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Amino acid composition, score and *in vitro* protein digestibility of foods commonly consumed in Northwest Mexico

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Abstract

A better knowledge of the amino acid composition of foods commonly consumed in different regions is essential to calculate their scores and, therefore, to predict their protein quality. This paper presents the amino acid composition, amino acid score and *in vitro* protein digestibility of fifteen foods that are commonly consumed in Northwest Mexico. The foods were prepared by the traditional methods and were analyzed by reverse-phase HPLC. The chemical score for each food was determined using the recommendations for children of 1-2 years of age, and the digestibility was evaluated using a multienzyme technique. Lysine was the limiting amino acid in cereal-based products (scores 15 to 54), and methionine and cysteine were limiting in legume products (scores 41 to 47), boiled beef (score = 75) and hamburger (score = 82). The method of preparation had an effect on the content of certain amino acids, some of them increased and others decreased their content. Meat products and regional cheese provided a high amino acid score (scores 67 to 91) and digestibility (80.7 to 87.8%). Bologna, a processed meat product, had a lower digestibility (75.4%). Data on the amino acid composition of foods commonly consumed in Mexico can be used to provide valuable information on food analysis and protein quality, and to contribute to nutrition and health research and health programs.

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Key words: *Amino acids. Food analysis. Mexican foods. Protein quality. In vitro protein digestibility.*

COMPOSICIÓN DE AMINOÁCIDOS, CALIFICACIÓN QUÍMICA Y DIGESTIBILIDAD PROTEÍCA *IN VITRO* DE ALIMENTOS CONSUMIDOS FRECUENTEMENTE EN EL NOROESTE DE MÉXICO

Resumen

El conocimiento adecuado de la composición de aminoácidos en los alimentos consumidos frecuentemente en las diferentes regiones, es esencial para calcular la calificación de aminoácidos y predecir la calidad proteica de dichos alimentos. Este manuscrito presenta la composición y calificación de aminoácidos, así como la digestibilidad de proteína *in vitro* de quince alimentos consumidos frecuentemente en el noroeste de México. Los alimentos se prepararon por los métodos tradicionales y se analizaron por cromatografía de líquidos de alta resolución en fase reversa. La calificación química para cada alimento se determinó utilizando las recomendaciones para niños de 1-2 años de edad, y la digestibilidad se evaluó utilizando un método multienzimático. La lisina fue el aminoácido limitante en los productos a base de cereales (calificación 15 a 54), y metionina y cisteína fueron limitantes en las leguminosas (calificación 41 a 47), carne cocida (calificación 75) y hamburguesa (calificación 82). El método de preparación tuvo un efecto en el contenido de ciertos aminoácidos, algunos de éstos aumentaron y otros disminuyeron su contenido. Los productos cárnicos y el queso regional tuvieron una calificación de aminoácidos (calificación 67 a 91) y digestibilidad (80,7 a 87,8%) elevadas. La bolonia, que es un producto cárnico procesado, tuvo la digestibilidad más baja (75,4%). Los datos de la composición de aminoácidos en alimentos consumidos frecuentemente en México pueden utilizarse para el análisis de alimentos y evaluación de su calidad proteica, y contribuir a la investigación en nutrición y salud.

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Introduction

Information on the nutritional content of foods brings the knowledge to bear on the goals of food analysis and food science, may contribute to the establishment of policies on food production and storage, the evaluation of the nutritional status, the formulation of therapeutic diets and investigations into the relationships between diet, health and disease.^{1,2} The analysis of nutrients in foods is incorporated into the food composition databases, which are essential tools for researchers, dietitians and other nutrition professionals but are never complete due to the constant introduction of new foods and food components that are important to human health.^{2,3}

Food analysis is expensive, is often complicated and requires substantial human and material resources. Due to financial constraints, data are often borrowed from the tables of developed countries and incorporated into the tables of developing countries. Such a practice, however, can be questioned considering that foods vary in their composition. Thus, analytical data obtained with food samples of one country may not be relevant for another country.⁴ A wealth of information on food composition has been gathered worldwide over decades of analytical efforts and research. Nonetheless, there are no data available on native foods or on a variety of regular foods consumed in different regions and countries.⁵

