A Note on the Effect of Water-Flour Ratio on Flour Protein Extracted by Employing a Paint Shaker[1]

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During the past 20 years considerable research has been conducted on the water-soluble fraction of flour. The approaches and techniques used to obtain the water-soluble fraction have varied, almost equivalent to the number of investigators (1 to 14). However, the role that "the water-soluble fraction" plays in dough behavior remains somewhat ambiguous, dependent upon the class and variety of wheat, grade of flour, and extraction technique employed to obtain the fraction. The heterogeneity of the constituents and their interactions also contribute to nature and amount of each component extracted.

This note presents a new technique for extracting water-soluble from a flour-water slurry and shows the effect of varying the flour-water ratio on the amount of protein extracted from hard red spring wheat flour using this technique.

MATERIALS AND METHODS

Flour

The flour used in this study was prepared on a Multomat[3] mill from hard red spring wheats (commercial varieties) of the 1963 crop. The data were 65.2% extraction, 14.4% protein, and 0.415% mineral content. These data were calculated to a 14% moisture basis.

Analytical Determinations

The moisture, protein, and mineral contents of the sample were determined as outlined in AACC Approved Methods (15).

Extraction of Water-Solubles

Two methods were employed to extract the water-solubles.

1. Waring Blendor[4]: One hundred and twenty-five milliliters of distilled water and 50 g. of flour were placed in a 1-qt. Waring Blendor for the 2½:1 ratio extraction. The slurry was mixed for 1 min. and transferred to a 250-ml. flat-bottom centrifuge.

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[3]A trademark of Miag North America, Inc., Minneapolis, Minn., and Miag, Braunschweig, West Germany.

[4]Waring Products Corp., Winsted, Conn.

2. Paint Shaker: A Red Devil Paint Conditioner, Model 30[5] was modified to hold a 2-liter jar. The cap of the jar was adapted with rubber stoppers to take up any air space after the slurry was added to the jar. The amount of flour and water placed in the jar to make up the slurry was dependent on the ratio desired.

One thousand two hundred and fifty milliliters of distilled water and 500 g. of flour were placed in the jar for 2½:1 ratio extractions. For 10:1 ratio extractions, 1,800 ml. of distilled water and 180 g. of flour were used. The aqueous-flour suspensions were shaken for 10 min. The suspensions were poured into chrome-plated centrifuge cups (250-ml. capacity) and centrifuged for 30 min. at 500 × g before decanting the supernatants. The residues were returned to the jar, and for the second extraction, sufficient distilled water was added to equal the original volume of the suspension, and the process was repeated. The supernatant solutions, as well as the residues, were lyophilized to 2% moisture content.

The water-solubles were determined by lyophilizing the water extract and weighing the residue.

3. Burrell Shaker[6]: One hundred and twenty-five milliliters of distilled water and 50 g. of flour were placed in a 250-ml. flat-bottom centrifuge bottle for the 2½:1 ratio extractions. For the 10:1 ratio extractions, 200 ml. of distilled water and 20 g. of flour were used.

The samples were shaken for 30 min., then centrifuged for 30 min. at 500 × g and decanted. For the second extraction, sufficient distilled water was added to equal the initial volume of the slurry, and the process was repeated.

Mixograms

The mixograms were determined with 30 g. of flour and 20 ml. of water. The sensitivity spring was set at 10. All mixograms were run with constant weight of flour and volume of water. Absorptions reported were adjusted according to the height of the mixogram.

Farinograms

The farinograms were determined by the 80-g. constant-dough weight method given in AACC Approved Methods (15), Method 54-21.

Amino Acid Analysis

Samples were hydrolyzed using 6N hydrochloric acid in refluxing boiling water for 24 hr. The samples were run according to the procedure outlined in Beckman Technical Bulletin A-TB-008, July 1964, in a Beckman Model 120 Amino Acid Analyzer[7], equipped with a long (53 cm. × 0.9 cm.) and a short (6 cm. × 0.9 cm.) column for the amino acid analysis. A Custom Research Resin Type AA-15 was used in the long column, and Type PA-35 in the short column.

Sodium citrate buffers having pH values of 2.2, 3.28, 4.25, and 5.28 were prepared as described in the Beckman Amino Acid Analyzer Instruction Manual AIM-2. Calculations on the chromatograms were made using the Integration-by-Height-Width Method outlined in the Manual AIM-2 for the amino acids. The standards were used at concentrations of 0.5, 1.5, and 2.0 μM.

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[5]Red Devil Tools, Union, N.J.

[6]Burrell Corp., Pittsburg, Pa.

[7]Beckman Instruments, Inc., Palo Alto, Calif.

