

## Nutritional evaluation of table bread fortified with defatted soybean and sesame meals

Sergio O. Serna Saldivar, Jesús R. Abril-Dominguez, Guadalupe López-Ahumada, Refugio Ortega-Ramírez

Universidad de Sonora, Hermosillo, Sonora, México

**SUMMARY.** The nutritional value of table bread fortified with 8% defatted soybean meal (DSBM), 12% DSBM and a mixture of 8% DSBM/4% defatted sesame meal (DSM) was assessed with *in vivo* and *in vitro* tests. Fortification with DSBM and DSM decreased protein digestibilities ( $P<0.05$ ) but improved essential amino acid scores (EAA) and overall nutritional value of the breads. Fortified breads contained twice as much lysine, and consequently a better protein efficiency ratio (PER) than the control bread. The PER of the 8% DSBM/4% DSM bread was similar ( $P>0.05$ ) to the 12% DSBM bread. The 8% DSBM fortified bread showed lower PER, amino acid and protein contents than breads fortified with 12% DSBM. *In vitro* procedures utilized to predict protein digestibilities and PER's provided a close estimation of *in vivo* results obtained from growing rats.

**Key words:** Fortified bread, soybean, sesame, protein quality.

**RESUMEN. Evaluación nutrimental de pan de mesa fortificado con harina desgrasada de soya y ajonjolí.** El valor nutrimental de pan de mesa fortificado con 8% de harina de soya desgrasada (DSBM), 12% de DSBM y una mezcla de 8% DSBM/4% harina de ajonjolí desgrasada (DSM) fue determinado mediante pruebas *in vivo* e *in vitro*. La fortificación con DSBM y DSM decremó significativamente la digestibilidad de la proteína ( $P<0.05$ ) pero mejoró los valores de aminoácidos esenciales y el valor proteico nutrimental de los panes. Los panes fortificados contuvieron el doble de lisina y consecuentemente una mejor razón de eficiencia proteica (PER) que los panes testigos. El PER del pan con 8% DSBM/4% DSM fue similar ( $P>0.05$ ) al del pan con 12% DSBM. El pan fortificado con 8% DSBM mostró un menor contenido de proteína, aminoácidos y PER que el pan fortificado con 12% DSBM. Los procedimientos *in vitro* utilizados para predecir las digestibilidades de la proteína y PER's estimaron adecuadamente a los resultados obtenidos *in vivo* con ratas de laboratorio.

**Palabras clave:** Panes fortificados, soya, ajonjolí, calidad proteica.

### INTRODUCTION

In underdeveloped countries, fortification of cereal-based foods with low cost protein sources can upgrade the nutritional status of the population, especially post weaned children. In Mexico, tortillas and bread are the main staple foods; therefore, these products are the best vehicles to improve the average diet. Recently, the large tortilla processors implemented a soybean tortilla fortification program that is gaining popularity. A similar program can be established using bread as a fortification vehicle. Previous reports have indicated the advantage of using oilseed protein meals to fortify cereal-based foods (1-8). The improved nutritional value is due to the superior essential amino acid profile, which in turn translates into increased weight gains. Generally sulfur-containing amino acids are the most limiting amino acids in oilseed flours. Among the different oilseed meals, sesame meal is reported to have adequate amounts of sulfur-containing amino acids. Therefore, when combined with soybean, sesame meal can improve even more the nutritional value of cereal based foods (9,10). Bread is one of the most difficult products to fortify, because of the detrimental effects of foreign (non-wheat)

proteins on loaf volume and color (11). However, recent reports have indicated that the use of dough conditioners can partially overcome the deleterious effects of protein fortification on bread volume (12-15). In a previous study, Serna-Saldivar et al. (12) produced and evaluated quality properties of breads fortified with defatted soybean and sesame meals and supplemented with sodium stearoyl-2-lactylate. Addition of the emulsifier improved properties (volume, crumb texture, density) of fortified breads. Fast, accurate and low cost *in vitro* techniques for nutritional value determinations are commonly used in food quality research. Hsu et al (16) developed an *in vitro* technique to predict protein digestibility using a multienzyme system, which correlated well with *in vivo* data. Methodologies have been developed to predict PER's using *in vitro* protein digestibility data and amino acid patterns of foods (17). The most common methods currently used are C'PER (calculated protein efficiency ratio) and DC'PER (discriminated calculated protein efficiency ratio) (18). The objectives of this study were to 1) investigate the nutritional value of breads fortified with defatted soybean and sesame meals using both *in vivo* and *in vitro* techniques and 2) to compare the *in vivo* and *in vitro* procedures.