Food protein quality is a key nutritional issue because it varies from one food protein to another, and it is important to consider in dietary protein requirements. The main determinant of food protein quality is the content and availability of essential amino acids. These nutrients have been shown to play an important role in the growth, reproduction and maintenance of the human body.^{6,7} Amino acid content in foods can be used to calculate the amino acid score, which provides a way to predict how efficiently protein will meet a person's amino acid needs. This concept assumes that tissue protein synthesis is limited unless all required amino acids are available at the same time and in appropriate amounts at the site of tissue protein synthesis. The method is based on comparison of the concentration of the first limiting essential amino acid in the test protein with the concentration of that amino acid in a reference (scoring) pattern. The reference amino acid scoring pattern expresses the amino acids requirements in milligrams/gram of dietary protein or as percentages in an "ideal" protein.⁶

The FAO/WHO/UNU^{6,8,9} has recommended that the composition of amino acids in local and regional diets be taken into consideration to determine the chemical composition of diets and to be able to estimate the protein quality of the diets. The Latin American Network of Food Data System (LATINFOODS) published a report highlighting the need to improve the strategies for selecting foods for analysis and inclusion in the databases.¹⁰ The foods consumed in México are different in the North and South regions. The consump-

tion of foods in the Northwest region is based on energy dense foods (fried meat and beans, whole milk, wheat and corn tortillas), with a poor consumption of fruits or vegetables. The foods consumed in the south region include fruits and vegetables, lower meat or fat intakes, and corn tortilla.¹¹

The analysis of the amino acid content in Mexican foods began approximately sixty years ago,^{12,13} but little has been published subsequently on the subject.⁵ Given the lack of information on the amino acid content of foods in Mexico, the objective of this study was to analyze the essential amino acid profile of fifteen different foods commonly consumed in Northwest Mexico in order to incorporate the data into the food composition tables used in the region. In addition, the amino acid score and *in vitro* protein digestibility were determined to evaluate the food protein quality.

Materials and methods

Selection and preparation of the samples

Fifteen different foods commonly consumed in the Northwest region of Mexico were selected based on their frequency of consumption.¹⁴ The foods were obtained from local supermarkets and were prepared according to traditional recipes. The foods included items of animal origin, cereals and legumes. After preparation, the dishes were homogenized in a Waring blender (VWR), dried at 50°C for 12 hours, defatted using a petroleum ether extraction for 16 h, and then ground in a Cyclotec (Tecator 1092) mill with an 80 mesh. The cheese used in the study was dried under vacuum at 60°C for 5 hours.¹⁵ The total nitrogen in the samples was determined by the Kjeldhal method.¹⁶ More information about food sampling and preparation, and macronutrient analysis of foods is provided by Grijalva-Haro and others.¹⁷

Amino acid hydrolysis

Duplicate samples of each food (0.1 g) were hydrolyzed using 6 N HCL (Merck) for 18 hours in an autoclave at 15 psi.¹⁸ Sodium thioglycolate (Sigma Chemical Co.) was added to the sample to prevent oxidation.¹⁹ Cysteine was determined by the performic acid oxidation method of Moore²⁰ with the following modifications: 20 mL of cold performic acid, freshly prepared, was added to 0.1 g of the sample; after incubating overnight at 0-5°C, 3 mL of cold 47% HBr (Merck) was added along with a few drops of 1-octanol as an antifoaming agent; the acid was evaporated at 40°C using a rotary evaporator (Buchi, Brinkman); the amino acids were then hydrolyzed according to the above described method. Tryptophan was determined colorimetrically according to the method of Vollmer,²¹ using 4 N sodium hydroxide for 14-16 hours at 110°C.

Amino acid analysis by HPLC

The technique used for the analysis of amino acids was standardized in our institution by Vazquez et al.²² and used in previous studies. A liquid chromatograph (Varian 5000, Varian Associates) was used for the determination of amino acids using a fluorescence detector (Varian Fluorichrom) connected to a Model Vista 401 data collection system (Varian). The sensitivity was set at a 1 μ A full scale. The sample was introduced using a Rheodyne Model 7120 valve equipped with a 10 μ l loop. Amino acid separations were performed on a Microsorb Shortones column (4.6 x 100 mm) packed with 3 μ m reversed-phase C-18 octadecyl dimethylsilane particles (Rainin Instrument Co.) and connected to a Microsorb (4.5 x 30 mm) precolumn packed with the same material (Rainin Instrument Co.). Pure amino acid standards were obtained from PIERCE Chemical Company.

