

Supplemental Effect of Wheat Protein Concentrate on the Protein Quality of White Wheat Flour

G. S. RANHOTRA, F. N. HEPBURN, and W. B. BRADLEY, Nutrition Laboratory, American Institute of Baking, Chicago, Illinois 60611

ABSTRACT

The effect on protein quality of incorporating increasing increments of wheat protein concentrate into white wheat flour was studied in the resultant blends and in breads prepared from each blend. Effects of protein concentrate on dough properties and baking characteristics were also examined. Substitution of flour with 0, 15, 30, and 45% of protein concentrate progressively lowered dough stability and adversely affected bread volume, texture, grain, and break and shred. Such substitution, however, resulted in a gradual but significant increase in food intakes, weight gains, and liver weights of weanling rats fed 10% protein diets prepared from substituted flour. For each 15% substitution of flour with protein concentrate, corrected protein efficiency ratio (PER) increased by about 0.2 (PER of flour, 0.9). Also, the net utilization of protein consumed by the animals increased progressively. The results of animal studies also revealed the loss of protein quality during baking in magnitudes which increased with the amount of protein concentrate in the blend. All essential amino acids except tryptophan showed some destruction during baking, which was more severe, ironically, for the amino acids most limiting in wheat protein, namely, lysine, threonine, etc.

Fibrous wheat mill-fractions, namely shorts, bran, and others, are high in essential amino acids—especially lysine, certain minerals, and vitamins such as phosphorus and niacin (1,2). The possibility of these fractions contributing in human dietary (3) recently led to their upgrading into wheat protein concentrate (WPC), a high-protein, low-fiber flour. The poor storage and baking qualities of WPC (4) have, however, interfered with its anticipated wide use in the fortification

of lysine-poor white wheat flour. While these problems are being examined in other laboratories, in present studies another aspect of WPC was investigated. This comprised a study of the effect of increasing replacement of white wheat flour with WPC on the protein quality of the resultant blends, and the effect of baking on WPC in conjunction with a brief study of the rheological and baking qualities of these blends.

MATERIALS AND METHODS

White flour from hard red winter wheat was replaced to the extent of 0, 15, 30, and 45% with WPC (Burrus Mill, Dallas) and the rheological baking qualities of the resultant blends were examined (Table I). Farinograph water absorption was determined by titrating 50-g. portions to peak consistency in a Brabender farinograph while dough water absorption and dough-mixing time (time taken to reach desired consistency) were measured during baking. Bread was baked by a modified straight-dough procedure (5) based on the formula listed in Table II. Loaf volume was measured by rapeseed displacement. Other bread characteristics examined included texture, grain, break and shred, crust and crumb color, and flavor.

Chemical analyses of flour and WPC were carried out by standard methods (6), except that boric acid modification of the Kjeldahl method (7) was used for the determination of nitrogen. Amino acids, except tryptophan, were determined in acid hydrolysate of protein (method B of Kohler and Palter (8) except that hydrolysis was carried out at 121°C. for 16 hr. at 15 p.s.i.) by a Beckman-Spinco Model 120 C amino acid analyzer. Tryptophan was determined by the method of Tkachuk and Irvine (9). Phytic acid was estimated by the method of Anderson (10) based on iron content of extracted phytate after its precipitation as ferric phytate.

Individually housed weanling male rats (Sprague-Dawley) averaging about 55 g. initially were fed the experimental diets (Table III), including the N-free diet, *ad libitum* for 14 days (6 rats per diet). All test diets contained 10% protein furnished by flour or blends in the form of bread ingredients or the resultant breads. Bread was air-dried and ground before use. A casein diet served as the reference control. All diets were offered mixed with water. Food-intake and weight-gain records were kept on individual rats. On day 15 all rats were sacrificed, their gastrointestinal contents washed out, and carcass nitrogen determined by the procedure described earlier (11). Nitrogen retention was calculated by the "net protein utilization" method of Miller and Bender (12).

