HORSEBEAN PROTEIN SUPPLEMENTS IN BREADMAKING. II. EFFECT ON PHYSICAL DOUGH PROPERTIES, BAKING QUALITY, AND AMINO ACID COMPOSITION¹

K. M. $PATEL^{2,3}$ and J. A. $JOHNSON^2$

ABSTRACT

Horsebean flour (HBF) and protein isolate (HBPI) were added to weak, medium, and strong wheat flours. The HBF was used to replace 5, 10, 15, and 20% of wheat flour while HBPI was added to wheat flour in amounts to provide equal quantities of horsebean protein. Effects of supplements on physical dough properties, bread quality, and amino acid composition were studied. HBF at all levels above 10% reduced mixing time and tolerance, created dough of low elasticity, and produced

inferior quality bread. HBPI, even with protein equivalent to 20% HBF, produced satisfactory physical dough properties as well as acceptable quality bread. Wheat flour of good quality obviously was needed. While increments of HBF or HBPI obviously increased the protein content of the breads, more significant were the marked increases in the amino acids lysine, histidine, arginine, threonine, and tyrosine.

Although the nutritional contribution of oilseed and legume proteins to wheat protein has been appreciated by many, acceptance has been limited of nonwheat proteins because of their detrimental effects on dough properties, baking qualities, or flavor of baked products (1–7). The acceptance of baked products supplemented with auxiliary proteins depends on source, amount, and type of supplement (4,6,8).

The partially purified protein supplements added to wheat flour usually have been more acceptable than raw or defatted supplements (8). During preparation of protein isolates, materials detrimental to baking may be removed. Furthermore, to achieve a given protein content with a protein concentrate in the baked product requires less dilution of gluten proteins than occurs with raw or defatted supplements.

Baking formulas or procedures usually are modified when nongluten proteins are used. Changes include using coarsely ground and steamed supplement, shorter fermentation time (5,9,10), relatively strong wheat flour, and surfactants (7,11,12).

Reported here are results of using HBF and HBPI as protein supplements in breadmaking. The preparation of HBPI was described previously (13).

MATERIALS AND METHODS

Three wheat flours representing strong, medium, and weak quality wheat flours were blended with 5, 10, 15, and 20% HBF or equivalent amounts of protein from HBPI. The strong and medium quality flours were milled from hard red winter wheats and had protein contents of 11.7 and 13.3%, respectively. The

¹Contribution No. 872, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan. Presented by senior author in partial fulfillment of requirements for the Ph.D. degree, Kansas State University, Manhattan. Presented in part at the 54th Annual Meeting, Miami Beach, Oct. 1972.

²Respectively: Research Associate and Professor, Kansas State University, Manhattan 66506.

³Present address: Department of Food Science and Human Nutrition, Michigan State University, East Lansing.

weak flour, having a protein content of 8.3%, was milled from a Western white wheat. The protein contents of both flours and breads were determined by the Kjeldahl method (14) using the customary factors of $5.75 \times N$ for calculating wheat flour protein and $6.25 \times N$ for calculating protein from horsebean.

Physical dough tests included farinograms and extensigrams made using AACC Methods (14).

Baking tests were performed by two methods: the straight-dough procedure with a lean formula and short fermentation time, and the sponge-dough procedure normally used in Morocco. The lean formula for both baking procedures follows:

Ingredient	g
Flour blend	400
Compressed yeast	8.0
Salt	8.0
L-ascorbic acid	1.0
Ethoxylated monoglyceride (EMG)	2.0
Water	Optimum

The Moroccan-type bread, using a strong wheat flour, was made by fermenting a 70% sponge for 90 min at 30°C before remixing to optimum

