Properties of Glutens from Doughs Containing Components of Cheddar-Cheese Whey[1]

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ABSTRACT

Glutens were isolated from bread-flour doughs and all-purpose-flour doughs containing lactose, whey proteins, lactose-plus-proteins, or dried whole whey. Lactose had no effect on the viscoelastic properties of gluten from either of the two flours. Whey proteins added to all-purpose-flour doughs increased yield of gluten and hardness of the gluten balls, and lightened the color. When added to bread-flour doughs, whey proteins decreased elasticity, increased tenderness and volume, and darkened the color. In general, properties of glutens from doughs containing both lactose and proteins were similar to those of glutens containing proteins alone. Adding dried whole whey to all-purpose-flour doughs decreased drip loss, extensibility of gluten, and volume of the gluten balls, but increased tenderness and lightness of color. In bread-flour doughs, dried whole whey decreased yield, drip loss, extensibility, and elasticity. Properties of lactose-plus-proteins glutens differed from dried whole-whey glutens in several respects. The two flours did not always react to the added components in the same manner.

Gluten properties may be markedly altered in the presence of other materials (1). Sugars have been shown to decrease the yield of gluten, increase drip loss, increase dough-development time, and, at higher levels, prevent dough formation entirely (2,3). The findings indicated that sugars had a peptizing action on gluten, inhibited heat coagulation, and decreased hydration. Ponte et al. (4) reported that glutens isolated from doughs containing various organic solvents exhibited decreased extensibility. Expansion properties of rehydrated commercial gluten containing the solvents were related to alkane chain length. Other workers have shown that glutens treated with D_2O became stronger and tougher as manifested by increased elasticity and decreased extensibility (5,6). The changes in viscoelastic properties were attributed to changes in hydrogen bonding.

The properties of gluten as related to its structure-forming role in bakery products may also be altered by ingredients in the formula. Earlier studies at this station on the use of dried whole whey and its two principal components—whey proteins and lactose—showed that whey proteins had a firming effect on dough and crumb of biscuits and doughnuts made from all-purpose flour, whereas lactose had a softening and tenderizing effect (7,8). It was not known whether these materials had altered the gluten, the starch, or both. The effects of these components of cheddar-cheese whey on properties of wheat-starch pastes have recently been reported by Hirai et al. (9). The present study examines the effects of the same materials on properties of glutens from all-purpose and from bread flours.

MATERIALS AND METHODS

A commercial, bleached all-purpose flour and a bleached, bromated bread flour were purchased locally. The dried whole whey and the lactose (α-monohydrate

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[1]Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 874.

form) were obtained commercially. The whey proteins were precipitated from cheddar-cheese whey as described by Hofstrand et al. (8), and frozen until used. Moisture, ash, and protein contents of the ingredients were as follows:

Ingredient	*Moisture* %	*Ash*[a] %	*Protein*[a,b] %
All-purpose flour	9.2	0.40	10.2
Bread flour	12.7	0.44	12.5
Lactose	0.5	0.15	0.2
Whey proteins	81.2	0.99	14.0
Dried whole whey	1.8	8.22	12.3

[a]On an "as-is" basis.
[b]N × 5.70 for flour; N × 6.38 for other ingredients.

The glutens were obtained from doughs prepared from ten experimental formulas (Table I). The levels of the components were similar to those used in a previous study on biscuits (7). The moisture level was adjusted to facilitate washing out the gluten. The ingredients were mixed for 10 min. in a KitchenAid® mixer, Model K-4. When whey proteins were used, they were dispersed with one-fourth of the water in a 1-pint jar of a Waring Blendor for 2 min. before use. Approximately 0.06% of the proteins were soluble in the water. The formed doughs were placed in a bowl containing 50 ml. distilled water, turned over to moisten the entire surface, covered with plastic, and allowed to rest 30 min.

