NUTRITIVE VALUE OF BREAD FORTIFIED WITH CONCENTRATED PLANT PROTEINS AND LYSINE

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ABSTRACT

Amino acid scores and rat growth assays were determined on wheat-flour bread and breads fortified with vital gluten and sufficient soy flour, sunflower concentrate, fababean concentrate, or field-pea concentrate to produce breads with at least 14% protein. The FAO Provisional Pattern (1973) was used as reference protein for calculating chemical score and essential amino acid index. The variations among diets were smaller by net protein ratio than protein efficiency ratio (PER), but these data were highly correlated with no obvious advantage for either measurement. The bread diets were limiting in lysine and its supplementation caused marked increases in protein quality. The lysine content of the diets gave significant correlations with both chemical and biological data. Soy, fababean, and field-pea bread gave PER ranging from 1.7 to 1.8, whereas sunflower and wheat bread had values of 1.3 and 1.1, respectively. Fababean and field-pea breads were given "excellent" protein ratings of more than 40, but soy bread was given a "good" rating of 37.5 due to the higher moisture content and therefore lower protein content on a fresh weight basis. Sunflower bread also received a "good" rating while wheat bread had a rating of less than 20. When supplemented with lysine the wheat bread had a protein rating of 37.8 and all high protein breads were given "excellent" ratings.

Wheat is a common crop throughout the world and yeast-leavened bread and unleavened chapatties are staple foods in many countries. Such universally consumed foods are well suited to protein fortification. The nutritive value of wheat-flour protein is low (1) but the amino acid balance has been improved by adding concentrated plant proteins (2,3) or L-lysine (4).

The baking procedure required to convert the dough into bread has been shown to cause destruction of lysine (5,6) which is the limiting amino acid for growth (7,8). As a result, baked bread has been reported to have a lower protein quality than bread dough (7,9). Further quality deterioration occurs with prolonged baking time, high temperatures, and increased proportions of crust (6,10). Rat growth assays have shown that supplementing bread diets with L-lysine (8), soy concentrate (11), or fababean (12) will markedly improve protein quality scores.

Since essential amino acids are destroyed in the baking process, it is critical that the protein supplement is added to the dough prior to baking if their complementary effect is to be evaluated experimentally. Bread which has been supplemented with lysine reportedly supports a larger rat growth response than unsupplemented bread (4,8,10). In addition, soy flour bread and field-pea concentrate bread, when fed to rats, gave protein efficiency ratio (PER) values of 1.45 and 1.81, respectively, compared to PER for wheat-flour bread of 0.7 to 1.0 (11,13).

Traditional methods of evaluating protein quality are based on chemical data or rat growth assays. To determine the contribution of different foods within a diet, an evaluation system known as protein rating which was based on both

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quality and quantity of protein was developed (14). Protein rating has been defined as the product of the PER and grams of protein in a daily intake of that food. Food with a rating of 20 to 39, inclusive, was designated as a "good dietary source of protein" while food with a rating of 40 or more was designated as an "excellent dietary source of protein".

In the present study, breads were baked from wheat flour: concentrated plant protein blends which included sufficient soy flour, sunflower concentrate, fababean concentrate, or field-pea concentrate to achieve 14% protein, as-is basis, in the final product (15). The formulations included 2% vital wheat gluten and 1% dough conditioners to ensure acceptable loaf quality characteristics. Each formulation was baked with and without supplemental lysine to assess the nutritive value and principal amino acid deficiencies. The nutritive values of the breads were evaluated by amino acid analysis, rat growth assays, and a protein rating procedure described by Campbell (14).

This study was designed to evaluate the following: the protein quality of breads supplemented with concentrated plant proteins and lysine; the possibility of producing a bread which could be rated as an "excellent" source of dietary protein; and the method of evaluation which could most easily be used to predict the protein quality of bread products.