TABLE I. PROXIMATE ANALYSES OF WHITE WHEAT FLOUR AND WPC

	Approximate Particle Size (U.S. sieve)	Proximate Composition				
		Protein (N X 5.7) %	Ether extract %	Fiber %	Ash %	Moisture %
Flour	150	11.83	1.28	0.33	0.43	11.07
WPC	100	15.35	3.18	1.22	1.97	11.52

TABLE II. BLENDS OF FLOUR AND WPC AND THEIR RHEOLOGICAL BAKING QUALITIES^a

	Blends			
	Flour	Blend A-1	Blend A	Blend A-2
	%	%	%	%
Flour	100	85	70	55
WPC	0	15	30	45
Farinograph water absorption, ml./100-g. sample at 14% moisture	61.8	62.2	62.6	62.8
Dough water absorption, ml./100-g. sample	65.0	65.0	67.0	67.0
Dough-mixing time, min.	10.5	6.5	5.5	5.0
Loaf volume, ml.	2,850	2,500	2,400	1,800
General bread characteristics, score ^d	3.8	3.3	2.3	0.8

^aBread was baked by a straight-dough procedure with the formula (%): flour or blend, 100; yeast, 2.5; yeast food, 0.5; salt, 2.0; lard, 3.0; sugar, 6.0; and water, 65-67. Baked at 400°F. for 25 min.

^bExcellent, 5; very good, 4; good, 3; fair, 2; and poor, 1. Characters evaluated included texture, grain, and break and shred.

TABLE III. PERCENTAGE COMPOSITION AND PROTEIN CONTENT OF EXPERIMENTAL DIETS

	Dextrin	Corn Oil		NaCl		Salt 446 ^a		VDFM ^b	N
Basal (N-free) diet	91.0	3.0		1.0		3.0		2.0	0.07
Diets ^c	Casein	Flour		Blend A-1		Blend A		Blend A-2	
		Bread Ingr.	Bread	Bread Ingr.	Bread	Bread Ingr.	Bread	Bread Ingr.	Bread
Protein source ^d , g.	11.53	91.00	84.39	89.28	80.65	85.10	76.92	80.97	74.07
Dietary protein ^e , %	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0

^aFrom General Biochemicals, Chagrin Falls, Ohio.

^bVitamin Diet Fortification Mixture from Nutritional Biochemicals, Cleveland, Ohio.

^{c,d}Dextrin in the basal diet was replaced with appropriate protein source (bread ingredients/ bread crumbs/casein) to obtain 10% protein diets. Yeast was inactivated (100°C., 30 min.) before addition to unbaked bread ingredients.

^eN X 6.25 for casein and N X 5.7 for others.

RESULTS AND DISCUSSION

Rheological Baking Qualities

The results in Table II show that although increasingly substituting flour with WPC did not appreciably affect either the farinograph or dough water absorption, stability of the dough was greatly reduced as revealed by dough-mixing time. Loaf volume decreased progressively with increasing replacement of flour with WPC. Such replacement also affected other bread qualities adversely.