The glutens were isolated by washing the doughs under tap water until the iodine test on the wash water was negative for starch. After draining for 15 min., the gluten was weighed. The percent yield of gluten was calculated from the following formula:

$$\frac{\text{Weight of gluten after draining} \times 100}{\text{Weight of flour}}$$

The gluten was then mixed again for 10 min., reweighed, pressed into a rectangular metal box lined with plastic wrap, covered, and refrigerated overnight. The next morning the gluten was weighed again. The losses in weight after mixing and

TABLE I. FORMULAS FOR DOUGHS FROM WHICH GLUTENS WERE ISOLATED

Flour					
Type	Amount g.	Lactose g.	Whey Proteins g.	Dried Whole Whey g.	Distilled Water ml.
Bread	525	...	...	...	336
Bread	525	50.4	...	...	336
Bread	525	...	44.3	...	327
Bread	525	50.4	44.3	...	327
Bread	525	...	...	73.2	336
All-purpose	643	...	...	...	370
All-purpose	643	60.1	...	...	370
All-purpose	643	...	52.8	...	382
All-purpose	643	60.1	52.8	...	382
All-purpose	643	...	...	87.4	370

refrigeration were designated as "drip loss" (2). Drip loss is considered to be a measure of the water-holding capacity. A similar measurement has been used to assess syneresis in starch gels (9,10).

The rectangle of gluten was then cut into eight equal portions. Six portions, trimmed with scissors to form rounded balls weighing 15 g. each, were used for baking. The remaining two portions were trimmed to form rounded rectangles of 15 g. each for extensibility tests. Unless used immediately, they were wrapped in plastic wrap and returned to the refrigerator until used. This period never exceeded 3 hr. The test pieces were always allowed to come to room temperature before the measurements were made.

Baking

The six gluten balls were placed on silicone-sprayed foil pans 4.5 in. in diameter and baked at 232°C. for 15 min. plus an additional 35 min. at 149°C. (3). After cooling for 30 min., the balls were weighed.

Objective Tests

The baked volume of the six gluten balls was measured by the rapeseed displacement method and was expressed as the mean volume per ball. Specific volume was computed by dividing the baked volume by the weight.

Tenderness measurements were obtained on each ball with an Instron Universal Testing Machine Model TM fitted with Load Cell CDM (load range, 0 to 500 kg.). The ball was placed in a stainless-steel box (10 X 9 X 6.5 cm.) on the platform of the compression cell (Fig. 1). A solid aluminum plate (9.2 X 8.2 X 1.5 cm.) crushed the ball at a speed of 2 cm. per min. The full-scale load setting was either 10 or 20 kg., depending on the firmness of the sample. The forces at the first peak, at 2-cm. descent, at half-height of the ball, and the maximum force were read directly from the graph. The mean of the values obtained on the six balls was considered representative of the batch.

A Gardner Automatic Color Difference Meter, standardized against a light yellow Gardner color standard tile No. CYL 0086 ($Rd = 59.7; a = -2.1; b = +22.7$) was used for color measurements. After the Instron test, the six crushed gluten balls from each treatment were ground in a food chopper. Two teaspoonsful of the well-mixed crumbs was evenly distributed in a glass-bottomed sample cup 3 mm. in depth, covered with a white tile, and presented to the instrument. The color of the samples was measured in terms of *Rd* values (white = 100% reflectance; black = 0% reflectance), since these data give an indication of lightness or darkness. The average of duplicate samples was taken as the reflectance value of the batch.

The method for measuring extensibility was essentially the same as that of Kaminski and Halton (11) adapted to a larger sample. Two metal hooks were carefully inserted into the exact center of each of the rounded rectangular samples of gluten, so that the diameter of the resulting loop would be as uniform as possible. The upper hook was hung from a slot in a plastic plate placed over a chromatography jar filled with distilled water at 24°C. The lower hook, weighing 35 g., was suspended from the gluten sample, and the whole assembly was lowered gently into the distilled-water bath (Fig. 2). The level of the bottom of the gluten loop relative to a transparent scale attached to the outside of the jar was read at the beginning of the test, at 5-min. intervals for 20 min., and at 10-min. intervals