MATERIALS AND METHODS

The wheat flour was an untreated baker's straight-grade flour, commercially milled from hard red spring wheat. Vital gluten, an undenatured wheat protein fraction, was obtained from Industrial Grain Products Ltd., Montreal, and the defatted soy flour (*Glycine max*) was obtained from a local supplier. The fababean concentrate (*Vicia faba*) and field-pea concentrate (*Pisum sativum*) were prepared by pin milling and air classification at the Prairie Regional Laboratory, National Research Council, Saskatoon. Dehulled sunflower kernels were purchased from Gardenland Packers Ltd., Altona, Manitoba. The sunflower concentrate was prepared by diffusing cracked sunflower kernels in an aqueous (pH 4.5) medium for 4 hr at 60°C to remove chlorogenic acid (16) and the dried seed was defatted with Skellysolve F (petroleum ether). All products were ground and sifted to pass a 100-mesh Tyler sieve.

The breadmaking formula included 100 g flour (14% moisture basis), 4 g nonfat dry milk, 3 g shortening, 3 g wet yeast, 5 g sucrose, 1.75 g salt, 0.1 g ammonium phosphate monobasic, 10 ppm potassium bromate, 0.3 g malt syrup, and water as needed. Where supplementary protein was added to the formula, the wheat flour was replaced by the protein component(s) so that the total flour blend was 100 g. The dough was mixed by the straight-dough procedure (17) and baked 25 min at 220°C in an experimental rotary oven.

Protein quality evaluations were conducted on blends of wheat flour: concentrated plant protein: vital gluten of 100:0:0 for the control wheat-flour bread, 83:15:2 for soy, fababean, and field-pea bread, and 86:12:2 for sunflower bread. In addition, these blends were baked with lysine monohydrochloride (95% L-lysine, 5% D-lysine, ICN Pharmaceuticals Inc., Plainview, NY) in sufficient quantity to add 4.0 g L-lysine/100 g protein. This addition was made to the flour ingredients prior to mixing the dough. ANRC casein (Hunko Sheffield Chemical Division, Krastco Corp., NY), with and without L-lysine supplementation, was used for comparative purposes.

After baking, freeze-drying removed moisture and preserved the bread. Proximate principles including protein, fat, ash, and fiber were determined by AOAC (18) procedures. The amino acid analyses were conducted on a Beckman Model 120C amino acid analyzer with separate hydrolyses for the sulfur-containing amino acids and tryptophan determinations (19). Methionine and cystine were measured as methionine sulfone and cysteic acid, respectively. Chemical scores (CS) and essential amino acid indices (EAAI) of the diets were calculated from the amino acid data (20,21). The 1973 FAO Provisional Pattern (22) was used as reference protein and included eight essential amino acids (isoleucine, leucine, lysine, methionine + cystine, phenylalanine + tyrosine, threonine, tryptophan, and valine). The data were subjected to analysis of variance and Duncan's multiple range test (23) to determine significant differences among diets.

The diets for the rat growth study were formulated to contain 10% protein (N × 6.25) as suggested by Campbell (14). Additional corn oil, cellulose (Alphacell, Nutritional Biochemicals Corporation, St. Pierre, Que.), vitamins (18) and minerals (USP XIV) were added so that each diet contained 8% fat, 5% fiber, 1% vitamins, and 5% ash (18). Corn starch (Nutritional Biochemicals Corporation) was added to make the diets isocaloric. The feedstuffs were stored at −15°C prior to use. The experimental design was a 6 × 2 × 7 factorial which involved feeding casein and five types of bread at two levels of lysine (unsupplemented or 4.0 g L-lysine/100 g protein) in seven replicates. The diets were fed to weanling male Wistar rats (40–50 g) under controlled environmental conditions. Throughout the 4-week feeding trial, rats in individual cages were provided with diets and water *ad libitum*. Feed consumption and weight gains were recorded at 7, 10, 14, 21, and 28 days for each rat. In addition, a group of rats was fed a N-free diet and the weight loss of each group was recorded after 10 days. Individual PER and net protein ratio (NPR) values were calculated from feed consumption, weight gains, and weight losses of the rats (24,25). Protein ratings were calculated by multiplying the adjusted PER by the grams of protein in 150 g of each bread (14). The data for feed consumption, weight gains, adjusted weight gains, NPR, and PER were subjected to analysis of variance and Duncan's multiple range test (23). The weight gains were adjusted by regression to equal feed intake in which weight gain was the dependent variable and feed intake was the independent variable.