## METHODOLOGY

### Bread preparation

A commercial 75% extracted hard wheat flour was used to prepare table bread, along with commercial defatted soybean meal (DSBM) and defatted sesame meal (DSM). Oilseed meals were ground in a pin mill to pass through a 100 US mesh. The bread formula was based on 300 g flour, 22.5 g sugar, 4.5 g salt, 5.1 g dry yeast and 9 g shortening. Control and fortified breads were produced according to the methods and procedures suggested by Serna-Saldivar et al. (12). Fortified flours contained 8% DSBM, 12% DSBM and a mixture of 8% DSBM/4% DSM. were utilized to produce breads.

### Sample preparation

Resulting breads were dried at 50°C for 24 hr in a drying tunnel with an air velocity of 290 m/min and then ground in a hammer mill to pass through a 2 mm screen. The ground dry bread was placed on the laboratory counter for 48 hr to equilibrate with the atmosphere and then bagged and stored in a freezer for further use.

### Analytical procedures

Proximate analyses of breads and casein were performed following AOAC (18) procedures. Amino acid composition was determined after a 6N HCl hydrolysis, and quantified by ion exchange chromatography using a modified Beckman 120 C Amino Acid Analyzer (19). Tryptophan analyses were quantified by a microbiological technique suggested by Ford (20,21). Essential amino acid compositions were compared and expressed as a percentage of the FAO/WHO/UNU (22) requirement. Rat feces were dried at 50°C, moisture equilibrated and analyzed for dry matter and protein following AOAC (18) procedures.

### Rat feeding trial

**Diet formulation.** Experimental diets were formulated following AOAC (18) procedures (Table 1). Diets were formulated to contain 1.6% nitrogen, 8% fat, 1% fiber, 5% supplemented vitamins and 1% supplemented minerals. Fiber and fat contents were adjusted by the use of cellulose and vegetable oil, starch was added to adjust the diet to 100%. The synthetic control diet contained ANRC casein as the sole source of protein. Five percent sucrose was added to improve palatability and enhance feed intake (Table 1).

**Feeding trial.** Thirty Sprage-Dawley weanling rats with an average initial weight of 53 g were used to perform the PER and in vivo protein digestibility studies. Rats had a two-day cage adaptation period in which they were fed laboratory chow diet. Rats were blocked by initial weight and sex and distributed at random in six blocks. Each block consisted of 5 experimental units in which all treatments were distributed at random. Therefore, each treatment consisted of six repetitions

composed of three males and three females. Rats were housed individually in wire cages under controlled room temperature (25°C) and light conditions (12 hr light/12 hr dark). The experiment consisted of four periods lasting one week each. Rats were fed daily and weighed at the end of each period. The amount of food consumed during each period was recorded after collecting food leftovers. During the second and third period, feces were collected daily and stored in a freezer. Later on, feces were cleaned using a 40 US mesh sieve to remove food particles and other foreign material and then dried in an air convection set at 50°C. The dry fecal material was air equilibrated, weighed, ground and stored in plastic bags. Apparent protein digestibilities were calculated by subtracting the amount of protein excreted in feces from the amount of protein intake and then dividing by the protein intake and expressed as a percentage. PER's were calculated by dividing weight gain by protein consumed. PER's were calculated for each period, and at the end, the average of the four periods was obtained. The PER's of the experimental diets were corrected based on the PER of the control-casein diet using the following equation:  $PER_c = (PER \text{ Test Protein}) (2.5) / PER \text{ Control-Casein}$ .

TABLE 1  
Composition and chemical analyses of diets used during rat bioassays<sup>1</sup>

Ingredients (%)	Wheat Bread	Wheat + 8% DSBM Bread	Wheat +12% DSBM Bread	Wheat + 8% DSBM + 4% DSM Bread	Control Casein
Ground bread	81.21	67.23	61.53	60.15	—
Starch	6.75	20.43	25.65	26.83	68.17
Casein <sup>2</sup>	—	—	—	—	11.83
Sucrose	—	—	—	—	5.00
Corn oil	5.28	5.54	6.01	6.20	8.00
Cellulose	0.76	0.80	0.81	0.82	1.00
Vitamins <sup>3</sup>	5.00	5.00	5.00	5.00	5.00
Minerals <sup>4</sup>	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Chemical Composition <sup>5</sup>					
Moisture	6.78	7.41	7.37	8.60	5.80
Protein <sup>6</sup>	10.38	10.05	10.48	10.14	10.44
Crude fiber	0.95	1.07	1.04	1.10	1.05
Ether extract	8.12	7.94	9.08	8.01	7.86
Ash	2.81	3.37	3.47	3.62	1.19

1 DSMB = deffated soybean meal; DSM = deffated sesame meal

2 ANRC standard casein

3 AIN vitamin premix

4 AIN mineral premix

5 Expressed on dry matter basis

6 Nitrogen x 6.25

**Statistical analyses.** Both protein digestibility and growth performance data results were statistically analyzed using Analysis of Variance Procedures with a randomized block

design (23). Treatment means were compared using Duncan's tests at a level of significance of 5%.