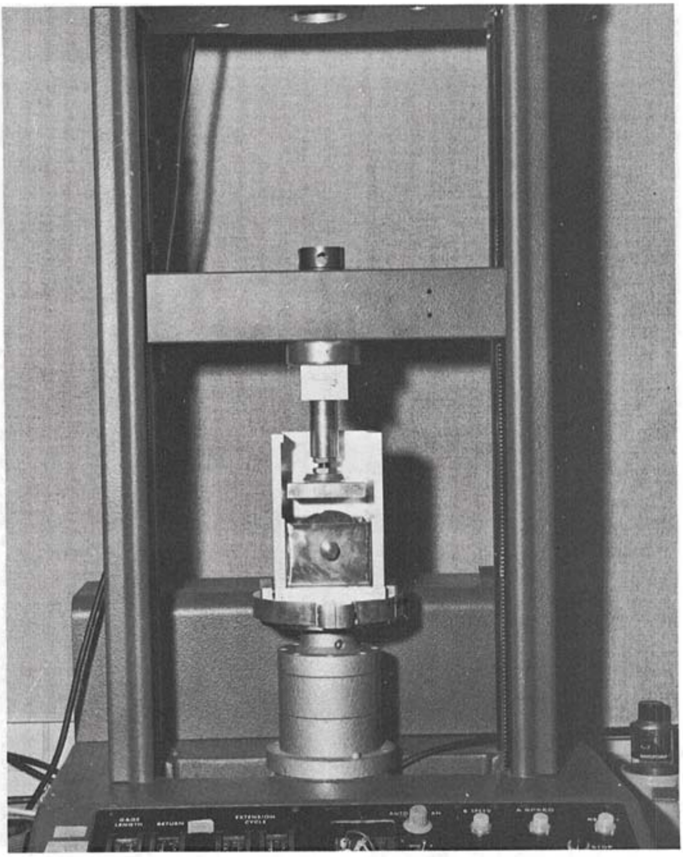

Fig. 1. Box and plate for crushing test.

thereafter. The lower hook was gently removed either when it approached 2 cm. above the bottom of the jar or when the loop threatened to break. In any case, the test was terminated after 1 hr. The parameter chosen to characterize extensibility was the slope of the curve at 10 min., i.e., the slope in units of cm. per min. (12).

After the hook was removed, the gluten loop was allowed to contract until it attained a constant length. Percent elasticity was calculated from the following formula:

$$\frac{\text{Decrease in length on contraction} \times 100}{\text{Increase in length on extension}}$$

Experimental Design

The two flours were studied separately, but the same experimental design was used in both studies–a balanced incomplete block; Plan 11.1 a, with five treatments, six replications, ten blocks, and three treatments per block (13). The data were subjected to the analysis of variance and the Duncan Multiple Range Test. Correlation coefficients among the various measurements were determined.