Bioevaluation of Protein Quality

Table IV summarizes the results of animal experimentation. Since this experiment was restricted to the 2-week feeding period when growth in weanling rats is most rapid, protein quality may be slightly over-evaluated for casein and other diets. Rats fed diets prepared with flour showed a progressive increase in their food intakes, weight gains, and liver weights as more and more of the flour was substituted with WPC, thus pointing toward the nutritional contribution of WPC. On the average, for each 15% replacement of flour with WPC, the protein efficiency ratio (PER) for diets prepared with bread ingredients increased by about 0.35 (0.20 corrected) from an initial value of 1.58 (0.93 corrected) for flour. This compares favorably with the PER of 3.37 or corrected PER of 1.90 reported for WPC (13,14). While growth and, as such, PER afford a satisfactory measure of protein quality, measurement of gain in body nitrogen and its efficiency provides a more precise assessment. Thus, in 10% protein diets, gradually increasing the percentage of WPC in the flour-based diet resulted not only in a progressive increase in nitrogen intake and in carcass nitrogen of rats but also in the efficiency of the rats in retaining nitrogen consumed. For example, 45% substitution with WPC increased the net utilization of protein (NPU) of flour by 17%. This apparently suggests that an increasingly better balance of essential amino acids was attained as more and more of the flour was substituted with WPC. In addition to bread ingredients, the resultant breads were also used to formulate diets to determine possible loss of protein quality during baking. Considering relative values for weight gains of rats, PER's, and NPU's, it becomes apparent that although the protein quality of bread improved through increasing the amount of WPC in the blend, loss during baking increased simultaneously. An exception was noted with blend A-2 (45% WPC) where improvement over blend A (30% WPC) did not result. This does not necessarily mean, however, that blends containing WPC over 45% would not improve protein quality of bread further.

Amino Acid Content

The capacity of WPC to improve protein quality of flour could be predicted from its amino acid composition (Table V). Compared to previous analysis of WPC (13) and also to values reported by others (14), the present lot of WPC was somewhat low in both protein and lysine, perhaps because its fiber was reduced still further to 1.22%. For ease of comparison and uniformity, the results of amino acid analyses are presented in percentages and on a moisture-free-sample basis. In addition to increases in protein content, replacement of flour with 15, 30, and 45% of WPC corresponds to increases of 0.071, 0.142, and 0.186 g. lysine, respectively. These increases in lysine, somewhat reduced since all diets were evaluated at 10%

TABLE IV. RAT-FEEDING EXPERIMENT (14 DAYS)

	Casein	Diets (10% Protein)							
		Flour (0% WPC)		Blend A-1 (15% WPC)		Blend A (30% WPC)		Blend A-2 (45% WPC)	
		Bread Ingr.	Bread	Bread Ingr.	Bread	Bread Ingr.	Bread	Bread Ingr.	Bread
Growth Data^a									
Food intake, g./day	13.59 ±0.60	9.28 ±0.96	8.95 ±0.83	9.40 ±0.54	9.24 ±0.50	11.11 ±0.66	10.49 ±0.70	12.21 ±1.12	10.41 ±0.81
Weight gain, g./day	5.71 ±0.48	1.46 ±0.32	1.43 ±0.16	1.75 ±0.21	1.71 ±0.13	2.55 ±0.32	2.10 ±0.20	3.20 ±0.57	2.14 ±0.19
Liver weight, g./rat	8.75 ±0.53	4.73 ±0.41	4.88 ±0.25	5.12 ±0.46	5.18 ±0.35	5.68 ±0.58	5.30 ±0.29	6.65 ±0.48	5.48 ±0.36
PER ^b (measured)	4.20 ±0.23	1.58 ±0.21	1.60 ±0.11	1.86 ±0.16	1.85 ±0.07	2.29 ±0.18	1.99 ±0.06	2.62 ±0.25	2.06 ±0.08
PER ^b (corrected to casein)	2.50 ±0.13	0.93 ±0.13	0.95 ±0.07	1.10 ±0.09	1.10 ±0.04	1.36 ±0.10	1.19 ±0.06	1.55 ±0.15	1.22 ±0.02
Nitrogen Retention Data^a									
N intake, g.	3.05 ±0.13	2.28 ±0.24	2.20 ±0.20	2.38 ±0.20	2.27 ±0.12	2.73 ±0.16	2.58 ±0.09	3.00 ±0.28	2.56 ±0.20
Carcass-N gain ^c , g.	2.23 ±0.16	0.66 ±0.12	0.61 ±0.10	0.75 ±0.10	0.65 ±0.06	0.91 ±0.10	0.78 ±0.07	1.01 ±0.13	0.74 ±0.08
NPU ^d , %	73.01 ±2.90	28.71 ±2.78	27.78 ±2.53	31.68 ±4.48	28.58 ±3.09	33.17 ±1.73	30.50 ±1.74	33.63 ±1.63	29.25 ±4.27
Relative^e									
Weight gain, %	391	100	98	120	117	175	144	219	147
PER, %	266	100	101	118	117	145	126	166	130
NPU, %	254.3	100.0	96.8	110.3	99.6	115.5	106.3	117.1	101.9