#### *In vitro* assays

**Protein digestibility.** The *in vitro* protein digestibility was determined according to the procedures suggested by Hsu et al. (16). The methodology was based on the incubation of the test sample with three proteolytic enzymes (trypsin, chymotrypsin and peptidase) in an aqueous solution adjusted to pH 8. The rate of protein digestion was proportional to the drop in pH after 10 min incubation at 37°C. The equation used to predict apparent protein digestibility was:  $210.464 - 18.103(x)$ ; where x was the pH after 10 min incubation.

**Calculated protein efficiency ratio (C'PER).** C'PER's were calculated using the procedure suggested by Satterlee et al. (17) and summarized by AOAC (18). The procedure was based on the utilization of the *in vitro* protein digestibility and essential amino acid compositions of the different bread systems and casein.

## RESULTS AND DISCUSSION

### Chemical composition

Fortified breads had a higher ash and fiber content than the control bread since oilseed meals provide more of these than the wheat flour alone. Fat content was similar among the different bread systems. The nitrogen free extract was highest for the control bread followed by the 8% DSBM bread and 12% bread mixtures. Contrarily, fortified breads had a higher protein and lysine content than the control bread (Table 2). Bread fortified with 8% DSBM, 12% DSBM and 8% DSBM/4% DSM had 24%, 35% and 38% more protein, respectively, than wheat bread. Tsen et al. (5) reported that bread fortified with 12% soybean meal had 35% more protein and twice as much lysine than wheat bread, which agrees with results obtained in this study. The higher protein content was reflected in higher amounts of essential amino acids. Lysine content increased from 0.3% in the control bread to 0.5% and 0.6% in the breads fortified with 8% and 12% DSBM, respectively. The higher lysine content obtained in fortified breads considerably improved their essential amino acid scores (Table 3). For all types of breads, lysine was the limiting amino acid. The addition of 8% DSBM improved the essential amino acid score from 42 to 54%. Breads fortified with 12% DSBM had higher scores than the bread fortified with 8% DSBM or 8% DSBM/4% DSM (Table 3). At higher levels of fortification, threonine could become either the first or second limiting amino acid and tryptophan the second or third. Therefore, in order to further improve the protein quality of fortified breads, it is recommended to use products that already have a high amount of lysine, threonine and tryptophan or to supplement the bread with the corresponding synthetic amino acids.

TABLE 2  
Chemical composition of wheat bread and breads fortified with defatted soybean and sesame meals<sup>1,2</sup>

(%)	Wheat Bread	Wheat + 8% DSBM Bread	Wheat + 12% DSBM Bread	Wheat + 8% DSBM Bread DSM 4% DSM Bread
Protein <sup>3</sup>	12.75	15.82	17.29	17.61
Ether extract	3.46	3.33	3.44	3.17
Crude fiber	0.26	0.37	0.46	0.42
Ash	1.96	2.44	2.57	2.73
NFE <sup>4</sup>	81.57	78.04	76.24	76.07
Lysine	0.31	0.50	0.61	0.55

1 Values are means of three observations. DSMB = defatted soybean meal; DSM = defatted sesame meal

2 All values are expressed on dry matter basis

3 Nitrogen x 6.25

4 Nitrogen free extract

TABLE 3  
Essential amino acid scores of wheat bread and breads fortified with defatted soybean and sesame meals

Amino Acid FAO/WHO Std. <sup>1</sup> (g AA/16 g N)	Wheat Bread	Wheat + 8% DSBM Bread	Wheat + 12% DSBM Bread	Wheat + 8% DSBM + 4% DSM Bread
% FAO / WHO/UNU Requirement <sup>1</sup>				
Lys	5.8	41.8*	54.0*	61.1*
Met+Cys <sup>2</sup>	2.5	79.3	89.7	92.6
Thr	3.4	60.2**	78.6**	78.2
Phe+Tyr	6.3	113.0	116.6	116.9
Trp <sup>3</sup>	1.1	101.4	82.3	77.6**
Leu	6.6	79.5	87.6	93.5
Ile	2.8	81.6	122.1	129.2
Val	3.5	71.0	107.2	110.1

1 Values are percentages of the FAO/WHO/UNU (22) suggested pattern of amino acid requirements for two-year old children. \* = Most limiting amino acid; \*\* = second limiting amino acid.