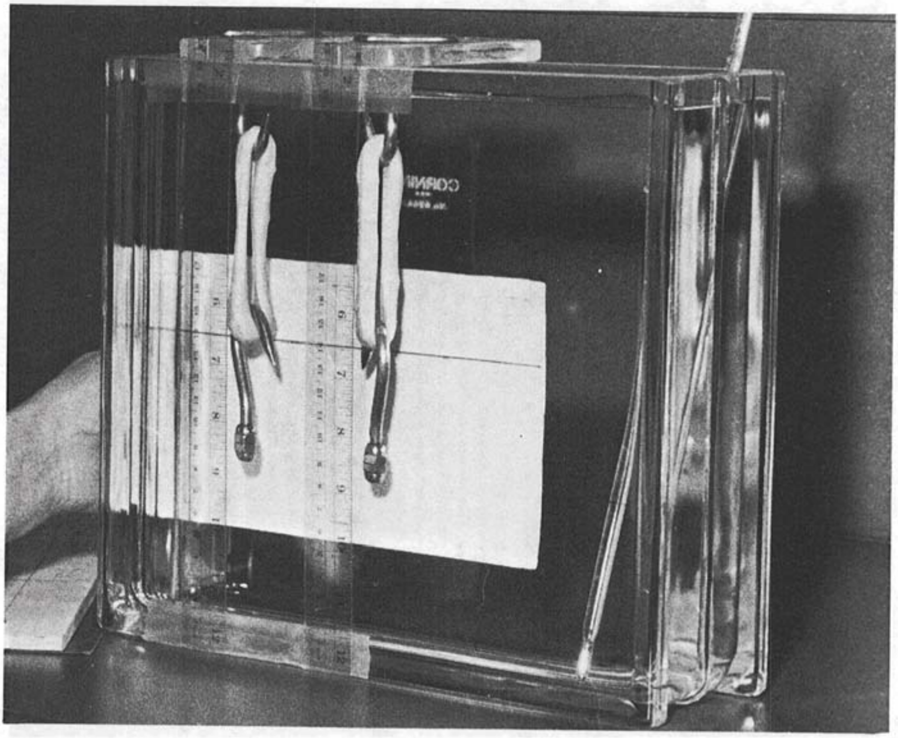

Fig. 2. Apparatus for extensibility test.

RESULTS AND DISCUSSION

Mean values for properties of raw gluten and baked gluten balls from doughs containing components of cheddar-cheese whey are given in Table II. All four measurements of force to crush were highly correlated and ranked the treatments in the same order within any given flour. Therefore, since maximum force was the easiest of the four to obtain, yielded the best discrimination among the treatments, and had the largest F ratio from the analysis of variance and the lowest coefficient of variation, only data for maximum force are presented.

All-Purpose Flour

The addition of lactose to all-purpose-flour doughs had no significant effect on any property of gluten. These findings agree with those of Meiske et al. (3) who found that α-lactose had no significant effect on viscoelastic properties of raw gluten or baked gluten balls.

The addition of whey proteins to all-purpose-flour doughs increased the yield of gluten, maximum force to crush, and the Gardner *Rd* value. The properties of glutens from doughs containing both lactose and proteins were similar to those of glutens from doughs containing proteins alone.

Adding dried whole whey to all-purpose-flour doughs decreased the drip loss and the extensibility of gluten (Fig. 3). These glutens were the most difficult to

TABLE II. MEAN VALUES FOR PROPERTIES OF RAW GLUTEN AND BAKED GLUTEN BALLS FROM DOUGHS CONTAINING COMPONENTS OF CHEDDAR-CHEESE WHEY[a]

Flour	Treatment[b]	Raw Gluten: Yield %	Raw Gluten: Drip loss %	Raw Gluten: Extensibility (slope at 10 min.) cm./min.	Raw Gluten: Elasticity %	Baked Gluten Balls: Baked volume cc.	Baked Gluten Balls: Specific volume cc./g.	Baked Gluten Balls: Maximum force to crush kg.	Baked Gluten Balls: Gardner Rd value[c]
All-purpose	C	31.0 d	6.4 e	0.07 e	77 de	142 de	28.5 e	2.12 d	20.9 d
	L	30.8 d	7.6 e	0.06 e	74 de	154 e	29.4 e	3.02 de	19.7 d
	P	33.3 e	8.0 e	0.06 e	66 d	154 e	28.9 e	4.25 ef	24.0 e
	LP	33.1 e	7.9 e	0.06 e	71 d	154 e	29.5 e	3.70 e	22.4 de
	W	30.7 d	4.0 d	0.02 d	92 e	124 d	22.7 d	5.83 f	28.5 f
Bread	C	42.2 e	7.6 e	0.55 e	92 f	124 d	23.2 d	6.40 e	35.1 f
	L	42.1 e	9.4 e	0.49 e	86 ef	125 d	23.4 d	7.30 e	30.6 e
	P	42.8 e	8.2 e	0.38 e	79 de	166 e	32.1 e	3.45 d	30.0 e
	LP	42.2 e	8.9 e	0.41 e	85 ef	157 e	30.2 e	3.98 d	26.8 d
	W	37.4 d	4.7 d	0.08 d	71 d	121 d	21.2 d	7.50 e	35.1 f

[a]Means in any given group with a common letter suffix are not significantly different. Those with different suffixes differ significantly ($P<1\%$).

[b]C = control; L = lactose; P = proteins; LP = lactose-plus-proteins; and W = dried whole whey.

[c]A higher value for Rd indicates a lighter product.

