaired crumb grain. Adding 3 g. shortening counteracted partly the deleterious effects of 16 g. soy flour; higher levels had no additional improving effect. Loaf volumes were increased and crumb grain was improved by adding 1.0 to 6.0 g. sucrose tallowate. The loaf volume of bread containing 3.0 to 6.0 g. sucrose tallowate was substantially above that of the control containing no added soy flour. Highest levels of tallowate increased mixing time and impaired rheological properties. Small amounts (0.5 to 1.0 g.) synthetic glycolipids and free lipids (rich in glycolipids) from wheat flour and *Briza spicata* improved volume and crumb grain of nutritionally improved bread as much as or more than 3 g. shortening. Synthetic glycolipids, but not shortening, rendered commercial wheat gluten functional in breadmaking.

Previous studies from our laboratory were concerned with improving breadmaking properties of soy products with glycolipids (1). Small amounts (0.25 to 2.0%) of synthetic sucroglycerides and polar wheat flour lipids (rich in glycolipids) counteracted the deleterious effects of up to 16% of various soy products on loaf volume, on crumb grain, and bread softness. The effects of sucroglycerides increased with increase in hydrophilic-lipophilic balance; i.e., with decrease in number and chain lengths of fatty acids attached to the sucrose molecule. This report is concerned with the beneficial effects of wheat flour polar lipids, lipids from *Briza spicata* seed that are rich in glycolipids (2), and sucroglycerides on production of bread nutritionally improved by several protein-rich products, including soy, cottonseed, sesame, wheat germ, wheat gluten, an air-fractionated protein-rich fraction from wheat flour, fish, Torula yeast, and NFDM.

MATERIALS AND METHODS

Flour Samples

Most of the experiments were made with an untreated flour, experimentally milled on an Allis mill from a composite grist of several wheat varieties grown at several locations throughout the Great Plains. This flour is designated RBS (Regional Baking Standard). In addition, three wheat samples grown in 1965 were milled at an extraction of about 70% on a Ming Multomat. The wheat samples were composited from equal portions of wheat as described previously for samples from the 1962 crop (3). Some chemical and breadmaking characteristics of the flours (baked by a formula including 4% milk solids and 3% shortening) are summarized in Table I.

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[1]Co-operative investigations between the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, and the Kansas Agricultural Experiment Station, Manhattan, Kansas. Approved May 24, 1968, for publication by the Director as Station Article No. 27[illegible]. Mention of a trademark name, proprietary product, or scientific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may also be available.

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TABLE I. SOME CHEMICAL AND BREADMAKING CHARACTERISTICS[a] OF FLOURS

Flour	Protein (N X 5.7) %	Ash %	Baking Abs. %	Mixing Time min.	Bromate Requirement p.p.m.	Loaf Volume cc.	Crumb Grain
RBS	12.9	0.42	65.5	3-1/4	30	935	S
Comanche	12.7	0.42	64.2	3-5/8	15	919	S
Thatcher	13.0	0.54	64.3	3-1/4	20	922	S
Senoca	8.5	0.39	54.2	3	10	740	G-S

[a] 14% moisture basis.

Lipids

The shortening used was a commercial product of vegetable origin, partly hydrogenated, with m.p. of 41°C. Free polar flour lipids were obtained by fractionating free lipids (petroleum ether-extracted) from the RBS flour by silicic acid column chromatography (4). Free lipids were also obtained by exhaustive extraction of seeds of *B. spicata* with petroleum ether. Bound lipids from the RBS flour and from *B. spicata* were extracted with water-saturated butanol after petroleum ether extraction. The wheat flour contained 0.8% free lipids (a mixture of 0.6% nonpolar components eluted from the silicic acid column with chloroform, and 0.2% polar components eluted with methanol after the chloroform elution) and 1.6% bound lipids. Extraction of *B. spicata* seeds gave 11.1% free and 1.4% bound lipids. The two sucroglycerides, sucrose monolaurate and sucrose tallowate, were commercial products.

Protein Concentrates

Nine protein concentrates were used. Some chemical characteristics of the concentrates are given in Table II. The soy flour used was a commercial, chemically treated product that contained 1.75% lecithin and had a dispersibility index corresponding to 70%. Defatted cottonseed flour, fish protein concentrate, NFDM, expeller-extracted sesame seed flour, wheat gluten, and Torula yeast (food grade) were commercial products. The wheat germ used was a fresh, granular product. The wheat germ was defatted by extraction with petroleum ether, ground to pass a 50-mesh sieve, and re-extracted with petroleum ether. The air-fractionated flour was prepared on a laboratory scale from defatted (petroleum ether-extracted) patent flour that was commercially milled from a mixed grist of HRW wheat. The original patent flour had a protein content of 10.3% and an ash content of 0.44%.

