EFFECT OF STRENGTH AND CONCENTRATION OF ACID ON THE FUNCTIONAL PROPERTIES OF SOLUBILIZED GLUTENS OF GOOD- AND POOR-QUALITY BREAD FLOURS[1]

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ABSTRACT

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Glutens from good- and poor-quality wheat flours were solubilized in acids that varied in strength and concentration. Flours (10 g) reconstituted with solubilized and neutralized glutens plus starch and water-solubles were made into bread. Acid solubilization was detrimental to loaf volume at pH values below 4 for glutens from good-quality flours and at pH below about 4.85 for glutens from a poor-quality flour. Impaired loaf volume was attributed to diminished hydrogen bonding caused by cleavage of amide groups from the gluten proteins during solubilization in acid.

There have been no extensive studies that compared the loaf-volume potentials of glutens that had been solubilized in acids that varied in strength and concentration. In any event, results cannot be meaningful unless the crude gluten protein can be reconstituted with the other flour constituents and baked into bread equal to that made from the original, unfractionated flour.

Vandevelde (1) found that wheat gluten lost its elasticity with various organic acids (concentration not given). Blish and Sandstedt (2) solubilized gluten proteins in 0.05*N* acetic acid with no destructive changes in their physical or chemical properties. Mangels and Martin (3) studied the solubilizing capacity of different organic and mineral acids (0.1–2*N*) on flour; mineral acids dispersed a lower percentage of the total N than organic acids. They concluded that the hydrogen-ion concentration affected the protein-dispersing power of organic acids. No baking data were reported. Lusena (4) purified gluten by dispersing washed gluten in 0.005*N* acetic acid (pH 4.8–5.2). Baking tests showed that the purified gluten was undamaged when used to fortify the original flour.

Shogren *et al.* (5) solubilized gluten protein with 0.005*N* lactic acid and precipitated the soluble gluten with 0.1*N* Na_2CO_3. Baking results for the flour reconstituted with purified gluten and starch plus water-solubles were essentially equal to those for the original flour. Hoseney *et al.* (6) found that loaf volume of reconstituted flour was impaired when the pH of gluten initially solubilized in 0.005*N* lactic acid was lowered to pH below 4.

The purpose of this study was to determine the optimum pH for solubilizing gluten, using acids that varied in strength and concentration, without impairing its baking properties, and to determine if pH optimum varied with gluten quality (loaf-volume potential).

[1]This work constitutes part of the dissertation submitted by D. R. Goforth to the Graduate Faculty of Kansas State University in partial fulfillment of the requirements for the Ph.D. degree.

Mention of specific instruments or trade names is made for identification purposes only and does not imply any endorsement by the U.S. Government.

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MATERIALS AND METHODS

Flours

Three flours were milled (Allis) from hard winter wheats that differed greatly in breadmaking quality. Regional baking standard (RBS-74) was a blend of typical varieties of hard winter wheats harvested at many stations in the Central, Southern, and Northern Great Plains of the U.S. in 1973. The flour contained 12.5% protein (N × 5.7) and 0.40% ash (14% mb), and had good loaf-volume potential and medium-long mixing requirement (bake mixing time, 3 3/4 min). C.I. 12995 (Quivera/Tenmarq//Marquillo/Oro), harvested at Manhattan in 1972, had good loaf-volume potential and strong physical-dough properties (bake mixing time, 7 min). The flour contained 12.2% protein (N × 5.7) and 0.40% ash (14% mb). KS501099 (Chiefkan/Tenmarq), harvested at Manhattan in 1973, had extremely poor loaf-volume potential and very weak physical-dough properties (bake mixing time, 5/8 min). The flour contained 13.8% protein (N × 5.7) and 0.50% ash (14% mb). Mixograms of the three flours, which varied widely in functional properties, are reproduced in Fig. 1.