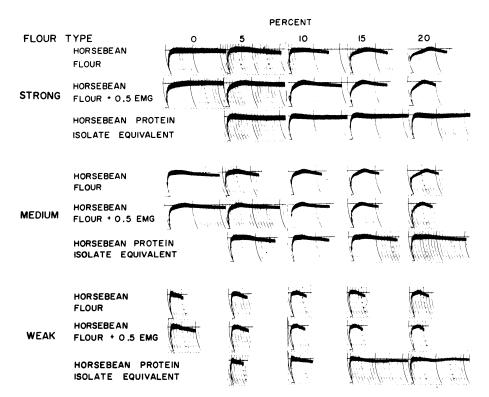


Fig. 1. Effects of wheat flour strength and amount and type of protein supplements on farinogram characteristics.

consistency with the remaining wheat flour, protein supplement, salt, L-ascorbic acid, ethoxylated monoglyceride (0.5% EMG), and 30% of the total water. After being mixed, the dough was scaled to 400 g, rested for 30 min, and sheeted to form 1/4 in. thick \times 7 in. diameter pieces, which were placed on silicone paper-covered cookie sheets for proofing at 30°C and 90% R.H. for 40 min. The breads were baked for 25 min at 211° C.

The straight-dough procedure used optimum dough-mixing and fermentation for 40 min at 30°C and 90% R.H. After fermentation, the dough was scaled to 150 g, rested 7 min, molded, and placed in low-form baking pans. The doughs were proofed for 55 min at 30°C and 90% R.H. The loaves were baked at 211°C for 25 min. Loaf volumes were measured by rapeseed displacement as soon as loaves were removed from the oven; other quality measurements were made 6 hr later. Each quality characteristic was rated 1 to 10 with each factor weighted as follows: crust color \times 1, break and shred, and symmetry, each \times 1/2, and crumb color, grain and texture, and loaf volume each \times 2. Maximum total score was 100.

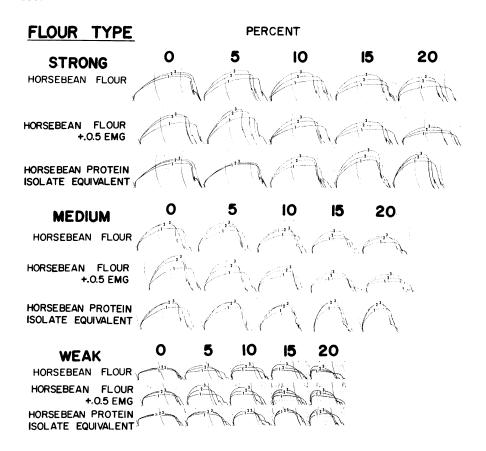


Fig. 2. Effects of wheat flour strength and amount and type of protein supplements on extensigram characteristics.

Crust color was measured by the Photovolt reflectometer, Model 210⁴, with standardized amber filter. Crumb color was measured with a Gardner color difference meter, Model As-2A⁵ using standardized white ceramic tile which gave reflectance (Rd) of 85%. Duplicate readings were made on two slices from the center of each loaf.

The flour blends and breads were analyzed for moisture, ash, crude fat, protein content, and crude fiber by AACC Methods (14). Bread crumb was partially dried at room temperature, then crushed in a Waring Blendor, ground with a micro-Wiley mill, then further dried in a forced-draft oven at 130°C for 1 hr. While the protein contents of the dried bread crumb were determined by the Kjeldahl method (14), corrections were made for the amount of protein contributed by the wheat flours and the horsebean supplements. Amino acid composition was determined with a Beckman automatic amino acid analyzer using Spackman's procedure (15). The essential amino acids were expressed as percentages of the FAO protein pattern (16).

RESULTS AND DISCUSSION

Effect on Physical Dough Properties

Effects of HBF and HBPI on physical dough properties are illustrated in Figs. 1 and 2. Results of analyses of variance on water absorption, dough stability, and resistance to extension are presented in Table I, and two-way interactions and least significant differences (LSD) are summarized in Table II.