TABLE II. CHEMICAL COMPOSITION OF PROTEIN CONCENTRATES

Protein Concentrate	Moisture %	Protein %	Petroleum Ether Ext. %	Ash %
Soy flour	8.3	52.7	0.5	6.91
Cottonseed flour	4.7	63.1	3.9	6.48
FPC	7.8	69.7	0.2	21.05
Milk powder	10.5	34.6	0.1	7.89
Sesame seed flour	8.8	46.1	5.6	11.75
Wheat gluten	7.8	57.0	0.6	0.89
Torula yeast	7.6	54.9	1.6	7.02
Wheat germ	11.0	30.0	0.2	5.15
Air-fractionated flour	6.8	27.0	1.3	0.78

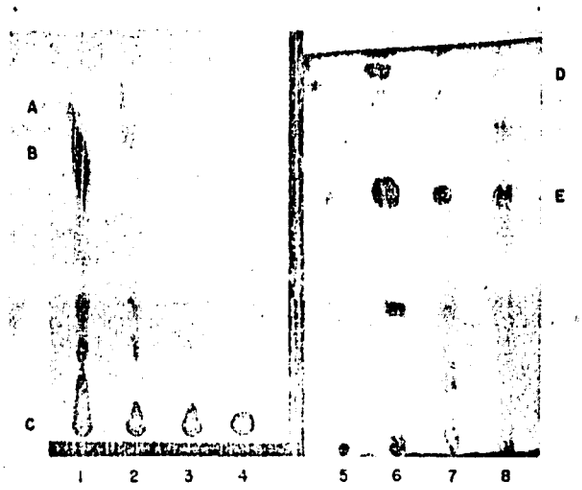

Fig. 1. Thin-layer chromatogram of lipids from wheat flour (1, 4, 5, and 8) and *B. spicata* (2, 3, 6, and 7); free lipids (1, 2, 5, and 6) and bound lipids (3, 4, 7, and 8). Spots 1 through 4 were developed with chloroform, 5 through 8 with chloroform-methanol-water (65:35:4). Spots of 100 γ lipids; visualized by charring with sulfuric acid; picture taken under ultraviolet light. Tentatively identified as: A, mixture of hydrocarbons and steryl esters; B, triglycerides; C, unresolved polar lipids; D, unresolved nonpolar lipids; E, digalactosyl diglyceride.

Analytical Determinations

Moisture, ash, crude fat, and Kjeldahl protein were determined by the AACC method (5). Percent nitrogen was converted to percent protein with the factor 5.7 in wheat products (including gluten) and the factor 6.25 in protein concentrates. Lipids were fractionated by thin-layer chromatography (TLC) as described elsewhere (4). The pure compounds used to identify lipid components included trilinolein, phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine, monogalactosyl diglyceride, and digalactosyl diglyceride. The TLC of lipids in wheat flour and in *B. spicata* in Fig. 1 shows that the latter contained substantial amounts of digalactosyl diglyceride.

Breadmaking

The breadmaking formula included 100 g. flour, 1.5 g. salt, 2 g. yeast, 4 g. NFDM, 6 g. sucrose, 0.50 g. 60°L. malt syrup, 3 g. shortening, water as needed, and optimum potassium bromate, except for these changes: In experimental baking with protein concentrates, NFDM was replaced by 8 or 16 g. protein concentrate. Similarly, in some experimental baking, shortening was omitted, added at various levels, or replaced by polar lipids. An optimum mixing time with the straight-dough procedure and a 3-hr. fermentation time at 30°C. were employed. Panning and punching were performed mechanically. Baking time was 24 min. at 218°C. Baking tests were replicated at least twice. Loaf volumes of bread baked from each whole dough mix were determined by dwarf rapeseed displacement immediately after the bread was taken from the oven. After cooling, the loaves were cut and their crumb grain and texture evaluated. This code was employed: S = satisfactory, Q = questionable, and U = unsatisfactory.