Analytical Procedures

Protein, ash, and moisture contents were determined by Methods 46-11, 08-01, and 44-15A, respectively, of the AACC Approved Methods (7). Mixograms were obtained as described by Finney and Shogren (8). The baking procedure has been

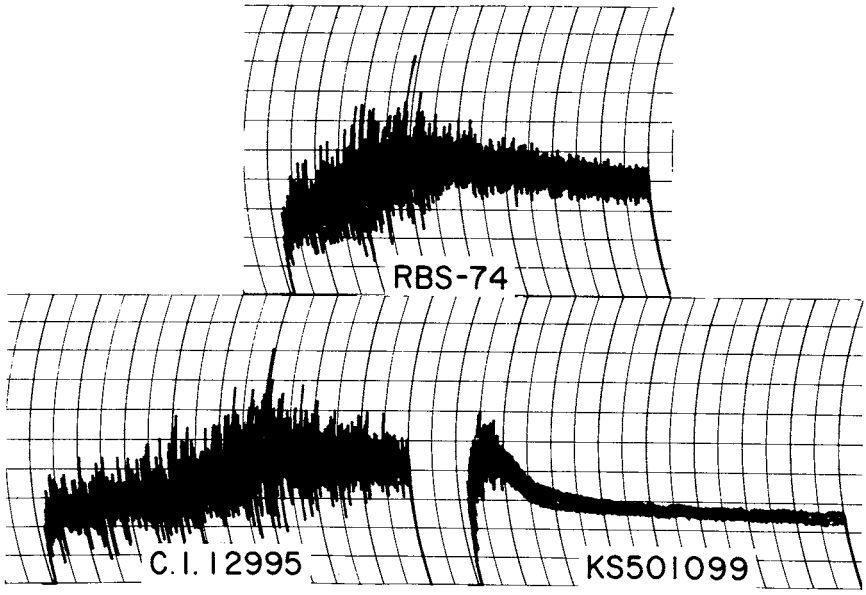

Fig. 1. Mixograms of typical hard winter wheat varieties that have strong (C.I. 12995), medium (RBS), and extremely weak (KS501099) mixing properties.

described by Finney and Barmore (9–11) and Finney (12) and adapted by Shogren *et al.* (5) for 10 g (14% mb) flour. The standard deviation for the average of duplicate loaf volumes was 1.80 cm^3. Loaf volumes were determined by dwarf rapeseed displacement after the bread had cooled to room temperature (25°C). Then, loaves were cut and their crumb grains evaluated by this code: S = satisfactory, Q = questionable; and U to U^4 = unsatisfactory to extremely unsatisfactory.

Fractionating and Reconstituting Flour

Crude gluten was handwashed from flour by the procedure of Shogren *et al.* (5). The crude gluten was lyophilized and ground, and then solubilized in 700 ml acid (Tables I–III) on a magnetic stirrer for 5 hr. The solubilized gluten was recovered as insoluble gluten according to the scheme in Fig. 2. The acid-solubilized and neutralized insoluble glutens were lyophilized, ground, reconstituted with the starch plus water-soluble fraction from RBS-74 flour, and made into bread.

Photography

Pictures of cut loaves were taken by an MP-4 polaroid camera (type 105 film) without filter and with side and back lighting.

TABLE I
Baking Data for the Original and Reconstituted Flours of RBS-74 Containing Gluten Protein Solubilized in Relatively Weak and Strong Acids of Various Normalities[a]

pH of Solubilized Gluten	Acid and Its Normality	Baking Absorption %	Mixing Time min	Loaf vol cm^3	Crumb Grain
Original flour	...	64.5	3 3/4	80	S
Crude gluten	...	64.5	3 1/4	80	S
	Acetic acid (*N*)				
4.90	0.005	63.0	2 1/2	80	S
4.70	0.0075	63.0	1 7/8	80	S
4.55	0.01	62.8	2 1/2	81	S
3.95	0.05	63.5	2 1/4	80	S
3.70	0.1	63.8	2 1/4	77	S
	Lactic acid (*N*)				
4.56	0.005	62.6	2 1/2	81	S
4.14	0.0085	62.6	2	81	S
3.95	0.01	62.6	2 1/8	80	S
3.55	0.025	63.0	2 5/8	70	Q
3.20	0.05	62.8	2 3/4	53	U^3
	HCl (*N*)				
5.17	0.0019	64.5	1 1/2	81	S
4.67	0.0039	64.5	1 1/2	81	S
2.61	0.019	65.0	2 1/2	48	U^3
	H_2SO_4 (*N*)				
4.44	0.0058	64.5	7/8	80	S
3.73	0.0116	64.5	5/8	74	S
1.67	0.0581	No 6.1 Insoluble gluten			

[a]Protein content of original and reconstituted flours was 12.5% (14% mb).