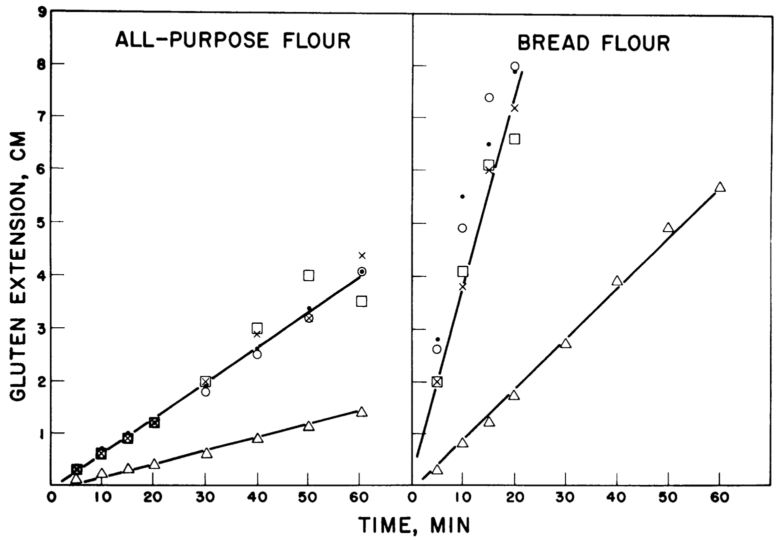

Fig. 3. Extensibility curves for gluten from all-purpose- and bread-flour doughs containing components of cheddar-cheese whey. Solid dots = control; open circles = lactose; crosses = whey proteins; open squares = lactose-plus-proteins; and triangles = dried whole whey.

wash out from the dough, and broke into many small fragments. When the gluten was finally isolated, it was tough and bucky in nature and tended to have discontinuities such as cavities and cracks. Baked volume of the dried whole-whey gluten balls was significantly lower than the control, and the balls were harder and lighter in color.

Although lactose and proteins are the principal components of cheese whey, the properties of the lactose-plus-proteins gluten differed from those of dried whole-whey gluten in several respects. The yield of the lactose-plus-proteins gluten was higher than that of the dried whole-whey gluten, as were drip loss and extensibility. The lactose-plus-proteins gluten balls had larger baked and specific volumes than the dried whole-whey gluten balls and were more tender and darker in color. The reasons for the differences in properties of the two glutens may have been due to changes in or possible denaturation of the protein as well as to the difference in ash contents of the added materials, i.e., 0.61 g. in the lactose-plus-proteins dough vs. 7.18 g. in the dried whole-whey dough. Since the method of preparing the whey proteins (8) differed from that for spray-dried whole whey, alterations in the state of the proteins could also have differed. The whey proteins were neutralized by the addition of sodium hydroxide and heated to 93° to 96°C. with constant agitation. Precipitation was accomplished by adding diluted glacial acetic acid with continued heating and agitation. The mixture was then cooled to 38°C. or below. After draining at 4°C. the whey proteins were frozen and stored at −18°C. until used.

The dried whole whey was prepared by the standard "high-heat treatment", i.e., heating liquid whey to 93° to 98°C. and concentrating it under vacuum to 30 to 40% solids. The temperature of the material at this point is about 57° to 60°C. The concentrate was then spray-dried at about 65°C.

Bread Flour

The addition of lactose to bread-flour doughs modified only one of the properties measured—color. The darker color, as indicated by a lower *Rd* value, may have been due to carmelization of the lactose.

The addition of whey proteins to bread-flour doughs significantly decreased elasticity, maximum force to crush, and Gardner *Rd* values, but increased baked and specific volume. The effects of lactose-plus-proteins were similar to those of proteins alone, except that Gardner *Rd* values were lower for the lactose-plus-proteins treatment.

Adding dried whole whey to bread-flour doughs decreased the yield, drip loss, extensibility, and elasticity (Fig. 3). As with all-purpose flour, the bread-flour doughs containing dried whole whey yielded glutens with properties different from those of glutens from doughs containing lactose-plus-proteins. The yield of gluten from lactose-plus-proteins doughs was higher than that from dried whole-whey doughs, as were drip loss, extensibility, elasticity, and baked and specific volumes. The dried whole-whey gluten balls required greater force to crush than the lactose-plus-proteins balls, and were lighter in color.