The dried hydrolyzates were diluted in sodium citrate buffer (pH 2.2) (Pierce Chemical Co.) and filtered through a glass microfiber filter (Whatman 934-AH). Subsequently, 100 ml of the hydrolyzate were mixed with 40 ml of α -aminobutyric acid as an internal standard and brought to 1 mL with sodium citrate buffer. For the sample derivatization, an orthophthalaldehyde (OPA) solution was prepared as follows: to 10 mg OPA dissolved in 250 μ L of methanol, 37.5 μ L 30% Brij 35, 25 μ L of 2-mercaptoethanol and 3 mL of 0.5 M potassium borate buffer pH = 10.4 were added. This solution was diluted to 10 mL with borate buffer and mixed well. It was then stored under refrigeration in the dark and allowed to stand for 24 h before use. The preparation was made one day prior to use. Immediately prior to loading the injection loop, a combination of 0.5 mL OPA solution and 0.5 mL of sample containing internal standard was prepared in a small tube and mixed.

The sample was injected in triplicate and was eluted at 1.5 mL/min with a mixture of 0.1 M sodium acetate buffer (pH = 7.2):methanol:THF 900:90:10 (v/v/v) as solvent A and methanol as solvent B. The following gradient was used: 20% B (at 0 min), 30% B (5-8 min), 50% B (10-15 min), 80% B (18-22 min) and 20% B (at 25 min). The amino acids were completely eluted at 22 minutes, and the column was equilibrated for 10 minutes. The amino acids were monitored using a Varian 430020-02 Fluorichrom fluorescence detector at excitation and emission wavelengths of 350 and 455 nm, respectively. The method was sensitive with detection limits of approximately 50 femtomoles.²²

Amino acid score

The amino acid score was calculated using the ratio of a gram of the limiting amino acid in the food to the same amount of the corresponding amino acid in the reference diet multiplied by 100. The scoring patterns

suggested by the FAO/WHO/UNU⁶ for children of 1-2 years of age were used for this purpose.

In vitro protein digestibility

The *in vitro* protein digestibility of the foods was measured using a multienzyme technique.²³ The enzymes used were trypsin, α -quimotrypsin, and peptidase (SIGMA Chemical Co.). The digestibility of each sample was calculated using the following regression equation: $Y = 234.84 - 22.56(X)$, where Y = % protein digestibility and X = pH of the protein suspension after 20 min of digestion with the enzyme solution.

Calculations and statistical analysis

The average amino acid contents were identified on the basis of the derivative retention times and were quantified using the internal standard method. The relative response factors (RRF) were determined by analyzing standards, and they were used to calculate the concentrations of amino acids in the samples. Each duplicate of hydrolysis was injected in triplicate, so that six amino acid replicates of each sample were obtained; the mean, the standard deviation and the coefficients of variation were calculated. The coefficients of variation of the replicates were less than 10% in all samples. The *in vitro* protein digestibility analyses were conducted using three replicates per sample; the coefficients of variation between replicates were less than 12%.

Results and discussion

Table I shows the list of ingredients and the methods of preparation for the fifteen foods analyzed in this study. We analyzed six cereal-based foods, three preparations of beans, four preparations of meat, regional cheese and bologna. The use of different ingredients of animal protein such as eggs in the bread products or milk in the tortillas is reflected in the analytical data. The amino acid composition of the foods in g/16 g N is presented in table II. It is difficult to compare our values to those reported in the literature due to the large differences in the composition of the Mexican foods in relation to the products from the United States or Europe. For simplicity, the foods were grouped into three categories: cereal products, pinto beans and meat and cheese products.