TABLE II
Baking Data for the Original and Reconstituted Flours of C.I. 12995 Containing Gluten Protein Solubilized in Acetic and Lactic Acids of Various Normalities[a]

pH of Solubilized Gluten	Acid and Its Normality	Baking Absorption %	Mixing Time min	Loaf vol cm^3	Crumb Grain
Original flour	...	64.9	7	75	S
Crude gluten	...	64.5	3 1/2	80	S
	Acetic acid (*N*)				
4.85	0.005	65.2	5	81	S
4.72	0.0075	64.6	3 1/4	80	S
4.58	0.01	63.5	2 3/4	79	S
4.18	0.025	62.7	3 1/2	79	S
3.61	0.1	61.3	1 7/8	62	U
	Lactic acid (*N*)				
4.53	0.005	65.9	6	79	S
4.28	0.0075	65.2	3 3/4	80	S
3.95	0.01	63.5	1 7/8	80	S
3.59	0.025	60.8	1 1/2	62	U
3.27	0.05	60.0	7/8	53	U^3

[a]Original flour contained 12.2% protein and the reconstituted flours 12.5%. The starch plus water-soluble fraction was from RBS-74 flour.

TABLE III
Baking Data for the Original and Reconstituted Flours of KS501099 Containing Gluten Protein Solubilized in Acetic and Lactic Acids of Various Normalities[a]

pH of Solubilized Gluten	Acid and Its Normality	Baking Absorption %	Mixing Time min	Loaf vol cm^3	Crumb Grain
Original flour	...	63.5	5/8	47	U^3
Crude gluten	...	61.3	5/8	58	Q-U
	Acetic acid (*N*)				
5.20	0.005	60.6	5/8	65	Q
4.96	0.0075	60.6	5/8	66	Q
4.72	0.01	58.7	7/8	61	Q-U
4.16	0.025	56.2	3/4	48	U^4
	Lactic acid (*N*)				
5.46	0.003 (L)[b]	57.8	5/8	48	U^5
5.37	0.003 (W)	60.5	3/4	67	Q
4.85	0.005	58.9	5/8	65	Q-U
4.25	0.0075	57.6	5/8	52	U^3
4.00	0.014	57.8	5/8	49	U^4

[a]Original and reconstituted flours had a protein content of 13.8% (14% mb). Glutens were reconstituted with the starch plus water-soluble fraction from RBS-74 flour.
[b]L and W are abbreviations for lyophilized and wet, respectively.

RESULTS AND DISCUSSION

Good-Quality Flours, RBS-74 and C.I. 12995

Loaf volumes of flours reconstituted from acid-solubilized glutens from the good-quality flours (RBS-74 and C.I. 12995) were adversely affected when the pH of the soluble gluten was below about pH 4 (Tables I, II, Fig. 3). Photographs of typical loaves baked from reconstituted flours containing C.I. 12995 acid-solubilized glutens appear in Fig. 4. At pH 3.2, crumb grain was heavy and open (Fig. 4, loaf 4) and typical of extremely poor-quality flours.

Washing the crude gluten from the flour of long-mixing C.I. 12995 (Table II) and reconstituting with the starch plus water-soluble-protein fraction improved loaf volume; thus, the flour reconstituted with crude gluten was the pertinent control for the acid-solubilized glutens. Acid-solubilization of gluten also improved loaf volume of the reconstituted flours, compared to that of the original flour. Improved volumes probably were due to the desirably increased dough extensibility as shown by shortened mixing times. Dough extensibility and loaf volume of long-mixing flours were similarly improved by cysteine hydrochloride (13).

