A Comparative Study of Protein Changes in Normal and Quality Protein Maize During Tortilla Making

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ABSTRACT

Protein changes were evaluated in two different maize genotypes of contrasting protein quality made into tortillas. In both types of maize, albumins, globulins, zeins, and glutelinlike components became insoluble after interacting with other biochemical entities catalyzed by the alkaline pH and the heat produced in tortilla-making. Increased nitrogen recovery with solvents having alkaline pH, a reducing agent, and sodium dodecyl sulfate indicate that hydrophobic interactions may have been involved in this change in solubility of proteins that are more easily solubilized in the unprocessed maize grain. In vitro protein digestibility with pepsin declined as nitrogen content increased in the glutelin fraction and in the residue after fractionation. Tortillas made from quality protein maize (QPM) had a superior amino acid score mainly because of their very high lysine and tryptophan content, which was not significantly affected during tortilla preparation. The superiority of the product obtained with the QPM sample was demonstrated by its high content of available lysine. Although the in vitro digestibility of protein with pepsin and the amount of available lysine changed during tortilla-making, no evidence was found of a specific detrimental effect on the protein quality of the original QPM grain.

Improving the protein quality of cereal grains has been a major concern of agricultural scientists for the last two decades. Mutant germ plasm with high levels of lysine has been identified in maize (Mertz et al 1964), sorghum (Singh and Axtell 1973), and barley (Munck et al 1970, Dolletal 1973), but the inherent agronomic defects of this germ plasm, particularly its low yield and high susceptibility to disease and insects, discourage many breeders from further investigation. The International Maize and Wheat Improvement Center (CIMMYT) has continued working for improvement of protein quality in maize under a project funded by the United Nations Development Program (UNDP). Through several cycles of recurrent selection, CIMMYT's maize breeders have combined the high-lysine potential of the *opaque*-2 gene with a sufficient number of modifier genes to change the original soft *opaque*-2 endosperm into a hard vitreous type (Vasal et al 1980). Quality protein maize (QPM) populations that have superior lysine content and yield and agronomic characteristics similar to those of normal maize are now available. The physical and biochemical changes that have occurred during the development of these QPM populations have been reported elsewhere (Ortega and Bates 1983). The first QPM variety to be released (Nutricta) was developed by the national agricultural program of Guatemala. Recently, another variety (Nutri-Guarani V-241) was released in Paraguay. Trials with similar materials are being conducted in other parts of the world.

Ways in which high-lysine maize might benefit human and animal nutrition have been thoroughly studied by several workers including Mertz et al (1965), Pradilla (1968), Bressani et al (1969), Manner (1975), and Valverde et al (1981). Nutritionists have, in addition, expressed an interest in learning how much of the original protein quality present in the grains is maintained in typical products (tortillas, arepas, porridges, etc.) that serve as staple foods in many developing countries.

Great amounts of maize tortillas are consumed daily in Mexico, Guatemala, and other countries of Central America. Fifty percent of the maize used in Mexico during 1982 went into tortillas, with per capita consumption of about 330 g per day (Tedaldi 1981). The techniques for making tortillas vary from one part of the region to another (Katz et al 1974), but all are based upon a lime-cooking process (Bressani et al 1958, Bedolla and Rooney 1982) known as "nixtamalization" that has been used for centuries.

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Various researchers have investigated nixtamalization and other aspects of tortilla making. Chemical changes that occur during the lime treatment of corn (Bressani and Scrimshaw 1958) and during preparation of tortillas (Bressani et al 1958) are reported. More recently, an extensive review of the role of lime in the alkaline treatment of corn for tortilla preparation was published (Trejo-Gonzalez et al 1982) as well as a review of tortilla production technology (Paredes-Lopez and Saharopulos 1983).

Some investigations focused upon the performance of *opaque*-2 maize in tortilla making. Bedolla and Rooney (1982) reported a higher degree of starch gelatinization during alkaline cooking of soft endosperm maize that produced a sticky masa not suitable for making tortillas. In another study (Sproule 1985) comparing the nutritional value of tortillas and chips made from QPM with those made from food-grade maize, QPM tortillas and chips were found to have good acceptability for flavor and color. It was reported that the protein efficiency ratio and feed efficiency of QPM products are much superior to those of products from normal maize (Sproule 1985).