TABLE III. LOAF VOLUME AND CRUMB GRAIN OF BREAD BAKED WITH 16 g. SOY FLOUR (PER 100 g. RBS FLOUR) AND VARIOUS LEVELS OF SHORTENING OR SUCROSE TALLOWATE

Lipid	Lipid Level	Loaf Volume	Crumb Grain
	g.	cc.	
		675	U.
Shortening	3	900	S-
	6	885	Q-S
	9	887	Q-S
Sucrose tallowate	1	875	Q
	2	938	Q
	3	993	S
	4	1,030	S
	6	1,100	S

RESULTS AND DISCUSSION

Effects of various levels of vegetable shortening and sucrose tallowate on loaf volume and crumb grain of bread baked with 16 g. soy flour (per 100 g. RBS flour) are summarized in Table III. Pictures of some loaves baked with 16 g. soy flour alone and with 4 g. sucrose tallowate are compared with the control baked with 4 g. milk solids and 3 g. shortening (Table I) in Fig. 2. Both shortening and sucrose tallowate increased loaf volume and improved crumb grain; but, whereas high levels of shortening (6 g. and 9 g.) were probably slightly detrimental, increasing the level of sucrose tallowate to 6 g. per 100 g. flour consistently increased loaf volume and maintained satisfactory crumb grain at high levels of tallowate. Loaf volumes of bread baked with 16 g. soy flour and 3 to 6 g. sucrose tallowate were substantially higher (993 to 1,100 cc., Table III) than loaf volume of bread baked by the complete formula without soy flour (935 cc., Table I). Increasing the level of sucrose tallowate from 0 to 6 g. gradually lengthened mixing time from 4 to about 6.5 min. Doughs containing 6 g. sucrose tallowate were tough, short, and difficult to handle. Crust color deepened significantly and consistently with increase in level of sucrose tallowate.

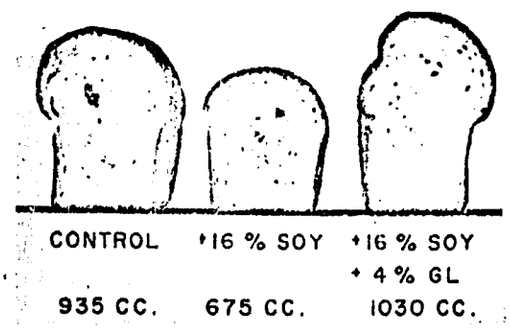

Fig. 2. Bread baked from 100 g. RBS flour. Left to right: with 4 g. milk powder and 3 g. shortening; with 16 g. soy flour; and with 16 g. soy flour and 4 g. sucrose tallowate.

TABLE IV. LOAF VOLUME AND CRUMB GRAIN OF BREAD BAKED WITH 8 g. SOY FLOUR OR 8 g. COTTONSEED FLOUR AND VARIOUS LIPIDS (PER 100 g. RBS FLOUR)

Lipid Source	Lipid Level g.	Loaf Volume cc.	Crumb Grain
	Soy flour		
None	...	755	Q
Shortening	3.0	870	S
Sucrose tallowate	0.5	915	S
B. spicata	0.5	883	S
	Cottonseed flour		
None	...	733	Q-U
Shortening	3.0	835	S
Sucrose tallowate	0.5	850	S
B. spicata	0.5	833	S
Polar-free (wheat flour)	0.5	900	S

Effects of various lipids on loaf volume and crumb grain of bread baked with either 8 g. soy flour or 8 g. cottonseed flour are summarized in Table IV. In bread enriched nutritionally with either of the two oilseed flours, loaf volume and crumb grain were improved by adding 3 g. shortening or as little as 0.5 g. polar lipids. Sucrose tallowate increased loaf volume more than shortening, and polar wheat flour lipids increased loaf volume most. The effects on loaf volume of lipids from *B. spicata* were smaller than the effects of sucrose tallowate.

Adding to three flours 8 g. (per 100 g. flour) of milk powder, air-fractionated wheat flour, or defatted wheat germ (without adding lipids), impaired loaf volume (Table V). Loaf volumes of bread baked from the three flours by the complete control formula, which included 4 g. NFDM and 3 g. shortening, were 919, 922 and 740, respectively, for Comanche, Thatcher, and Seneca (Table I). Addition of commercial shortening or sugar (sucrose) increased loaf volume and improved

TABLE V. LOAF VOLUME OF BREAD BAKED WITH MILK POWDER, AIR-FRACTIONATED FLOUR, OR DEFATTED GERM AND 3 g. SHORTENING OR 0.5 g. SUCROSE MONOLAURATE

	NFDM 4 g.[a] cc.	8 g.[a] cc.	Air-Frac. Flour 8 g.[a] cc.	Defatted Germ 8 g.[a] cc.
Commancho Flour				
No lipid added	...	870	870	658
Shortening	919	950	945	850
Sucr. Monolaurate	...	940	990	815
Thatcher Flour				
No lipid added	...	793	890	605
Shortening	922	938	923	875
Sucr. Monolaurate	...	915	945	813
Seneca Flour				
No lipid added	...	655	680	--
Shortening	740	780	715	--
Sucr. Monolaurate	...	760	680	--

[a]Concentrate per 100 g. flour.