The effect of acid-solubilized glutens on mixing times of RBS-74 reconstituted flours was more drastic for mineral than for organic acids at the same pH (Table I). Loaf volume, however, was not impaired by any acid unless pH was below 4. When the solution of RBS gluten in 0.0581 *N* H_2SO_4 was centrifuged, there was an almost complete aggregation of the proteins, probably because the increase in

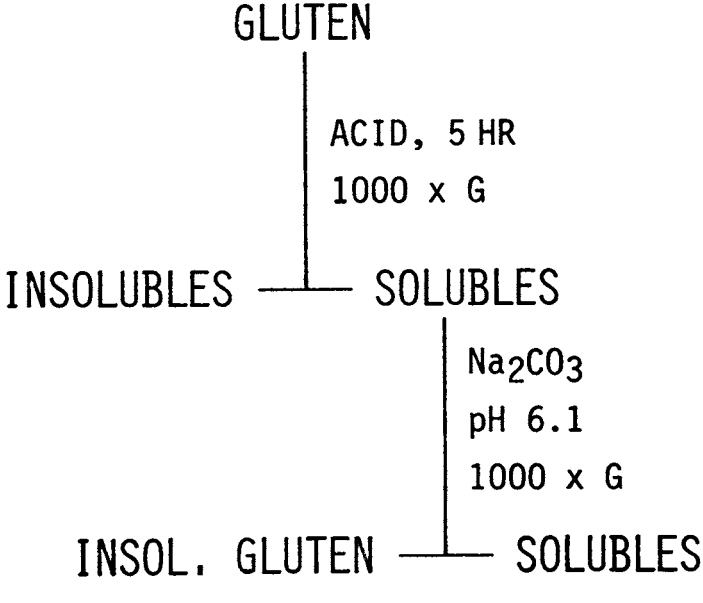

Fig. 2. Scheme for acid-solubilization of crude gluten.

ionic strength created favorable conditions for attraction between protein molecules (14).

Poor-Quality Flour, KS501099

Reconstituted KS501099 flour containing crude gluten had a loaf volume of 58 cm^3 (still extremely poor), 11 cm^3 above that (47 cm^3, extremely poor) of the original (Table III), because the starch plus water-soluble fraction of RBS-74 flour was used in place of that from KS501099. Thus, flour reconstituted with crude gluten was the logical control for the acid-solubilized glutens. Preliminary studies (data not given) showed that the starch fraction of the extremely poor-quality KS501099 was inferior to that of previous samples. Previous studies (6,15) showed that the starch and water-soluble fractions of KS501099 were equal to those from good-quality varieties, but the quality of that KS501099 sample was only very poor compared to the extremely poor quality of the KS501099 sample in Table III.

Loaf volume was increased 7–9 cm^3 over that for flour reconstituted with crude gluten, for the flours reconstituted with glutens solubilized at pH 4.85 and above (Table III, Fig. 3). Apparently the crude gluten proteins of the extremely poor-quality KS501099 flour were more sensitive to acid treatment than those of

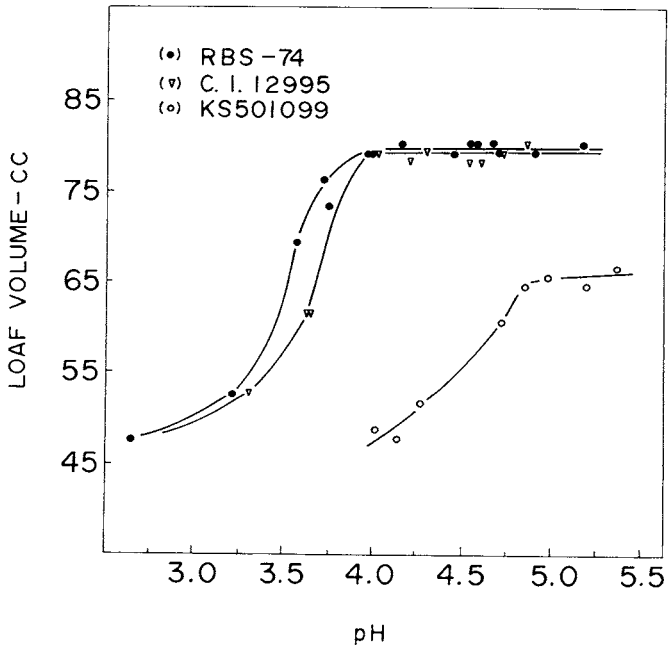

Fig. 3. Loaf volumes of reconstituted flours containing glutens (of varying quality) solubilized at different pH levels. Loaf volumes of the flours reconstituted with the three different crude glutens and the starch plus water-soluble fraction of RBS-74 were 80 cm^3 for RBS, 80 cm^3 for C.I. 12995, and 58 cm^3 for KS501099.

the good-quality flours. The optimum pH was about 5.0 for solubilization of KS501099 crude gluten (also note crumb grains) and about 4 for solubilization of those from the good-quality flours, RBS-74 and C.I. 12995. Photographs of typical loaves baked from control flours and from reconstituted flours containing KS501099 acid-solubilized glutens appear in Fig. 5. The decrease in loaf volume with decreasing pH below 4.85 was accompanied by impaired crumb grain.