The purpose of this research was to investigate the protein fractions and protein quality changes that occur during the different stages in tortilla making, and to compare QPM with its normal maize counterpart for lysine and tryptophan content. An additional aim was to obtain information on quality parameters such as amino acid score, protein digestibility, and available lysine in tortillas made with QPM and normal maize.

MATERIALS AND METHODS

Maize Samples

Tortillas were prepared from seed of a widely grown southeastern Mexican open-pollinated variety (Tuxpeño-1) and from the QPM version of this same material. The QPM version (Blanco Dentado-1, QPM) was developed through several cycles of selection both for agronomic performance in the field and lysine content in the laboratory.

Nixtamalization Process and Tortilla Making

For the preparation of masa and tortillas from the two materials tested, we adopted the traditional process used in central Mexico (Paredes-Lopez and Saharopulos 1982). Whole kernel maize and water (1:2 ratio) were cooked with lime (1% in water) at 92° C for 50 min. The resultant nixtamal was maintained at room temperature (18° C) for 16 hr, then washed three times with distilled water before grinding. About 50 g of dough (masa) was flattened and cooked on both sides on a hot iron plate (approximately 200° C) for 2 min to produce tortillas.

Sample Preparation

Subsamples of both types of maize were taken at each of the

stages in the tortilla-making process: uncooked whole grain, whole grain after nixtamalization, masa, and tortilla. The subsamples were air-oven dried for 24 hr at 65° C, finely ground through a Cyclotec mill with a 0.5-mm screen, and defatted for 8 hr with hexane. Endosperm samples from the original and nixtamalized maize were obtained by dissection and were prepared similarly to the whole kernel material.

Chemical Analysis

Protein content (% N × 6.25) was determined by the micro-Kjeldahl method (AOAC 1980) for total nitrogen. Tryptophan was determined by the colorimetric method of Opienska-Blauth, modified by Hernandez and Bates and used for rapid screening in the Protein Quality Laboratory at CIMMYT (Villegas et al 1984). Amino acids were determined by ion-exchange chromatography following hydrochloric acid hydrolysis for 24 hr at 110° C (Moore and Stein 1951). Lysine was analyzed on a 6-cm Aminex-AS resin bed. The full amino acid profiles were determined by single-column ion-exchange chromatography (Kremen and Vaughn 1972). Protein fractionation of duplicate 200-mg samples was performed by following sequence D of the Landry-Moureaux procedure (Landry-Moureaux 1970). Five different protein fractions—fraction I (components soluble in 0.3*M* NaCl), fraction II (true zeins), fraction III (zeinlike), fraction IV (glutelinlike), and fraction V (true glutelins)—and a solid residue were obtained. During each extraction step, the weight-to-volume ratio was maintained at 1:10, with an initial sample of 0.2 g and 2 ml of extractant solutions. For the two materials tested, in vitro pepsin digestibility (Axtell et al 1981) was determined from duplicate samples of whole kernels, nixtamal, masa, and tortillas. Average soluble nitrogen values, determined by the micro-Kjeldahl method after enzymatic treatment, were adjusted using casein as the standard protein with 100% digestibility. Available lysine was determined in the raw grain and in tortillas of both maize samples by the official AOAC method (AOAC 1980).

RESULTS AND DISCUSSION

The protein content of masa and tortillas, whether prepared from QPM or normal maize, was slightly higher than that of the original grain (Table I), even though, as previously reported (Bressani and Scrimshaw 1958), some nitrogen was lost to the cooking liquor during nixtamalization. This protein increase may have been caused by removal of the seed pericarp (which consists of highly cellulolytic material) during nixtamalization. Protein changes brought about by processing followed the same trend as reported in the literature (Bressani et al 1958), except that the original maize samples discussed here had a higher protein content.