Cereal products

The Mexican diet consists mainly of a mixture of cereals (corn and wheat) and legumes. The eating habits of most of the Northwestern population include

Table I
The commonly consumed foods of Northwest Mexico analyzed in this study

<i>Food</i>	<i>Ingredients</i>
Wheat flour tortillas, low fat (tortilla de agua)	Wheat flour, water, salt, vegetable fat (hydrogenated vegetable oil)
Wheat flour tortillas, high fat (tortilla de manteca)	Wheat flour, salt, milk, vegetable fat (hydrogenated vegetable oil)
Flour tortillas, medium fat (tortilla de harina)	Wheat flour, water, salt, vegetable fat (hydrogenated vegetable oil)
Corn tortillas, lime-treated (tortilla de maiz nixtamalizada)	Corn flour, water, lime
White bread (virginia)	Wheat flour, sugar, malt, salt, and yeast
Breakfast roll (conchita)	Wheat flour, sugar, egg, margarine
Cooked beans, whole	Pinto (<i>Phaseolus vulgaris</i>) beans and water (1:5), salt
Refried beans	Cooked pinto beans mashed with bean broth and fried in vegetable oil
Refried beans without broth	Cooked pinto beans mashed and fried in hydrogenated vegetable oil
Cooked beef	Beef boiled in water and salt
Charcoal-broiled beef, (chuck steak)	Beef, salt, grilled over charcoal
Steak, fried (home-prepared)	Beef, salt (1%), fried in vegetable oil
Hamburger, fried (home-prepared)	Ground beef, salt, and vegetable oil
Bologna	Beef, pork, chicken, corn starch, salt, spices and soy protein
Regional white cheese	Made from milk curd, contains residual whey and salt

Table II
Essential amino acid profile in fifteen Mexican foods

<i>Food</i>	<i>Essential amino acids (g/16 g nitrogen)</i>									
	<i>Lys</i>	<i>Met</i>	<i>Cys</i>	<i>Ile</i>	<i>Leu</i>	<i>Phe</i>	<i>Tyr</i>	<i>Val</i>	<i>Thr</i>	<i>Trp</i>
Flour tortillas with water	1.01	1.14	0.70	2.47	4.70	1.88	1.41	2.51	1.86	0.92
Flour tortillas with extra oil	1.36	1.16	0.86	1.88	4.49	2.18	2.24	2.13	1.80	1.13
Flour tortillas, commercial	1.56	1.30	0.72	2.56	5.33	1.60	1.47	2.48	2.36	0.44
Corn tortillas	2.79	1.79	0.93	2.47	10.52	3.02	5.15	3.24	2.62	0.62
White bread (virginia)	0.77	0.83	0.67	2.25	4.44	0.78	0.46	2.47	2.22	1.07
Breakfast roll (conchita)	2.05	2.02	0.31	2.75	6.48	3.34	5.22	3.20	3.28	1.06
Cooked beans, whole	4.59	0.75	0.31	2.69	5.25	1.87	1.40	2.57	2.57	0.83
Refried beans	5.98	0.95	0.27	2.83	5.93	3.33	2.69	3.24	2.93	0.92
Refried beans without liquid	6.47	1.06	0.17	2.46	6.07	2.53	3.20	2.60	2.11	0.98
Boiled beef	6.77	1.71	0.24	2.98	5.40	1.98	2.17	3.17	2.75	1.33
Charcoal-broiled beef	8.62	2.05	0.32	3.27	6.20	1.47	1.82	3.21	3.42	1.08
Steak, fried	7.49	1.95	0.27	3.17	6.13	1.56	1.54	3.27	3.35	1.35
Hamburger, fried	6.02	1.74	0.40	3.28	5.83	2.13	2.20	3.48	3.27	1.21
Bologna	4.47	2.64	0.51	3.75	8.17	2.63	7.99	4.36	4.77	1.30
Mexican white cheese	6.14	2.36	0.16	3.48	7.33	3.12	3.48	4.23	2.45	1.29

Lys: Lysine; Met: Methionine; Cys: Cysteine; Ile: Isoleucine; Leu: Leucine; Phe: Phenylalanine; Tyr: Tyrosine; Val: Valine; Thr: Threonine; Trp: Tryptophan.

wheat and beans as central elements.^{14,24,25} Therefore, three different preparations of wheat tortillas and two preparations of wheat bread were analyzed in this study. As expected, the lysine content was low in all of the cereal products, with white bread being the lowest. In the breakfast roll that contained eggs, a slightly higher value of lysine was observed. A decrease in the availability of amino acids containing free amino groups caused by the process of baking due to the reaction of the amino groups with sugars (Maillard reaction) has been reported in bakery products.^{26,27}