RESULTS AND DISCUSSION

The modified paint shaker and augmented jar cap which eliminated the air pocket in the jar minimized foaming during extraction of the sample. Essentially no crust formation occurred after the sample was centrifuged. This helped to eliminate the possibility of denaturation of the water-soluble proteins during the extraction period, important because denaturation of protein may occur due to surface action when mixed violently.

To illustrate the formation of crust, which has a high protein content, a sample was extracted independently with a Waring Blendor and a paint shaker. The crust formation on the sample agitated in a Waring Blendor can be seen clearly in Fig. 1. However, no crust formed for the same flour sample agitated in the paint shaker. The sample was shaken for 10 min. in the paint shaker but mixed for only 1 min. in the Waring Blendor. A time study showed no advantage in shaking the slurry longer than 10 min. in the paint shaker.

Table I shows the marked differences in the amount of amino acids in the protein composition of "the water-solubles" extracted by the two techniques. Approximately half as much glutamic acid, proline, and leucine was found in the protein extracted with the paint shaker rather than the Waring Blendor. This would indicate either that different proteins were extracted or proteins were materially changed to effect their extraction.

Even the use of the Burrell shaker, in which the air pocket was replaced by nitrogen, indicated some denaturation when the sample was shaken for 30 min. For example, the mixogram data in Table II show a larger range in absorption and less decrease in PK length between the extracted and reconstituted samples for the Burrell series than the paint shaker series. Samples extracted on the Burrell shaker also exhibited crust formation.

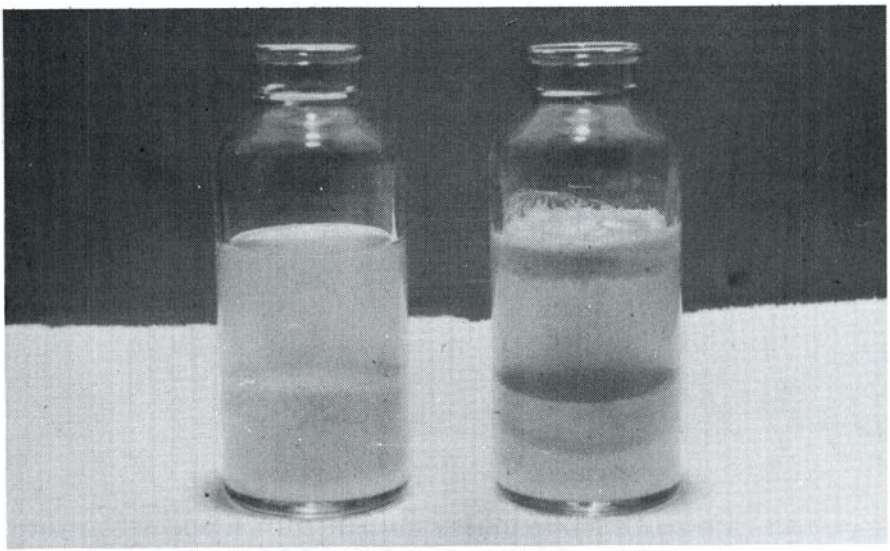

Fig. 1. Effect of the type of agitation for an extraction procedure after centrifugation for a water:flour ratio of 10:1 (v./w.). Sample on right, Waring Blendor. Sample on left, paint shaker.

TABLE I. COMPARISON OF SIX AMINO ACIDS IN PROTEINS FROM "WATER-SOLUBLES" EXTRACTED BY TWO TECHNIQUES AT 10:1 RATIO WATER:FLOUR (v./w.)

Technique	Amino Acid[a]					
	Lysine	Aspartic acid	Glutamic acid	Proline	Glycine	Leucine
Waring Blendor	1.3	3.2	46.8	41.7	4.7	14.2
Paint Shaker	0.8	3.1	34.4	17.1	3.5	7.5

[a]g.A.A./16 g. N. (Note: The same percentage of the original flour protein was extracted by both procedures, i.e., 25%.)

TABLE II. COMPARISON OF EXTRACTION METHODS AT 10:1 WATER:FLOUR RATIO (v./w.) ON MIXOGRAMS OF A HIGH-PROTEIN AIR-CLASSIFIED FRACTION FROM A HARD RED SPRING WHEAT 14.4% PROTEIN PATENT FLOUR[a]

Extraction Method	Sample Protein[b] %	Water-Solubles		Solids		Mixograms	
		Reconstituted[c] %	Protein[b] %	Reconstituted[c] %	Protein[b] %	Absorption[b] %	PK cm.
Burrell	17.4	...	...	100.0	17.4	69.8	35.8
Paint shaker	17.3	...	...	100.0	17.3	73.2	30.3
Burrell	18.4	9.0	28.9	91.0	17.4	77.8	23.5
Paint shaker	18.3	9.4	28.2	90.6	17.3	77.2	14.0
Original flour	18.5	...	...	...	...	77.5	13.4

[a]Patent flour ground in an Alpine Kolloplex Laboratory Mill, Model 160 Z, at 14,000 r.p.m. and air-classified in an Alpine Microplex, Model 132 MP (Alpine American Corp., Natick, Mass.).
[b]14% moisture basis.
[c]Percent in reconstituted flour.