RESULTS AND DISCUSSION

Bread Composition

The chemical compositions of the protein ingredients used in the bread formulations were similar to the products previously described (15). While the protein content of the wheat flour was only 15% (N × 6.25), vital gluten was 89% protein and the other concentrated plant proteins ranged from 50 to 76%. Wheat flour and vital gluten contained lower levels of fat, fiber, and ash than the other products.

The breads fortified with concentrated plant proteins had 21.9–23.3% protein compared to only 15.9% for the control wheat-flour bread (Table I). The lysine-supplemented breads had higher protein contents since the lysine was incorporated into the standard formula and was not used to replace other ingredients. Fat and fiber showed little change but the ash contents were increased.

Chemical Evaluation

Compared to raw casein, the control bread was low in most essential amino acids, especially lysine and tryptophan (Table II). The amino acid levels in the control bread were lower than those previously reported (6) but the patterns were the same. Sunflower bread was also low in lysine while fababean and field-pea bread had low levels of methionine + cystine. These patterns resemble the data previously reported for 50:50 protein blends from wheat flour and legumes (26).

Supplementation of diets with L-lysine resulted in marked increases of that amino acid but caused the percentages of all other essential amino acids to be reduced. This was expected since lysine was added to the blends and would, therefore, have a diluting effect on the other essential amino acids when expressed in terms of total protein content. Since 4.0 g L-lysine was added per 100 g protein, the lysine content for casein was increased by 3.8%. However, the lysine value was only increased by 3.31% for the control bread and 2.77–3.07% for sunflower, soy, fababean, and field-pea breads indicating that destruction had occurred in the baking process.

The CS of casein was much higher than the scores for the breads. Since each bread was limiting in the essential amino acid, lysine, the chemical scores were proportional to the quantity of that amino acid. While the scores for the control

TABLE I
Proximate Composition of Casein and Baked Bread (%, Dry Basis)

Protein Ingredients	Crude Protein[a]	Crude Fat	Crude Fiber	Ash
Casein	90.7	0.1	0.1	1.9
Casein and lysine	89.8	0.1	0.1	2.8
Breads: no lysine				
Control (100:0:0)[b]	15.9	3.2	0.4	2.5
Sunflower (86:12:2)	23.2	3.9	1.0	2.6
Soy (83:15:2)	21.9	4.1	0.8	3.3
Fababean (83:15:2)	23.3	4.2	0.6	3.2
Field pea (83:15:2)	22.3	4.5	0.7	3.2
Breads: with lysine				
Control	16.3	3.2	0.4	2.7
Sunflower	23.9	3.9	1.0	2.8
Soy	22.9	4.1	0.8	3.5
Fababean	23.6	4.2	0.6	3.5
Field pea	23.1	4.5	0.7	3.4

[a]N × 6.25.
[b]Ratio of wheat flour:concentrated plant protein:vital gluten.

and sunflower bread were less than 50, soy and field-pea breads gave scores of more than 60. Supplementing each diet with lysine increased the level of that amino acid in each case, but since threonine then became the limiting amino acid, the calculated chemical scores were not increased for fababean or field-pea breads. The EAAI values were high for all diets and were further increased by the addition of lysine. Only small differences among diets were noted using this measure, and the values of 79.7 and 82.9 shown for the control and sunflower bread (no lysine supplementation), respectively, were higher than those calculated for fababean and field-pea breads.