The concentration of lysine and tryptophan, essential limiting amino acids in maize, was determined for both genotypes. The protein quality of QPM was decidedly superior to that of the normal genotype in the original whole kernel samples. QPM had 80% more tryptophan and 64% more lysine. The percentage of tryptophan in protein was slightly diminished by tortilla making in both genotypes, the reduction measuring about 11% for normal maize and 15% for QPM (Table II). Loss of total lysine content (percentage of lysine in protein) during alkaline treatment and cooking of masa was minimal in both the normal maize and QPM samples (Table II). Similar results were reported for normal protein quality maize samples (Bressani and Scrimshaw 1958). The stability of lysine indicates that very little lysinoalanine (Sanderson et al 1978) or other degradation products form during tortilla processing. The moist, alkaline heat treatment used in tortilla making is quite distinct from other food processes using higher temperatures or longer process times, during which lysinoalanine has been reported, e.g., soy protein texturization (Wooddard and Short 1973).

Changes in the protein fractions that took place during nixtamalization and tortilla making were observed for both maize types after total nitrogen analysis of the five liquid fractions and of the residue. The percentage of total nitrogen detected in each fraction is given in Table III. The values listed are averages for duplicate samples. In both of the maize materials, more than 90% of the total nitrogen was solubilized into five protein fractions. Lower figures have been reported by other researchers (Trejo-Gonzalez et al 1981). The distribution of the protein components in these five fractions was different for the two materials. Changes in the protein fraction reported elsewhere (Misra et al 1972,

TABLE I
Protein Content[a] of Grain, Nixtamal, Masa, and Tortilla from Tuxpeño-1 and Blanco Dentado-1 QPM[b]

Maize Form	Tuxpeño-1 (%)	Blanco Dentado-1 QPM (%)
Whole grain	9.8 c (9.1)	9.8 c (9.1)
Nixtamal	10.4 a (5.8)	10.2 b (5.6)
Masa	10.4 a (5.0)	10.6 a (5.1)
Tortilla	10.2 b (6.2)	10.5 a (6.3)

[a] Determined as percent nitrogen × 6.25; all values are means of two replications. Values are presented on a dry basis followed by wet/as is values in parentheses.
[b] QPM = quality protein maize.

TABLE II
Changes in Lysine and Tryptophan Contents[a] Through the Tortilla-Making Process for two Different Maize Genotypes

Maize Form	Tuxpeño-1		Blanco Dentado-1 QPM[b]	
	Lysine	Tryptophan	Lysine	Tryptophan
Whole grain	2.57 a	0.56 ab	4.22 a	1.01 a
Nixtamal	2.42 a	0.56 a	3.92 b	0.93 b
Masa	2.63 a	0.54 ab	4.15 ab	0.89 c
Tortilla	2.54 a	0.50 a	4.06 ab	0.86 c

[a] Average values of two replications are presented as percentage of each amino acid in the protein. Mean values in a column followed by the same letters are not significantly different at the 5% level. (LSD 0.05).
[b] QPM = quality protein maize.

TABLE III
Changes in the Protein Fraction Distribution[a] of Tuxpeño-1 and Blanco Dentado-1 QPM[b] Through the Different Stages in Tortilla Making

Maize Form	Tuxpeño-1 Fraction I	II	III	IV	V	Residue	Blanco Dentado-1 QPM Fraction I	II	III	IV	V	Residue
Whole grain	3.21 (16.0)	6.18 (30.8)	2.74 (13.7)	2.39 (12.0)	4.08 (20.4)	1.44 (7.1)	6.65 (31.5)	1.25 (5.9)	1.98 (9.4)	3.72 (17.6)	5.74 (27.2)	1.76 (8.3)
Nixtamal	1.86 (9.0)	5.91 (28.3)	3.47 (16.6)	2.24 (10.9)	5.88 (28.2)	1.51 (7.0)	2.75 (13.5)	0.79 (3.9)	2.08 (10.2)	3.02 (14.8)	9.54 (47.0)	2.11 (10.6)
Masa	2.25 (10.5)	5.82 (27.2)	3.09 (14.4)	2.26 (10.5)	6.71 (31.2)	1.34 (6.3)	2.95 (13.6)	0.85 (4.0)	2.22 (10.3)	3.11 (14.4)	10.53 (48.8)	1.91 (8.9)
Tortilla	1.43 (7.2)	2.60 (13.0)	4.30 (21.4)	1.90 (9.5)	7.22 (36.1)	2.58 (12.8)	2.51 (12.2)	0.61 (2.9)	1.56 (7.6)	2.58 (12.6)	9.02 (44.1)	4.18 (20.6)

[a] Determined by the Landry-Moureaux (1970) fractionation scheme. Values are of two replications in milligrams of protein (N × 6.25); values in parenthesis refer to percentage of total protein.
[b] QPM = quality protein maize.