^aAverage values for six rats per diet ± standard deviation.^bPER = g. weight gain per g. protein consumed.^cOver carcass-N (1.33 g.) of rats fed N-free diet.^dNPU = carcass-N gain of test rats/ their N intake × 100.^eRelative to diet of flour (bread ingredients).

TABLE V. ESSENTIAL AMINO ACID COMPOSITION^a

Amino Acid	Flour	WPC	Flour (0% WPC)			Blend A-1 (15% WPC)			Blend A (30% WPC)			Blend A-2 (45% WPC)		
			Bread Ingr.	Bread	Loss %	Bread Ingr.	Bread	Loss %	Bread Ingr.	Bread	Loss %	Bread Ingr.	Bread	Loss %
Lys	0.285	0.673	0.272	0.265	2.6	0.343	0.303	11.7	0.414	0.365	11.9	0.458	0.372	18.8
His	0.283	0.436	0.276	0.265	4.0	0.297	0.288	3.1	0.334	0.310	7.2	0.348	0.325	6.7
Arg	0.484	0.961	0.474	0.473	0.2	0.560	0.540	3.6	0.637	0.608	4.6	0.695	0.635	8.7
Thr	0.373	0.555	0.376	0.342	9.1	0.390	0.369	5.4	0.426	0.371	13.0	0.458	0.406	11.4
Cys	0.228	0.179	0.216	0.202	6.5	0.191	0.175	8.4	0.175	0.165	5.8	0.172	0.172	...
Val	0.654	0.883	0.649	0.639	1.6	0.673	0.640	5.0	0.706	0.674	4.6	0.741	0.691	6.8
Meth	0.216	0.240	0.206	0.208	...	0.217	0.202	7.0	0.217	0.206	5.1	0.225	0.199	11.6
Ileu	0.526	0.656	0.536	0.515	4.0	0.551	0.530	3.9	0.574	0.569	0.9	0.589	0.548	7.0
Leu	1.023	1.257	1.043	0.971	6.9	1.049	1.004	4.3	1.079	1.028	4.8	1.129	1.063	5.9
Tyr	0.336	0.369	0.336	0.321	4.5	0.341	0.316	7.4	0.342	0.333	2.7	0.349	0.341	2.3
Phe	0.686	0.719	0.679	0.636	6.4	0.692	0.674	2.7	0.697	0.664	4.8	0.701	0.649	7.5
Trp	0.147	0.224	0.147	0.144	2.0	0.146	0.159	...	0.151	0.174	...	0.174	0.193	...
Protein ^b , % (N X 5.7)	13.30	17.34	12.22	12.63		12.52	13.23		13.10	13.82		13.72	14.33	
Phytic acid phosphorus ^c			13.6	0.0		76.8	34.7		130.7	71.0		190.4	132.2	

^aExpressed as g. per 100 g. moisture-free sample.^bOn moisture-free-sample basis.^cExpressed as mg. per 100 g. moisture-free sample.

protein level, were accompanied by 20, 75, and 119% increases in weight-gain of rats; 18, 45, and 66% increases in PER; and 10.3, 15.5, and 17.1% increases in NPU, respectively (Table IV). The loss in protein quality that occurred during baking can readily be explained by comparing the amino acid composition of bread ingredients with that of the corresponding bread, especially with regard to lysine—destruction of which, as also of most other less limiting amino acids, tended to increase with increasing substitution of WPC for flour. The most severe destruction of lysine in bread baked with blend A-2 perhaps explains the inability of this bread to improve rat performance over bread of blend A. Jansen et al. (15) reported that some 30% of added lysine became nutritionally unavailable during baking.