Rat Growth Assay

Analyses following the 10-day growth trial showed that the addition of lysine to the bread diets caused a significant increase in NPR and PER measurements. The casein diets gave the highest NPR and PER values regardless of lysine supplementation while all the breads when fortified with lysine were rated second (Table III). The unsupplemented wheat-flour control bread received the lowest rating by the NPR measurement, but was not less than the sunflower bread when PER was used. The soy, fababean, and field-pea breads gave intermediate values ranging from 2.85 to 3.05 for NPR and 1.68 to 1.86 for PER.

Averaged over all diets, lysine caused a significant increase in all measurements taken after 28 days on diet. Specifically, lysine increased feed consumption for the control, sunflower, soy, and fababean breads. Weight gains for diets with no additional lysine varied from 24.9 g for wheat-flour bread to 114.6 g for casein (Table III), but the addition of lysine narrowed the range (61.7 to 101.5 g) and, with the exception of casein, increased weight gains of all diets.

TABLE II
Essential Amino Acid Distribution in Diets and Reference Standards (g Amino Acid/100 g Protein)

Protein Source	Ile	Leu	Lys	Met + Cys	Phe + Tyr	Thr	Try	Val	CS	EAAI
Reference protein (FAO, 1973)	4.0	7.0	5.5	3.5	6.0	4.0	1.0	5.0		
			Diets							
Casein	5.20	9.62	8.15	2.80	11.28	4.35	2.59	7.06	80.0	98.3
Control bread	3.46	6.62	2.36[a]	2.84	7.14	2.74	0.85	3.98	42.9	79.7
Sunflower bread	3.70	6.46	2.63[a]	2.52	6.88	2.85	0.90	4.26	47.8	82.9
Soy bread	3.62	6.80	3.46[a]	2.61	7.61	3.01	1.03	4.09	62.9	87.4
Fababean bread	3.53	6.62	3.37[a]	2.24	7.32	2.88	0.85	4.05	56.0	78.9
Field-pea bread	3.53	6.59	3.42[a]	2.35	7.09	2.85	0.77	3.94	62.2	78.3
Lysine supplemented										
Casein	5.10	9.42	11.93	2.58	9.88	4.15	2.50	6.70	73.7	97.5
Control bread	3.21	6.23	5.67	2.70	7.03	2.54[a]	0.83	3.71	63.7	85.3
Sunflower bread	3.37	5.92	5.70	2.52	6.70	2.72[a]	0.94	4.11	68.0	88.3
Soy bread	3.34	6.16	6.30	2.43	6.99	2.80[a]	1.10	3.75	70.0	88.8
Fababean bread	3.34	6.26	6.25	2.14	6.72	2.70[a]	0.75	3.95	53.0	80.2
Field-pea bread	3.33	6.21	6.19	2.12	6.59	2.65[a]	0.69	3.74	52.9	78.6

[a]Denotes limiting amino acid in diet.

TABLE III
Influence of Lysine and Protein Source on F-Ratios and on Means for Feed Consumption, Weight Gain, NPR, and PER[a]

Source of Variation	10-Day Trial NPR	10-Day Trial PER	28-Day Trial Feed intake g	28-Day Trial Weight gain g	28-Day Trial Adjusted weight gain g	28-Day Trial PER	28-Day Trial Adjusted PER[b]
Lysine ($s_{\bar{x}}$)	0.030	0.537	5.21	1.09	2.23	0.013	
No lysine	3.02b	1.79b	292.2b	56.6b	63.4b	1.74b	
With lysine	3.59a	2.32a	309.3a	77.0a	70.1a	2.35a	
Protein × lysine ($s_{\bar{x}}$)	0.074	0.131	10.8	1.7	5.46	0.03	
No lysine							
Casein	3.93a	3.07a	398.1a	114.6a	104.8a	2.60a	2.50
Control bread	2.56d	1.04f	212.7g	24.9f	32.3f	1.13f	1.09
Sunflower bread	2.80c	1.34e,f	235.1f,g	32.0f	41.0e,f	1.32e	1.27
Soy bread	2.85c	1.77d	298.5c,d	55.2e	66.3c,d	1.76d	1.69
Fababean bread	2.91c	1.68d,e	297.1c,d,e	56.1e	65.3c,d	1.74d	1.67
Field-pea bread	3.05c	1.86c,d	311.7c,d	56.8e	71.0c	1.88c	1.81
With lysine							
Casein	3.91a	2.86a	356.5b	101.5b	88.5b	2.68a	2.58
Control bread	3.54b	2.08b,c,d	259.3e,f	61.7d,e	50.5d,e	2.24b	2.15
Sunflower bread	3.55b	2.23b,c	286.3d,e	66.9d	61.1c,d	2.23b	2.14
Soy bread	3.42b	2.26b	330.0b,c	82.1c	78.1b,c	2.30b	2.21
Fababean bread	3.62b	2.31b	322.0b,c,d	80.0c	75.0b,c	2.33b	2.24
Field-pea bread	3.51b	2.17b,c,d	302.1c,d	69.5d	67.2c,d	2.34b	2.25