Type of protein supplement had no effect on water absorption. Effect of the supplements on dough-mixing stability was particularly significant. Increasing levels of HBF greatly reduced dough-mixing time and stability, but HBPI tended to increase the mixing stability and reduce the mixing time (Fig. 1, Tables I and II). Increasing levels of HBF reduced the resistance to extension and caused the dough to have putty-like properties. These properties were not corrected by addition of 0.5% EMG (Fig. 2, Table II).

TABLE I

Analyses of Variance for Physical Dough Properties and Baking Quality Characteristics (F Values)

Source of Variance	Farinograph Absorption	Dough Stability	Maximum Resistance 135 min	Specific Loaf Volume	Crumb Color Reflect.	Crumb Tender- ness	Bread Score
(F) Flour	69*	765*	465*	1399*	20*	60	329*
(P) Type protein	9*	1485*	76*	64*	24*	21*	30*
(C) HB protein level	1	125*	6*	324*	18*	34*	70*
$F \times P$	2	299*	12*	27*	6*	4	6*
$F \times C$	1	67*	6*	8*	0	3	. 1
$P \times C$	1	329*	39*	44*	2	14	6*
$F \times P \times C$	1	96*	6*	6*			
Error mean square	45	37	329	2.0	5	56	6
Error degrees of							
freedom			•••	72	6	6	6

⁴Photovolt Corporation, New York, NY.

⁵Gardner Color Meter, Gardner Laboratories, Bethesda, Md.

⁶Beckman Instrument Co., Fullerton, Calif.

The stronger the flour, the greater were the absorptions, dough-mixing times and stability, extensibility, and resistance to extension (Fig. 2 and Tables I and II).

Effect on Breadmaking Qualities

Effects of HBF and HBPI supplements on bread qualities are illustrated in Figs. 3 and 4 and Tables I and III. Strength of the flour was the most dominant factor influencing specific volume and total quality. That a strong flour is needed to carry auxiliary protein supplements was particularly evident (Figs. 3 and 4 and Tables I and III). However, HBPI at protein levels equivalent to HBF produced a far superior quality bread, perhaps because detrimental constituents of HBF were removed during processing and gluten protein was diluted less when HBPI was used.

Bread crumbs containing HBPI were much whiter than those made with HBF. However, bread crusts from HBF supplement were much darker than from HBPI supplement, suggesting that free amino acids were removed during HBPI processing (Table III).

Breads containing HBPI had a more tender crumb than those containing HBF, which might be expected because of the significantly detrimental effect on loaf volume of HBF when supplemented at 15 or 20% (Table III).

Characteristics of the Moroccan bread made by the sponge method were similar to the results using the straight-dough procedure. The effects of increasing levels of HBF and HBPI were nearly alike for the two baking methods.

TABLE II
Two-Way Interaction Means and Least Significant Differences
of the Physical Dough Properties

Treatment Combinations		arinograpl Absorption			Dough Stability min		Max. Resistance to Extension after 135 min BU			
	Strong	Medium	Weak	Strong	Medium	Weak	Strong	Medium	Weak	
	Whea	t Flour St	rength >	Type of	Protein S	uppleme	ent ($F \times P$)		
Control	Whea 58.6	t Flour St 61.4	rength > 56.5	Type of 27.0	Protein S 10.5	uppleme 4.2	ent (F × P 689	685	290	
Control HBF			Č					,	290 339	
	58.6	61.4	56.5	27.0	10.5	4.2	689	685		

Type of Protein Supplement \times Content of Protein Supplement (P \times C)

Equiv. (%)	HBF	HBPI	HBF	HBPI	HBF	HBPI
0	58.8	58.8	13.9	13.9	555	555
5	59.0	58.9	8.0	8.8	551	509
10	59.6	58.4	6.0	10.3	504	524
15	59.2	58.3	4.4	16.5	448	571
20	58.9	57.8	3.6	24.8	398	568
LSD	n	.s.	1	.0	2	20

an.s. = Not significant.