2 Cysteine was obtained from table values.

3 Calculated by a microbiological assay according to Ford (20,21).

### *In vivo* rat assays

**Protein digestibilities.** *In vivo* and *in vitro* apparent protein digestibilities were similar for all fortified breads. Wheat bread had a higher protein digestibility ( $P < 0.05$ ) than fortified breads containing 12% DSBM or 8% DSBM/4% DSM (Table 4). An analysis of soybean's trypsin inhibitor activity showed that the inhibitor was inactivated by the desolventizing-toasting process. As expected, casein had the highest value of protein digestibility which agrees with previous data obtained by Hsu et al. (16). The *in vitro* technique utilized to predict protein digestibility produced results that were similar to the corresponding *in vivo* values although the *in vitro* technique slightly underestimated the values (Table 4).

TABLE 4  
Protein digestibilities and protein efficiency ratios of wheat bread and breads fortified with defatted soybean and sesame meals<sup>1</sup>

	Wheat Bread	Wheat + 8% DSBM Bread	Wheat +12% DSBM Bread	Wheat +8% DSBM + 4% DSM Bread	Control Casein
<b>Protein Digestibility</b>					
<i>In vivo</i> %	88.96 c	87.74 bc	86.38 ab	85.82 a	95.58 d
<i>In vitro</i> <sup>2</sup> %	86.05 c	84.87 b	84.47 b	83.17 a	94.11 d
<b>PER study</b>					
Food intake, g/day	7.95 a	8.02 a	9.52 bc	9.06 b	10.41 c
Protein intake, g/day	0.83 a	0.83 a	0.96 b	0.95 b	1.03 c
Weight gain, g/day	0.81 a	1.28 b	1.60 c	1.53 c	2.41 d
<i>In vivo</i> PER <sup>3</sup>	1.05 a	1.64 b	1.78 c	1.72 bc	2.50 d
<i>In vitro</i> PER <sup>4</sup>	0.87	1.22	1.35	1.31	2.50

- 1 DSMB = defatted soybean meal; DSM = defatted sesame meal; PER = protein efficiency ratio. Values of *in vivo* data are the average of six observations. Means with different letters in the same row are statistically different (Duncan's test  $P < 0.05$ ).
- 2 *In vitro* protein digestibility values are the average of three replicates. Estimated according to Hsu et al. (16).
- 3 Corrected PER. Correction factor 1.068.
- 4 Calculated according to Satterlee et al. (17).

**Protein efficiency ratios (PER).** The improved essential amino acid patterns or scores observed in all fortified breads (Table 3) clearly improved rat performance (Table 4). As expected, rats fed the 100% wheat bread had the lowest average daily gains, feed intake and PER'S (Table 4). This occurred despite its higher protein digestibility. Due to the higher feed intake and better amino acid composition, rats fed the 12% fortified mixtures gained twice as much weight as rats fed wheat bread alone. Consequently, rats fed wheat bread had lower PER's (1.05) than rats fed fortified breads (1.64 to 1.80). Marnett et al. (24) reported PER's of 1.0 and 1.95 for wheat bread and bread fortified with 12% DSBM, respectively. The PER of wheat bread was higher than the one for wheat flour alone (0.7) because the bread contains yeast. Yeast protein has been known to have a good biological value and adequate amounts of lysine. The PER at the 8% DSBM/4% DSM mixture was similar ( $P > 0.05$ ) than the 12% DSBM bread although DSM did not contain as much lysine as DSBM (Table 3). Brito and Nuñez (10) mentioned that sesame soy blends may be as useful as soy alone for addition to corn based foods. Wheat bread and the average of the breads had 42% and 70% of the PER value found for the casein based diet. Fortified bread diets had more added starch than the bread diet in order to balance the protein content to 10% or produce isonitrogenous diets (Table 1). Thus, even better nutritional value can be expected in these products because their protein content will actually be higher in a practical scenario. PER's obtained by the use of *in vitro* protein digestibilities and amino acid underestimated the values obtained from rats. However, the absolute differences between treatments were similar, indicating that the CIPER technique predicted the same

differences observed in the rat bioassay. Therefore, it is recommended to use the *in vitro* techniques as fast, accurate indicators of protein digestibilities and PER's.

## CONCLUSIONS

Fortified breads can upgrade the nutritional status of many people who consume bread daily due to their higher protein content and better protein quality or essential amino acid composition. Growing rats fed fortified breads with 12% DSBM gained twice as much weight than their counterparts fed wheat bread. The dramatic improvement in the nutritional value of the fortified products is easily worth the small increment in their production cost. In a previous study (12) it was proved that fortified breads with good processing properties and overall characteristics can be produced if sodium stearoyl 2 lactylate is utilized in the formulation. Furthermore, the baking operation per se does not require the extensive modification of existing equipment in producing establishments. With a fortification program such as the one proposed in this study, oilseed meals can be incorporated into much-needed human nutritional programs in developing countries, instead of being channeled into animal feeding.

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