The patent flour was extracted using the paint shaker technique with water:flour ratios of 2½:1, 5:1, 10:1, 25:1, and 50:1, respectively. Standard deviations derived from quintuplicate determinations for the 2½:1 and 10:1 ratios were ±0.228 and ±0.214%, respectively. Protein contents of the extracted solids were within ±0.75%.

The data revealed that protein content and yield of water-solubles increased with increased dilution. The ratios (water:flour):yield and yield:protein content were both found to be semi-logarithmically related as shown in Figs. 2 and 3. No doubt, this relation would not continue to exist for extremely dilute suspensions. However, the 50:1 ratio was an adequate limit for this study, since the material extracted at the ratio of 10:1 (water:flour) sufficed to influence the mixing characteristics of the flour as shown by the farinograms in Fig. 4.

The increase in protein content of the water-solubles with an increased dilution demonstrated protein partitioning, as shown by other workers (3,5,6,9,10). Holme (5) extracted soft wheat flour at 2:1, 4:1, and 8:1 water:flour ratios and found that the precentage of flour protein extracted for each ratio was 19, 42, and 55%, respectively. The rate at which the protein was extracted from the spring wheat flour with increasing ratios of water:flour was considerably less as shown in Fig. 5. The percentage of flour protein extracted appears to depend on at least two factors: The initial protein content of the flour and the type of flour.

The results showed that the ratio of water:flour used to extract the flour

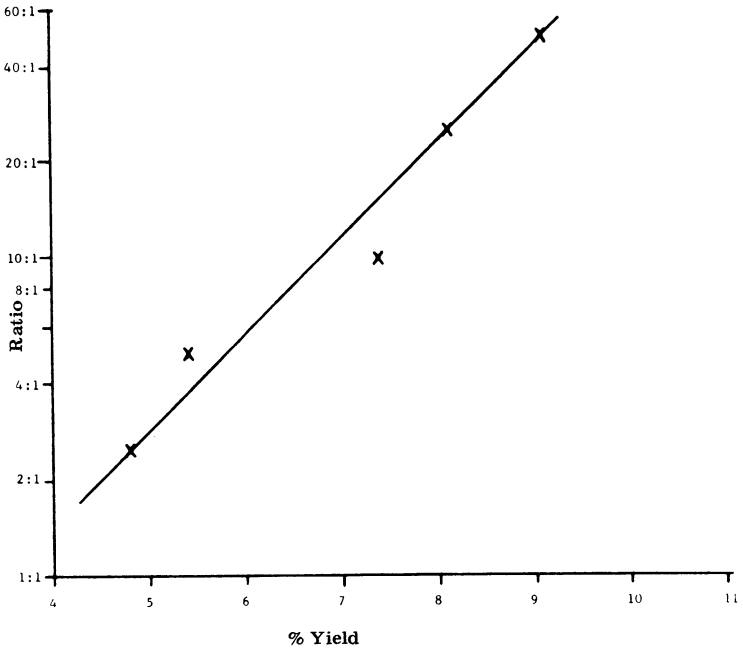

Fig. 2. The semi-logarithmic relation of the ratio of water:flour and yield of water-solubles extracted.

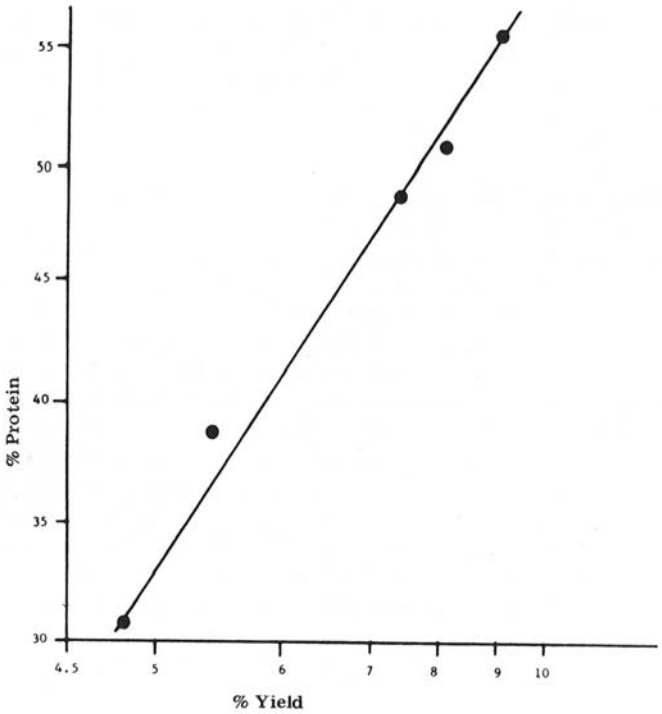

Fig. 3. The semi-logarithmic relation of protein and yield content.