CONCLUSIONS

The results suggest that appreciable nutritional improvement of white wheat flour requires its substitution with a substantial amount, 30% or better, of WPC. While this adversely affects baking qualities somewhat, changes in formulation and baking technology can perhaps alleviate this still further than has been attempted thus far (5). In spite of loss during baking, owing to destruction of lysine and other limiting amino acids, protein quality of bread continued to improve with increasing substitution of flour with WPC. Since such substitution also increases protein content of the bread and since baking also results in a sizable hydrolysis of phytic acid (Table V) and possibly of bound niacin, the over-all nutritional value of bread baked with WPC-substituted flour should increase substantially.

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Literature Cited

1. WAGGLE, D. H., LAMBERT, M. A., MILLER, G. D., FARRELL, E. P., and DEYOE, C. W. Extensive analyses of flours and millfeeds made from nine different wheat mixes. II. Amino acids, minerals, vitamins, and gross energy. *Cereal Chem.* 44: 48 (1967).
2. HEPBURN, F. N., CALHOUN, W. K., and BRADLEY, W. B. The distribution of the amino acids of wheat in commercial mill products. *Cereal Chem.* 37: 749 (1960).
3. BRADLEY, W. B. Bread as a food. *Northwest. Miller* 272(4): 17 (1965).
4. FELLERS, D. A., and BEAN, M. A. Flour blend A: II. Storage studies. *Baker's Dig.* 44(6): 42 (1970).
5. KULP, K. Wheat protein concentrate in baked goods. In: Report of Sixth National Conference on Wheat Utilization Research. Agricultural Research Service, USDA, ARS 74-54 (August 1970).
6. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official methods of analysis (9th ed.). The Association: Washington, D.C. (1960).
7. SCALES, F. M., and HARRISON, A. P. Boric acid modification of the Kjeldahl method for crop and soil analysis. *Ind. Eng. Chem.* 12: 350 (1920).
8. KOHLER, G. O., and PALTER, RHODA. Studies on methods for amino acid analysis of wheat products. *Cereal Chem.* 44: 512 (1967).
9. TKACHUK, R., and IRVINE, G. N. Amino acid compositions of cereals and oilseed meals. *Cereal Chem.* 46: 206 (1969).
10. ANDERSON, G. Effect of iron/phosphorus ratio and acid concentration on the precipitation of ferric inositol hexaphosphate. *J. Sci. Food Agr.* 14: 352 (1963).
11. RANHOTRA, G. S., and JOHNSON, B. C. Effect of feeding different amino acid diets on growth rate and nitrogen retention of weanling rats. *Proc. Soc. Exp. Biol. Med.* 118: 1197 (1965).

12. MILLER, D. S., and BENDER, A. E. The determination of the net utilization of proteins by a shortened method. *Brit. J. Nutr.* 9: 382 (1955).
13. RANHOTRA, G. S., HEPBURN, F. N., and BRADLEY, W. B. Effect of fiber on availability of protein from wheat shorts. *Cereal Chem.* 48: 9 (1971).
14. FELLERS, D. A., SHEPHERD, A. D., BELLARD, NANCY J., MOSSMAN, A. P., JOHNSTON, P. H., and WASSERMAN, T. Protein concentrates by dry milling of wheat millfeeds. II. Compositional aspects. *Cereal Chem.* 45: 520 (1968).
15. JANSEN, G. R., EHLE, S. R., and HAUSE, N. L. Studies on the nutritive loss of supplemental lysine in baking. II. Loss in water bread and in breads supplemented with moderate amounts of nonfat dry milk. *Food Technol.* 18: 114 (1964).

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