[a]NPR = net protein ratio; PER = protein efficiency ratio.
[b]Adjusted PER values were calculated with casein = 2.50.
Lower case letters denote significant differences at P = 0.05.

Growth of rats was lowest on sunflower and wheat-flour bread diets but showed the largest responses to lysine supplementation. Soy, fababean, and field-pea breads showed similar weight gains, but the field-pea bread showed a lower response to supplemental lysine. By removing variations in weight gain caused by such factors as palatability, the adjusted weight gains are usually considered a better measure of growth promoting properties than the unadjusted gains (27). The adjusted gains for bread diets with no lysine supplementation were higher than the recorded gains while the diets with lysine showed the opposite trend. Supplemental lysine in casein or field-pea bread diets had a negative influence on adjusted weight gains while wheat-flour and sunflower bread showed the largest positive effects.

Statistical analysis of PER calculated after 28 days ranked the diets in generally the same order as NPR and PER after 10 days. However, the longer feeding time ranked unsupplemented field-pea bread higher than either soy or fababean, and sunflower bread gave a significantly better score than the wheat-flour control. All breads, when supplemented with lysine, showed equivalent PER values.

The high feed intakes, weight gains, and PER of the breads containing soy, fababean, and field pea suggest that no toxic components or antinutritive factors were present. Heat treatment has been found to destroy the antinutritive factors of soybean (28) and fababean (29). Previous reports (3) indicated that in blends of wheat flour and legumes (50:50, protein basis) field pea gave the highest PER followed by soy and fababean. However, the results in Table III show no significant difference between soy and fababean bread. This may be due to the influence of the baking process which is required for bread preparation.

The PER values were adjusted to 2.50 for casein in accordance with accepted procedures (14,18) to permit comparisons with results of other workers. The PER for wheat-flour bread of 1.09 (Table III) agrees with the value of 1.06 by

TABLE IV
Calculation of Protein Rating for Breads

Lysine	Bread	Moisture[a] %	Protein (Dry Basis, N × 6.25) %	Protein in 150 g Bread g	PER[b]	Protein Rating
No lysine	Wheat control	28.1	15.9	17.2	1.09	18.7
	Sunflower	29.9	23.2	24.2	1.27	30.7
	Soy	32.1	21.8	22.2	1.69	37.5
	Fababean	25.6	23.3	26.0	1.67	43.4
	Field pea	28.5	22.3	23.9	1.81	43.2
With lysine	Wheat control	28.1	16.3	17.6	2.15	37.8
	Sunflower	29.9	23.9	25.1	2.14	53.7
	Soy	32.1	22.9	23.3	2.21	51.5
	Fababean	25.6	23.6	26.3	2.24	58.9
	Field pea	28.5	23.1	25.7	2.25	57.8

[a]As determined 1 hr after baking.
[b]PER = protein efficiency ratio; PER adjusted to 2.50 for casein.

some workers (4) but is somewhat higher than those reported by others (8, 14). The higher values are probably due to the 4% nonfat dry milk in the formula which has been found to have a significant influence on protein quality of bread (10).