The two flours did not always react in the same manner to the added components. In some cases, the components had opposite effects on gluten properties. Whey proteins or lactose-plus-proteins made gluten balls from all-purpose flour harder than the control. When these materials were added to bread-flour doughs, the gluten balls were more tender. In other cases, the added component had a significant effect on only one of the two flours. Dried whole whey or whey proteins decreased the elasticity of bread-flour gluten but not that of all-purpose-flour gluten. Whey proteins increased the baked volume and specific volume of bread-flour gluten balls, but did not affect these properties of balls from all-purpose flour. This was also true for the lactose-plus-proteins addition. Dried whole whey had no significant effect on *Rd* values or on tenderness of gluten balls from bread flour, but increased the *Rd* values and the force needed to crush balls from all-purpose flour.

Gluten, the only continuous structure in dough, is predominant in influencing the mechanical properties of dough (1,14). The gluten phase is bound together by various types of forces including amide, thioester, and salt linkages; hydrogen and hydrophobic bonds; and the very important disulfide bonds. That the physiochemical and viscoelastic properties of gluten are modified in the presence of disulfide (SS) and sulfhydryl (SH) groups has been reported by a number of workers and discussed in several reviews (1,15,16,17,18). The SS bonds are important in maintaining the strength and structure of the dough. Exchange reactions between the SS and SH groups provide mobility to the dough and reduce strains and breakdowns. Gluten structure is best stabilized when a proper balance exists between free SH groups and SS bridges (19).

SH groups may arise not only from flour but also from substances added to doughs, e.g. milk. The literature on the deleterious effects of certain milk proteins on bread quality has been recently reviewed by Swanson and Sanderson (20). In whey, the principal protein component is β-lactoglobulin. It is the source of most, if not all, of the SH groups in milk (21).

Although the determination of SH-SS interchange reactions was outside the scope of this study, it is quite possible that such reactions could account in part for changes in viscoelastic properties of gluten caused by the protein constituents of whey. In addition, the high ash content of whey (8.22%) could also have modified the viscoelastic properties of gluten. Electrolytes may influence dough properties by altering the hydration capacity, the colloidal state of the proteins, or possibly the protein-lipid interaction (1).

Although conditions in doughs from which bakery products are made differ from those in the simpler flour-water doughs used for the isolation of gluten, it is tempting to examine relationships which might logically be expected to ensue between properties of the gluten as measured by the various tests and the physical properties of the end products For example, glutens with superior water-holding capacity might be expected to foster moistness in the baked products. Gluten extensibility and elasticity and volume of baked gluten balls might influence volume or compressibility; tenderness of the baked gluten balls might be reflected in tenderness of crust or crumb. However, properties exhibited in the isolated gluten could be either enhanced or reversed in the baked products as a result of interactions with other ingredients in the formula or from differences in extent or rate of mixing. Whey, for example, has been shown to affect dough systems in at least two directions. In their rapid breadmaking process, Henika and Rodgers (22) reported that whey tempered both the depressing effect of cysteine and the improving effect of bromate. Although many workers have reported that whey proteins lowered bread quality, Swanson and Sanderson (20) found that neither loaf volume nor bread score was adversely affected by the major components of whey.

The results of the present study on all-purpose flour and of the studies on wheat-starch pastes (9) suggest that the changes in volume or tenderness (or both) of doughnuts and biscuits (7,8) may have been due to the effects of components of cheese whey on properties of starch rather than on properties of gluten. However, before this question can be answered conclusively, additional studies are needed to characterize more fully the interactions among components of cheese whey and the basic food materials in baked products.

Acknowledgments

The author thanks Foremost Dairies, Inc., San Francisco, Calif., for the dried whole whey and lactose; John E. Montoure, Department of Food Science, for the whey proteins; and Franklin P. Parks, Department of Agricultural Biochemistry and Soils, for the nitrogen and ash determinations. Grateful acknowledgment is made of the technical assistance of Marian M. Manis and Goldie Brausen in the preparation, baking, and testing of the gluten, and of Helen H. Cunningham, in the measurement of color.