Nutritive Value of Breads

Proximate analyses of breads made with 0, 5, 10, 15, or 20% HBF or equivalent protein from HBPI are summarized in Table IV. The average protein content increased from 12.6% for the control to 16.2 and 18.7% for 20% HBF and 20% protein equivalent from HBPI breads, respectively. The higher protein content of the breads made with HBPI, although added in amounts of equivalent horsebean protein, was due to the lesser diluting effect on the wheat protein by the smaller percentage of replacement when HBPI was used. Ash and crude fat of bread made with HBF exceeded their counterparts in bread made with HBPI.

Amino acid compositions of the breads are summarized in Table V and essential amino acid contents are compared with the FAO reference pattern in Table VI. The control bread contained 42% lysine, 70% total sulfur amino acids, 75% isoleucine, and 79% tryptophan of the FAO reference pattern, respectively.

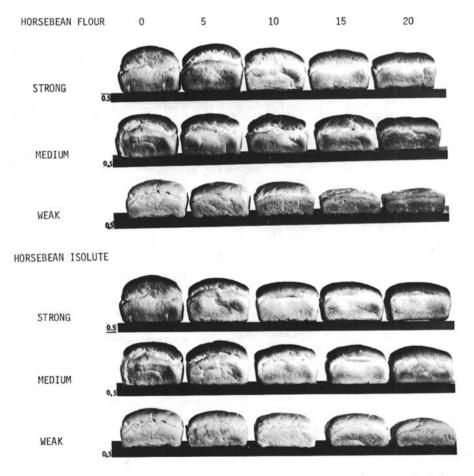


Fig. 3. Effects of various concentrations of horsebean flour and horsebean protein isolate on baking results with strong, medium, and weak wheat flours.

Adding 5, 10, 15, or 20% HBF increased lysine contents to 52, 58, 67, and 72% of the FAO reference pattern, respectively. Adding equivalent protein from HBPI gave lysine contents of 47, 56, 62, and 71% of the FAO reference pattern, respectively. Other essential amino acids also increased as HBF or HBPI supplements were increased.

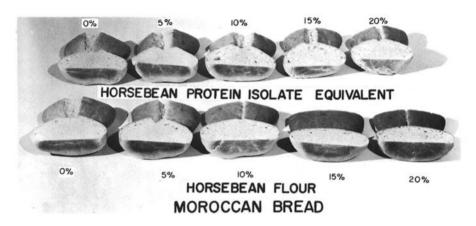


Fig. 4. Effects of increasing horsebean flour and horsebean protein isolate content on characteristics of Moroccan bread.

TABLE III
Two-Way Interaction Means and Least Significant Differences of Baking Quality Characteristics

Treatment Combinations	Specific Loaf Volume cc/g			Crumb Color Reflectance %			Crumb Tender- ness (Fresh) g/-1 mm			Bread Score		
	Strong	Med.	Weak	Strong	Med.	Weak	Strong	Med.	Weak	Strong	Med.	Weak
	Wh	eat Fl	our Str	ength ×	Туре	of Pro	tein Su	pplem	ent (F	× P)		
Control	6.6	5.9	4.5	54	51	49	405	402	295	89	81	61
HBF	5.7	5.0	3.6	46	42	36	293	275	244	74	65	39
HBPI	5.6	5.4	4.0	36	48	43	295	294	265	75	71	49
LSD			0.1		4.0				n.s.a		4.0	

Type of Protein Supplement × Content of Protein Supplement (P × C)

Equiv. (%)	HBF	НВРІ	HBF	HBPI	HBF	HBPI	HBF	HBPI
0	5.7	5.7	52	52	300	300	77	77
5	5.6	5.4	47	49	293	295	71	72
10	5.1	5.1	42	46	289	286	64	66
15	4.4	4.9	40	45	271	281	56	64
20	3.9	4.5	35	42	231	277	46	58
LSD	0	.1	4	.0	15	0.0		5.0

an.s. = Not significant.