The results for tryptophan, isoleucine, leucine and lysine in wheat products analyzed in our study were

comparable to those reported for wheat bread and flour in central Mexico.⁵ Corn is still an important food in the Mexican diet, accounting for 50% of the energy intake and 40% of the protein intake of the average diet. Therefore, corn tortilla was analyzed in this study and showed a high content of leucine (10.5 g/16 g N), which is similar to that obtained for nixtamal flour (10.7 g/16 g N) and opaque-2 corn (10.1 g/16 g N).⁵ The lysine content in corn tortilla was similar to the obtained in corn tortilla from the Central region of Mexico (2.25 g/16 g N).⁵ This amino acid was higher in corn tortilla than in the wheat products, because corn may be a better source of lysine than wheat, i.e. in the study by Morales

Table III
Amino acid score of fifteen foods consumed in Northwest Mexico based on the essential amino acid content and the pattern for children of 1-2 years of age

<i>Food</i>	<i>Limiting amino acid</i>	<i>Amino acid score using the pattern for children 1-2 years*</i>
Flour tortillas with water	Lysine	19
Flour tortillas with extra oil	Lysine	26
Flour tortillas, commercial	Lysine	30
Corn tortillas	Lysine	54
White bread (virginia)	Lysine	15
Breakfast roll (conchita)	Lysine	39
Cooked beans, whole	Methionine + Cysteine	41
Refried beans	Methionine + Cysteine	47
Refried beans without liquid	Methionine + Cysteine	47
Boiled beef	Methionine + Cysteine	75
Charcoal-broiled beef (chuck steak)	Phenylalanine + Tyrosine	72
Steak, fried	Phenylalanine + Tyrosine	67
Hamburger, fried	Methionine + Cysteine	82
Bologna	Lysine	86
Mexican white cheese	Threonine	91
FAO/WHO/UNU 2007		100

*FAO/WHO/UNU (2007) reference pattern suggested for children of 1-2 years of age in g/16 g N: cysteine + methionine, 2.6; valine, 4.2; isoleucine, 3.1; leucine, 6.3; tyrosine + phenylalanine, 4.6; lysine, 5.2; threonine, 2.7; tryptophan, 0.74.

de Leon and others,⁵ lysine values of nixtamal flours ranged from 2.41 to 4.38 g/100 g N, while the lysine content in wheat flour was 2.68 g/16 g N.

Common beans

Common beans (*Phaseolus vulgaris*) are the main legumes of the Northwest diet and are combined with corn or wheat. For hundreds of years, they have formed the basic complementary cereal-legume system in the Mexican diet. Beans are prepared in several ways, the most common being “beans in their own broth” or beans fried in oil, animal or vegetable fat. Although beans are reported to be relatively high in protein,²⁸ they are low in sulfur amino acids. The methionine content in the beans analyzed in this study (0.75-1.06 g/16 g N) was lower than that published for cooked black beans (1.36 g/16 g N) and light brown beans (1.24 g/16 g N).⁵ According to the results, the method of preparation may have some effect on the content of certain amino acids in beans; as an example, the values for lysine, methionine, leucine and tyrosine were higher for the refried beans without liquid than for the cooked and refried beans with broth. The cooked beans had the lowest values of almost all the amino acids (except cysteine, isoleucine and threonine). This can be explained because the cooked beans have more water and less quantity of beans compared to the refried beans (with or without liquid). Therefore, the protein and hence the amino acids, are less concentrated in the cooked beans.

Meat and cheese products

The eating habits in the Northwest region of Mexico include significant amounts of animal products, mainly beef, meat products such as sausages, bologna and ham, and cheese. The lysine content in meat products and cheese was higher than in cereal products or beans. The highest value of lysine was found in the charcoal-broiled beef and the lowest value was found in the hamburger (ground beef). The low lysine content for bologna reveals the lower meat quality used in the formulation of this product, which uses primarily viscera and other parts of the animal that contain excessive connective tissue;²⁹ this trend is more evident by observing the value of glycine in bologna (7.02 g/16 g N, data not shown in table), which was higher than that obtained in the other foods (1.72 to 6.94 g/16 g N, data not shown). In general, the amino acid content that we found in the meat products was different from those published for bovine dried muscle⁵ because the values for some of the amino acids, such as lysine, isoleucine and leucine, were higher than those reported (3.85, 2.34 and 4.89 g/16 g N, respectively) for these foods. The amino acid content in fresh white cheese was similar to that published for “asadero cheese” in central Mexico.⁵