When ground lyophilized crude gluten of KS501099 was solubilized in 0.003 *N* lactic acid (Table III), the proteins aggregated and formed an insoluble complex (pH 5.46). Only 55% of the gluten protein was solubilized and when the flour

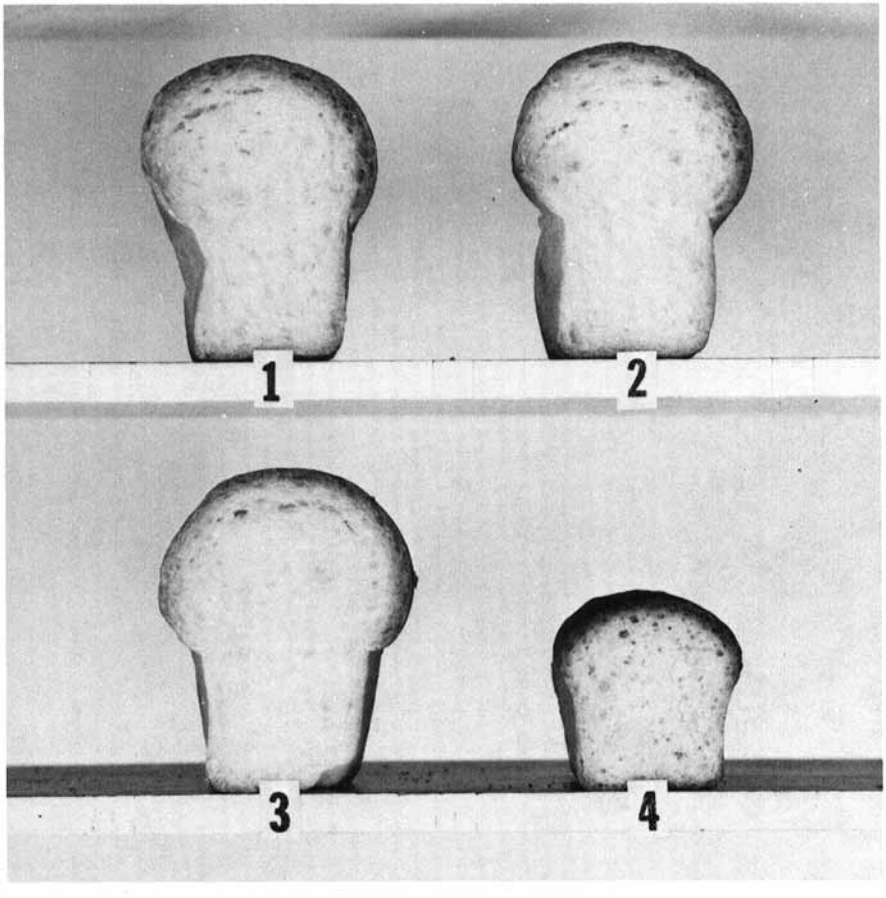

Fig. 4. Cut loaves baked from original flour of C.I. 12995 (1), and reconstituted flours containing crude gluten (2), and gluten solubilized in lactic acid at pH 4.53 (3), and pH 3.27 (4). All flours were reconstituted with the starch plus water-soluble fraction of RBS-74 flour.

reconstituted with that soluble gluten was baked into bread, loaf volume was 48 cm^3 and crumb grain was extremely poor. When wet crude gluten of KS501099 was scissored into 0.003*N* lactic acid, however, the gluten behavior was normal (pH 5.37). For the flour reconstituted with that gluten, loaf volume was 67 cm^3 and crumb grain was improved and better than that for crude gluten. One explanation is that wet gluten proteins are loosely structured within a continuous phase of water. Then, unfolding increases as the gluten proteins become associated with more water, depending on the concentration of the surrounding acid molecules, and finally are solubilized. When the water is removed, however, those proteins, now compactly structured, form intermolecular associations that cannot be broken until the hydrogen-ion concentration of the media is increased enough to cause an unfolding and/or loosening of the protein-protein associations.