Ingredient	Moisture %	Protein % db	Ash % db	Crude Fat % db
Horsebean flour	7.2	30.9	3.7	3.2
Horsebean protein	· ·-			
isolate	10.4	85.4	2.5	
Control bread	31.2	12.6	1.9	0.2
5% HBF bread	31.1	13.3	2.0	0.2
10% HBF bread	31.6	14.9	2.2	0.3
15% HBF bread	31.6	15.5	2.2	0.3
20% HBF bread	28.5	16.2	2.3	0.3
5% HBPI equiv.	32.0	14.3	1.9	0.2
10% HBPI equiv.	32.1	15.6	1.9	0.2
15% HBPI equiv.	31.5	16.8	1.9	0.2
20% HBPI equiv.	32.4	18.1	1.9	0.2

TABLE V

Amino Acid Compositions of Moroccan-Type Breads with
Horsebean Flour and Horsebean Protein Isolate

Amino Acids	0% HBF	5% HBF	10% HBF	15% HBF	20% HBF	2% HBPI	4% HBPI	6% HBPI	8% HBPI
	g Amino Aci	d/100 g	Protein (Corrected	l to 100%	6 Protein	Recover	·у	
Lysine	1.78	2.18	2.42	2.80	3.01	1.96	2.35	2.59	3.00
Histidine	1.85	2.05	3.35	3.31	2.52	1.77	2.14	2.79	2.02
Arginine	2.97	4.14	4.48	5.29	5.78	3.40	4.10	4.71	4.90
Aspartic acid	3.54	4.32	4.29	5.39	6.10	4.57	5.45	5.65	6.75
Threonine	2.57	2.64	3.09	2.84	3.19	2.67	2.75	2.87	3.04
Serine	4.62	4.76	4.55	4.70	4.75	4.86	4.76	4.87	5.26
Glutamic acid	36.64	33.70	33.06	31.04	30.40	35.05	32.87	32.91	28.55
Proline	10.74	10.33	10.51	9.54	8.29	10.39	9.64	8.61	9.54
Glycine	3.33	3.46	3.28	3.48	3.64	3.54	3.55	3.42	3.89
Alanine	2.75	2.97	2.86	3.18	3.21	3.06	3.07	2.80	3.47
Cystine ^a	1.71	1.45	1.15	1.36	1.42	1.30	1.47	1.30	0.92
Valine	4.06	4.24	3.92	3.51	3.91	4.51	4.03	4.04	4.46
Methionine ^a	1.23	1.13	1.14	1.15	1.20	1.01	1.00	1.11	0.97
Isoleucine	3.14	3.22	3.19	3.36	3.50	3.41	3.79	3.49	3.83
Leucine	6.55	6.70	6.56	6.88	6.96	7.04	7.25	7.04	7.78
Tyrosine	2.93	2.96	2.90	2.98	3.32	2.88	3.07	3.14	3.03
Phenylalanine	4.45	4.45	4.37	4.33	4.46	4.78	4.88	4.51	4.92
Tryptophan	1.10	1.08	1.08	1.07	1.06	1.10	1.10	1.10	1.10

^aCystine and methionine by oxidation.

TABLE VI
Essential and Related Amino Acids in Horsebean Flour and Horsebean Protein IsolateSupplemented Moroccan-Type Breads Expressed as Percentages of FAO Reference Protein Pattern^a

Wheat Flour	Horsebean Flour						Horsebean Protein Isolate				
+ Protein Supplement	100%	95% + 5%	90% + 10%	85% + 15%	80% + 20%	98% + 2%	96% + 4%	94% + 6%	92% + 8%	FAO Pattern g/16 g N	
Protein ^b	14.2	14.9	15.6	16.9	17.8	15.8	16.3	18.6	20.0		
Amino acids											
Lysine	42	52	58	67	72	47	56	62	71	4.2	
Threonine	92	94	110	101	114	95	98	102	109	2.3	
Cystine											
Methionine	56	51	52	52	54	46	46	50	44	2.2	
Total S-amino acids	70	61	54	60	62	55	59	57	57	4.2	
Phenylalanine	159	162	156	155	159	171	174	161	176	2.8	
Tyrosine	105	106	104	106	119	103	110	112	108	2.8	
Isoleucine	75	77	76	80	83	81	90	83	91	4.2	
Leucine	134	137	134	141	143	144	149	144	159	4.8	
Tryptophan	79	79	79	79	79	79	79	79	79	1.4	
Valine	97	101	93	84	93	107	96	96	106	4.2	

^aReference 16.