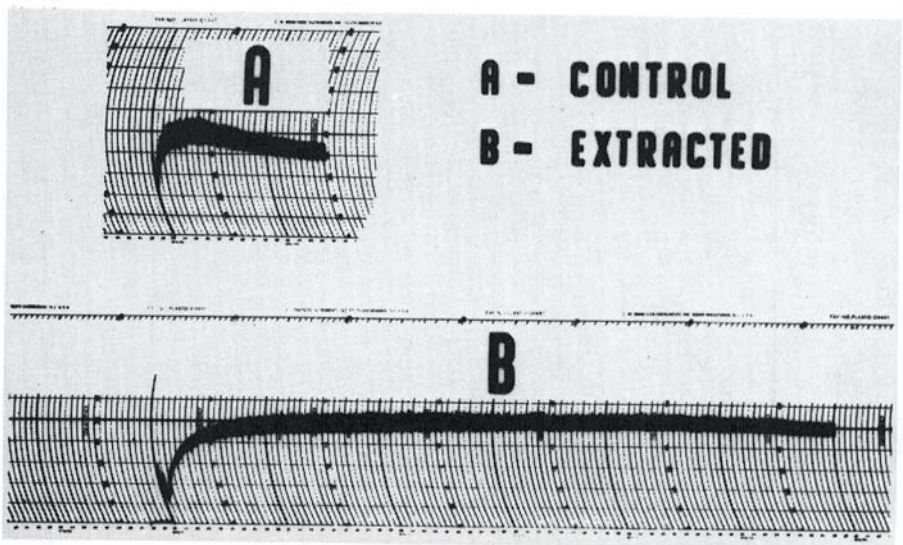

Fig. 4. Farinograms: Original flour (A); water-solubles removed using a ratio of 10:1 water:flour (v./w.) (B).

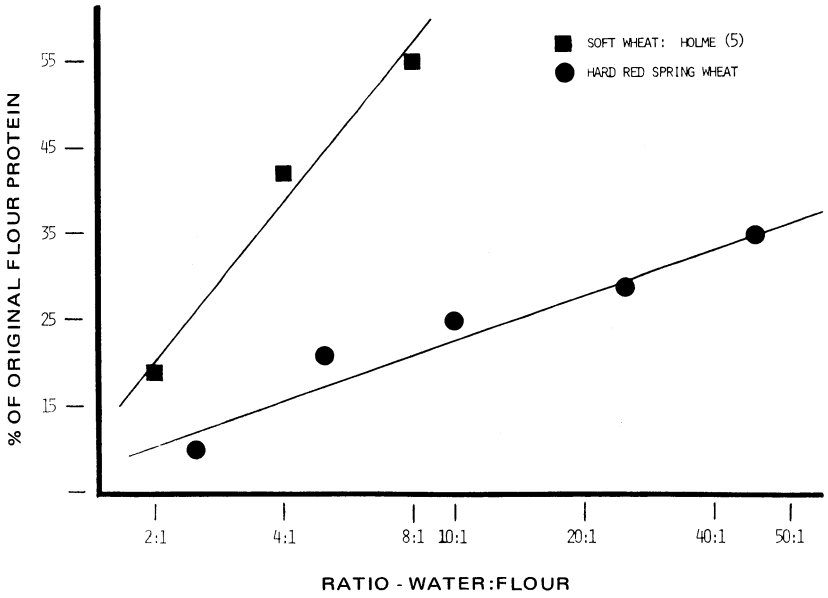

Fig. 5. The semi-logarithmic relation between water:flour ratio and percent of the original flour protein extracted. ■ = data obtained for soft wheat, Holme (5). ● = data for hard red spring wheat.

influenced quantity, composition, and properties of the water-solubles extracted. The amount of protein extracted as well as the type was affected. This was exhibited by the difference in the farinograms, mixograms, and amino acid analysis of the extracted flour.

Using a paint shaker to extract the water-solubles afforded a worthwhile method. Since foaming was minimized and gluten strands, as such, were not formed, denaturation of the protein would appear to be minimized. The action is sufficiently violent to extract the water-solubles, yet the duration of extraction time was short enough to minimize enzyme activity.

The effect of the removal of water-solubles on the extracted flour and the percentage of flour protein removed was not only dependent upon the ratio of water:flour used in the extraction procedure, but also on the protein content of the flour. This study implies that extraction procedures must be clearly defined to facilitate proper evaluation of the physical and chemical effects of an extract itself on the experimental results.

Acknowledgments

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