Literature Cited

1. BLOKSMA, A. H., and HLYNKA, I. Basic considerations of dough properties. In: Wheat: Chemistry and technology, ed. by I. Hlynka; p. 465. American Association of Cereal Chemists: St. Paul, Minn. (1964).
2. BAXTER, ELMA J., and HESTER, E. ELIZABETH. The effect of sucrose on gluten development and the solubility of the proteins of a soft wheat flour. Cereal Chem. 35: 366 (1958).
3. MEISKE, DONNA POLAND, JONES, MARY FRANG, and JONES, EVELYN M. The effect of various sugars on the formation and character of gluten. Cereal Chem. 37: 483 (1960).

4. PONTE, J. G., Jr., DE STEFANIS, V. A., TITCOMB, S. T., and COTTON, R. H. Study of gluten properties as influenced by certain organic solvents. Cereal Chem. 44: 211 (1967).

5. VAKAR, A. B., PUMPYANSKII, A. YA., and SEMENOVA, L. V. Effect of D_2O on the physical properties of gluten and wheat dough. Appl. Biochem. Microbiol. 1: 1 (1965). [Abstr. in J. Sci. Food Agr. 18: part i, 139 (1967).]

6. TKACHUK, R., and HLYNKA, I. Some properties of dough and gluten in D_2O. Cereal Chem. 45: 80 (1968).

7. POTTER, GRETCHEN L., and ZAEHRINGER, MARY V. Functional properties of two components of cheddar cheese whey in bakery products: 2. Biscuits. Cereal Sci. Today 10: 215 (1965).

8. HOFSTRAND, JOYCE T., ZAEHRINGER, MARY V., and HIBBS, R. A. Functional properties of two components of cheddar cheese whey in bakery products: 1. Cake doughnuts. Cereal Sci. Today 10: 212 (1965).

9. HIRAI, JOY, ZAEHRINGER, MARY V., and WIESE, A. C. Properties of wheat starch pastes containing components of cheddar cheese whey. Food Technol. (Chicago) 24: 79 (1970).

10. HESTER, E. ELIZABETH, BRIANT, ALICE M., and PERSONIUS, CATHERINE J. The effect of sucrose on the properties of some starches and flours. Cereal Chem. 33: 91 (1956).

11. KAMINSKI, E., and HALTON, P. Stretching characteristics of gluten washed from air-classified fractions of English and Manitoba wheat flours. J. Sci. Food Agr. 15: 625 (1964).

12. MATSUO, R. R., and IRVINE, G. N. Effect of gluten on the cooking quality of spaghetti. Cereal Chem. 47: 173 (1970).

13. COCHRAN, G., and COX, GERTRUDE M. Experimental designs (2nd ed.). Wiley: New York (1957).

14. WALL, J. S., and BECKWITH, A. C. Relationship between structure and rheological properties of gluten proteins. Cereal Sci. Today 14: 16 (1969).

15. MATSUO, R. R., and McCALLA, A. G. Physicochemical properties of gluten from three types of wheat. Can. J. Biochem. 42: 1487 (1964).

16. SOKOL, H. A., and MECHAM, D. K. Review of the functional role and significance of the sulfhydryl group in flour. Baker's Dig. 34(12): 1 (1960).

17. SULLIVAN, BETTY. Wheat protein research: Fifty years of progress. Cereal Sci. Today 10: 338 (1965).

18. POMERANZ, Y. Relation between chemical composition and bread-making potentialities of wheat flour. Advan. Food Res. 16: 335 (1968).

19. LEE, C. C., and LAI, T-S. Studies with radioactive tracers. XV. Some observations on the sulfhydryl groups in dough. Cereal Chem. 46: 598 (1969).

20. SWANSON, A. M., and SANDERSON, W. B. Milk proteins responsible for deleterious effects in continuous mix bread. Cereal Sci. Today 12: 363 (1967).

21. LARSON, B. L., JENNESS, R., and GEDDES, W. F. The effect of various milk serum proteins and their sulfhydryl groups on bread quality. Cereal Chem. 29: 440 (1952).

22. HENIKA, R. G., and RODGERS, N. E. Reactions of cysteine, bromate, and whey in a rapid breadmaking process. Cereal Chem. 42: 397 (1965).

[Received July 21, 1971. Accepted December 21, 1971]