CONCLUSION

HBPI supplement had much less detrimental effect than did HBF supplement on physical dough properties and bread quality. Medium-to-strong wheat flour is recommended as a carrier of nongluten protein supplements. Adding either HBF or HBPI greatly increased protein contents of breads and improved essential amino acid compositions compared with control breads. Breads containing HBPI with superior quality characteristics were more desirable than those made with HBF as protein supplement.

Literature Cited

- 1. BOHN, R. T., and FAVOR, H. H. Functional properties of soya flour as a bread ingredient. Cereal Chem. 22: 296 (1945).
- 2. BAILEY, L. H., CAPEN, R. G., and LeCLERC, J. A. The composition and characteristics of soybeans, soybean flour and soybean bread. Cereal Chem. 12: 441 (1935).
- 3. BAYFIELD, E. G., and SWANSON, E. C. Effect of yeast, bromate, and fermentation on bread containing soy flour. Cereal Chem. 23: 104 (1946).
- BACIGALUPA, A., AGUILAR, T. S., LUNA DE LA FUENTE, R., and VALLE RIESTRA,
 J. Bread enrichment with Protal-peruvian cottonseed flour. Cereal Sci. Today 12: 431 (1967).
- MATTHEWS, R. H., SHARPE, E. J., and CLARK, W. M. The use of some oilseed flours in bread. Cereal Chem. 47: 181 (1970).
- ROONEY, L. W., GUSTAFSON, C. B., CLARK, S. P., and CATES, C. M. Comparison of the baking properties of several oilseed flours. J. Food Sci. 37: 14 (1972).
- 7. AIDOO, E. S. High protein breads: Interaction of wheat proteins and soy proteins with surfactants in doughs and in model systems. Ph.D. Thesis, Kansas State University,

^b% protein, moisture-free basis. Wheat = $N \times 5.7$, HBF and HBPI = $N \times 6.25$.

Manhattan (1972).

- MARINEZ, W. H., BECHTEL, W. G., and LEHMAN, T. T. Glandless cottonseed protein products in bread. (Abstr. No. 163) Cereal Sci. Today 14: 110 (1969).
- LORENZ, K., and MAGA, J. A. The production of high-protein breads under reduced atmospheric pressures. Cereal Chem. 49: 522 (1972).
- TSEN, C. C., and TANG, R. T. K-State process for making high-protein breads. I. Soy flour bread. Baker's Dig. 45(5): 26 (1971).
- 11. FINNEY, K. F., and SHOGREN, M. D. Surfactants supplement each other, make foreign proteins more compatible in breadmaking. Baker's Dig. 45(1): 40 (1971).
- TSEN, C. C., and HOOVER, W. J. The shortening sparing effects of sodium stearoyl-2-lactylate and calcium stearoyl-2-lactylate in breadmaking. Baker's Dig. 45(3): 38 (1971).
- PATEL, K. M., and JOHNSON, J. A. Horsebean as protein supplement in breadmaking. I. Isolation of horsebean protein and its amino acid composition. Cereal Chem. 51: 693 (1974).
- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Approved methods of the AACC. Methods 54-10, 54-21. The Association: St. Paul, Minn. (1962).
- SPACKMAN, D. H., STEIN, W. H., and MOORE, S. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30: 1190 (1958).
- NATIONAL ACADEMY OF SCIENCES. National Research Council Committee on Protein Malnutrition, Evaluation of Protein Quality. NRC Pub. 1100 (1963).

[Received July 12, 1974. Accepted March 1, 1975]