Amino acid score

Table III shows the amino acid scores for the fifteen foods analyzed in this study based on the essential

amino acid content and the pattern for children of 1-2 years of age (% of the FAO/WHO/UNU 2007 recommendation). As expected, lysine was the first limiting amino acid in cereal-based products. White bread and flour tortillas with water provided less than 20% of the recommended amount. This result is in agreement with Bressani,³⁰ who published a lack of lysine in cereals. Gonzalez and Serna³¹ reported similar results for wheat flour tortillas using the FAO/WHO (1973) recommendations for adults, and Morales and others⁵ published similar results for corn products that were limiting in lysine (56%). Beans have been reported to have a low nutritive value because of the low values of certain essential amino acids, particularly sulfur-containing amino acids such as methionine and tryptophan. Independent of the preparation method, methionine and cysteine were the first limiting amino acids in beans.

The results of the amino acid score in meat products indicated that phenylalanine and tyrosine were the limiting amino acids in charcoal-broiled beef and fried steak, while methionine and cysteine were the limiting amino acids in boiled beef and hamburger. According to the FAO/WHO/UNU recommendations published in 1991,⁹ all of the meat products in this study had been classified as limiting in phenylalanine and tyrosine. However, according to the FAO/WHO/UNU recommendations in 2007,⁶ the requirements for phenylalanine and tyrosine decreased from 6.3 to 4.6 g/16 g N and therefore, the sulfur-containing amino acids have become limiting in the two aforementioned meat products. Bologna had a relatively high score, which is probably due to its meat content; however, it was low in lysine due to its cereal content, in accordance with Vázquez and González.²⁹ Threonine was the limiting amino acid in white cheese and provided 91% of the recommendation; regional cheese had the highest amino acid score.

In vitro protein digestibility

The *in vitro* protein digestibilities of the foods are presented in table IV. The lowest values were observed for bologna (75.4%) and refried beans (77.5%). Bologna is one of the processed meat products consumed in the Northwest region, and similar values of protein digestibility (70.6 to 74.02%) were published for several brands of bologna that were available in the region.³² Bologna had a relatively high amino acid score due to its meat content. However, protein quality is not only based on the amino acid profile, but also on the protein digestibility, and bologna had a low digestibility due to the high content of collagen in the product.²⁹ The values for the digestibility of beans were lower than those reported for the *Jatropha curcas* plant (78.6-87.2%),³³ faba beans (83.1%) and lentils (82.5%), but higher than those for chickpeas (78.3%) and dry beans (73.8%).³⁴ The different values of protein digestibility obtained

Table IV
The in vitro digestibility of protein from fifteen foods consumed in Northwest Mexico

<i>Sample</i>	<i>% Digestibility*</i>
Flour tortillas with water	87.8
Flour tortillas with extra oil	85.9
Flour tortillas, commercial	84.9
Corn tortillas	77.9
White bread (virginia)	83.8
Breakfast roll (conchita)	85.1
Cooked beans, whole	80.3
Refried beans	77.5
Refried beans without liquid	81.5
Boiled beef	87.8
Charcoal-broiled beef (chuck steak)	85.0
Steak, fried	80.7
Hamburger, fried	84.4
Bologna	75.4
Mexican white cheese	83.8

*Based on a multienzyme technique²².
Values are means of duplicate determinations.

for the three preparations of beans indicated that the preparation method may have an effect on their digestibility.

Data on the composition of foods consumed in the different regions are important for determining the nutritive value of diets and can be useful for improving nutrition through intervention programs or public health policies. Data on food composition from other countries can sometimes pose limitations in the interpretation of the nutritive value of single foods or dishes, given the wide variation of food items and preparation methods. This study provided information on the amino acid content of foods traditionally consumed in the Northwest region. This is new information that was not available in the region, and is now accessible to be able to use in studies based on the dietary assessment of the population.

Conclusion

The protein quality of foods for human consumption through the use of amino acid profiles, amino acid scores and protein digestibility can provide added value to the national food composition tables and international food databases. Although cereals and legumes had low amino acid profiles, the nature of the Mexican diet in the Northwest region is a natural combination of foods (tortillas and beans) that results in a diet with a mixture of relatively high-quality proteins.

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