TABLE VI. LOAF VOLUME AND CRUMB GRAIN OF BREAD BAKED WITH 8 g. OF VARIOUS PROTEIN CONCENTRATES (PER 100 g. RBS FLOUR) AND COMMERCIAL VEGETABLE SHORTENING OR SUCROSE TALLOWATE

Protein Concentrate	Shortening g.	Sucrose Tallowate g.	Loaf Volume cc.	Crumb Grain
Torula yeast	...	...	773	Q-S
	3	...	803	S
	...	1	850	S
Sesame flour	...	...	678	U
	3	...	776	Q
	...	1	848	Q
Fish meal	...	...	710	U
	3	...	843	Q
	...	1	860	Q
Wheat gluten	...	...	805	Q
	3	...	845	Q
	...	1	990	S

crumb grain (data not given) of bread baked with 8 g. of any of the three protein concentrates. There was no significant or consistent difference between the improving effects of 3 g. shortening or 0.5 g. sucrose monolaurate. Loaf volume was restored (to that of bread baked by the complete control formula) in bread baked with either 8 g. milk powder or 8 g. air-fractionated flour and either shortening or sucrose monolaurate. Loaf volume of bread baked with 8 g. defatted germ and added lipids continued to be lower than that of bread baked by the complete control formula.

Loaf volume and crumb grain of bread baked with 8 g. of four protein concentrates and 3 g. commercial vegetable shortening or 1.0 g. sucrose tallowate (per 100 g. RBS flour) are summarized in Table VI. Bread baked with 8 g. of any of the protein concentrates and without added lipids was inferior to that of bread baked by the complete control formula (935 cc., Table I). In each case, addition of shortening or sucrose tallowate increased loaf volume and improved crumb grain. The improvement was consistently larger from adding 1 g. sucrose tallowate than from adding 3 g. vegetable shortening. The difference is most significant in bread baked with 8 g. functionally impaired (in breadmaking potential) commercial gluten. The results indicate that adding synthetic glycolipids (but not shortening) might restore functional properties of commercial gluten. More-detailed results of similar investigations will be reported shortly.

Acknowledgment

The following generously provided samples used in this study: G. A. White, USDA, Beltsville, Maryland, seed of *B. spicata*; Archer-Daniels-Midland Co., Minneapolis, Minnesota, soy flour; Traders Protein Division, Fort Worth, Texas, cottonseed flour; and Colonial Sugars Co., Gramercy, Louisiana, sucroglycerides.

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[Received August 5, 1968. Accepted October 18, 1968]

An Evaluation of Natural vs. Synthetic Substrates for Measuring the Antitryptic Activity of Soybean Samples[1]

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ABSTRACT

The casein digestion method of Kunitz for the measurement of trypsin inhibitor activity of soybean extracts has been modified to obtain more accurate and reproducible results. The modification involved the use of 2% casein (instead of 1%) and the mathematical transformation of absorbance readings (A) at 280 mμ to $A^{3/2}$. The use of the synthetic substrate, benzoyl-DL-arginine-p-nitroanilide (BAPA), proved to be a convenient and reliable method of assaying trypsin inhibitor activity provided one takes into account the competitive nature of the inhibition. The latter effect could be largely compensated for by extrapolating the trypsin inhibitor activity, expressed as trypsin units inhibited per ml. extract, to zero concentration of the inhibitor. Although a series of soybean samples could be ranked in the same relative order of inhibitor activity by using either casein or BAPA, the quantity of trypsin inhibited was consistently higher when BAPA was employed as the substrate.

Casein has been widely used as a substrate for measuring the trypsin inhibitor activity of natural trypsin inhibitors such as those which occur in soybeans and other legumes. The most common method employing casein is the one originally described by Kunitz (1) which involves the spectrophotometric determination of the breakdown products produced by a given concentration of trypsin in the presence and absence of the inhibitor. However, as pointed out by several investigators (2,3) and according to our own experience, the fact that the rate of hydrolysis of casein by trypsin does not follow zero order kinetics under the conditions defined by Kunitz (1) detracts from the reliability of this method and poses problems of reproducibility.

On the other hand, the tryptic hydrolysis of a synthetic substrate such as benzoyl-DL-arginine-p-nitroanilide (BAPA), first introduced by Erlanger et al. (4) does follow a zero order reaction, and one obtains, within broad limits, a linear relationship between the quantity of p-nitroaniline released and the concentration

[1]This study was supported by Agricultural Research Service, U.S. Dept. of Agriculture Grant No. 12-14-100-9180 (71), administered by the Northern Utilization Research and Development Division, Peoria, Illinois.