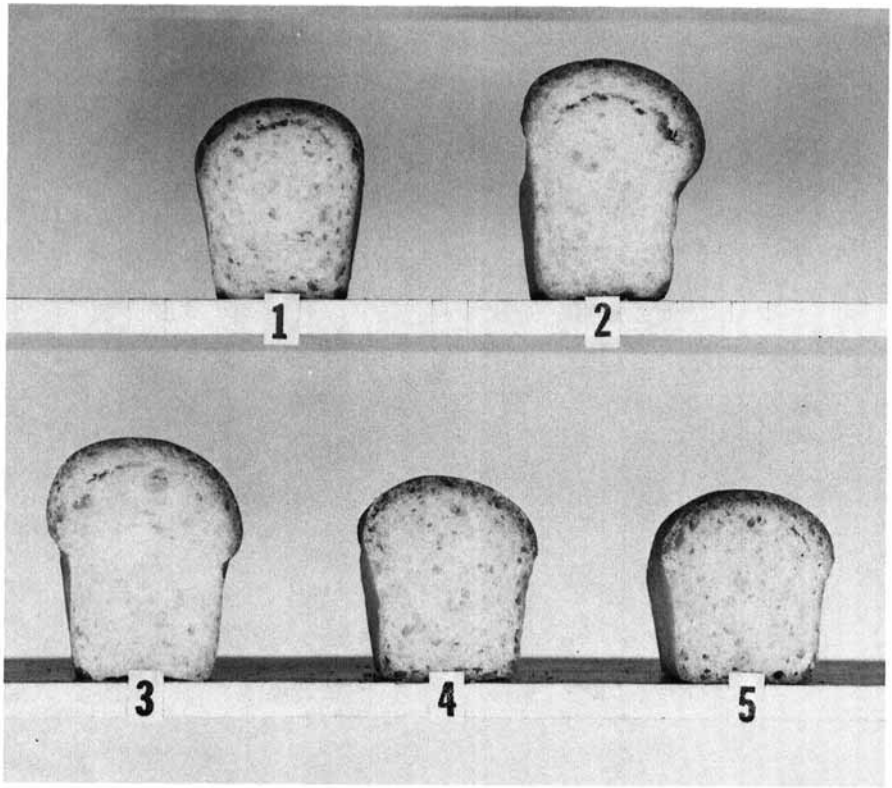

Fig. 5. Cut loaves baked from original flour of KS501099 (1), and reconstituted flours containing crude gluten (2), and glutens solubilized in lactic acid at pH 4.85 (3), pH 4.25 (4), and pH 4.00 (5). All flours were reconstituted with the starch plus water-soluble fraction of RBS-74 flour.

Reasons for Impaired Glutens

Two factors can be instrumental in decreasing the loaf volume of flours reconstituted with acid-solubilized and neutralized glutens. First, a critically low pH might cause hydrolysis of some of the amide groups from the gluten proteins. An increasing number of amide bonds probably were cleaved as pH was further decreased. The amide group participates in hydrogen bonding in the dough system and thereby facilitates the formation of a stable matrix for gas retention (16). Second, partial protein hydrolysis could critically simplify the gluten matrix and thereby impair its gas-retaining properties. Holme and Briggs (14) presented evidence indicating that the sites of intermolecular hydrogen cross-linkages in gluten involve the amide groups of the proteins. Beckwith *et al.* (16) found that cleaving the amide group with acid increased the solubility of gluten in methanol, but reduced its solubility in urea solutions, thus showing the influence of hydrogen bonding on gluten properties. Those results would tend to support the theory that loss of amide nitrogen was the cause of impaired loaf volume.

Beckwith *et al.* (16), in addition to confirming the work of Holme and Briggs (14) on gluten protein amide groups, found that conversion of the amide group to an ester caused gluten to lose its cohesive, elastic properties. They also found that solubilization of gluten with 0.6*N* HCl at 30°C for 48 hr caused an appreciable cleavage of peptide bonds near the ends of peptide chains, thereby yielding amino acids or short-chain peptides. Unpublished data from this laboratory indicated that proteins were hydrolyzed even with the acid concentrations used in this study.

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