The experimental results showed that adjusted PER increased from 1.09 for the control to 1.69 when 15% of the wheat flour was replaced with soy flour (Table III). However, other workers (10) found bread containing 6% nonfat dry milk to have a PER of 0.9 and bread with 16% soy flour but no nonfat dry milk had a PER of 1.45. The inclusion of 4% nonfat dry milk in the present study would explain the somewhat higher PER for soy bread. Wilding *et al.* (11) replaced wheat flour with 15% soy concentrate and noted an increase in PER from 0.98 for white bread to 1.70 for soy bread. Bread formulated to contain 80% wheat flour, 15% field-pea concentrate, and 5% wheat gluten was found to have a PER of 1.87 (13). Although only 2% gluten was added in the present study, the PER for field-pea bread of 1.81 is in good agreement with the previously reported value.

Protein Rating

Unsupplemented wheat-flour bread gave a protein rating of only 18.7, which was less than required for a "good" protein rating (Table IV). Of the breads containing no supplemental lysine, soy and sunflower bread received "good" ratings (20–40). Fababean and field pea gave "excellent ratings" as dietary sources of protein and had scores of 43.4 and 43.2, respectively. Although soy bread had a PER equivalent to fababean, the higher moisture level reduced the quantity of protein in a reasonable daily intake of bread and, as such, reduced the protein rating.

The addition of lysine had a marked effect on protein ratings due to the large increase in PER values (Table IV). All breads containing concentrated plant proteins were given "excellent" ratings ranging from 51.5 to 58.9, which are in the range reported for egg or milk, and only slightly less than that reported for beef (14). The wheat-flour control received a rating only slightly less than "excellent" when supplemented with lysine.

Correlation Analysis

Chemical score gave highly significant correlations with the dietary lysine level and with all measures of protein quality derived from rat growth assays (Table V). However, EAAI showed less significant correlations with PER values, 28-day feed intake, and adjusted weight gains. The amount of lysine in the diet gave highly significant correlations with most rat growth measures. The high correlation between lysine content of bread and PER ($r = 0.923^{**}$) has been previously reported by others (6,10). All rat growth parameters were highly significantly correlated with the exception of 10-day intake with NPR and 28-day PER values.

CONCLUSIONS

The chemical (CS, EAAI, and lysine content) and biological (feed intake, weight gain, NPR, and PER) measures of protein quality were closely correlated in this study. Therefore, the relatively simple measures of CS or lysine content

TABLE V
Correlation Matrix for Chemical and Biological Evaluation of Protein Quality[a]

				Rat Growth—10 Days				Rat Growth—28 Days		
	CS	EAAI	Lysine	Feed intake	Weight gain	NPR	PER	Feed intake	Weight gain	Adjusted weight gain
EAAI	0.895**									
Lysine	0.791**	0.742**								
10 Days—Feed intake	0.854**	0.708**	0.666*							
Weight gain	0.909**	0.805**	0.862**	0.925**						
NPR	0.753**	0.642*	0.943**	0.608*	0.844**					
PER	0.873**	0.735**	0.930**	0.799**	0.951**	0.955**				
28 Days—Feed intake	0.831**	0.637*	0.761**	0.930**	0.937**	0.753**	0.905**			
Weight gain	0.867**	0.730**	0.913**	0.839**	0.965**	0.914**	0.986**	0.944**		
Adjusted weight gain	0.832**	0.638*	0.761**	0.915**	0.937**	0.752**	0.905**	1.000**	0.944**	
PER	0.775**	0.598*	0.923**	0.646*	0.841**	0.968**	0.962**	0.819**	0.934**	0.819**

[a]CS = chemical score; EAAI = essential amino acid index; NPR = net protein ratio; PER = protein efficiency ratio.

could be used to predict the protein quality of breads fortified with high levels of concentrated plant proteins or lysine. Since the PER of diets fed for 10 and 28 days were highly significantly correlated, a 10-day rat feeding study could also be used in place of the more time-consuming 28-day trial.

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