Gentinetta 1975, Ortega and Bates 1983) as being a consequence of the *opaque*-2 gene were clearly manifested in the QPM sample. The superior protein quality (high tryptophan and lysine content) of this genotype resulted from the low amounts of zeins and higher content of albumins, globulins, and glutelins. About the same amount of nitrogen was recovered from the original grain samples of the two maize genotypes, but quite different results were obtained with the tortillas made from them; only 79.4% of the total nitrogen was extracted from the QPM tortillas, compared with 87.2% for those made from Tuxpeño-1, the non-QPM counterpart.

From the changes in protein solubility that occurred during tortilla-making, we made four principal observations (Table III). First, protein components soluble in 0.3*M* NaCl (albumins, globulins, amino acids, and small peptides) were either lost during the process or converted into an insoluble form. In Tuxpeño-1 the reduction was approximately 55%, whereas in QPM it was around 62%. Most of this reduction results from the heat-alkali treatment. Other researchers have detected this change during the alkali treatment of corn (Bressani and Scrimshaw 1958) and identified the main components lost as globulin proteins (Trejo-Gonzalez et al 1982). From fractionation data on protein in the nixtamalized endosperm, we can conclude that, because endosperm fraction I from both materials shows only a slight decrease after nixtamalization (around 13%), germ proteins are probably the main components made insoluble or lost after alkaline and heat treatments. The decrease of fraction I components may have resulted from the loss not only of insoluble globulins, but also of soluble nitrogen components, such as free amino acids, that are present in much higher concentrations in QPM materials containing the *opaque*-2 gene than in normal genotypes (Mertz et al 1974).

A second finding was that in both types of maize the true zeins (fraction II) and the glutelinlike substances in fraction IV also became insoluble during the process. The solubility of true zeins decreased 58% in the normal maize and 52% in the QPM tortillas. Bressani and Scrimshaw (1958) found that the solubility of zein in the alcohol-soluble fraction decreased constantly, first from the original grain to masa and again during tortilla making. On the contrary, in this study major changes in these proteins occurred only during tortilla production using masa from normal maize. The QPM had so little true zeins initially (6%) that it was difficult to ascertain the actual trend of the decrease. The amount of glutelinlike components present in soluble form diminished in Tuxpeño-1 (21%) and in QPM (31%) from the original grain to tortillas. Under alkaline conditions, particularly after the masa had been heated, these proteins may have interacted with other insoluble polypeptides, becoming soluble only after sodium dodecyl sulfate (SDS) treatment in the true glutelin (fraction V) extraction. This conclusion is suggested by the instability and initial breakdown of cystine in the glutelin fraction of maize during alkaline processing (Sanderson et al 1978), by the increase in the true glutelin (fraction V), and by the appearance of glutelin subunits of higher molecular weight detected by SDS-PAGE in alkali-treated maize samples (Paredes-Lopez et al 1982). The amount of zeinlike components extracted from fraction III varied greatly during different steps of the process for both maize samples. The increased amounts of these polypeptides in tortillas made from normal maize could be attributable to certain true prolamins (which may have cross-linked by disulfide bonding during the heat-alkali process), becoming soluble only after reduction with 2-mercaptoethanol (2-ME) used for extracting zeinlike proteins.

The third observation was that the amount of nitrogen solubilized by borate buffer (pH 10.0) + 0.6% 2-ME and 0.5% SDS in fraction V almost doubled from the original raw maize samples. It increased 77% with normal maize and 83% with QPM. In the former, an increase occurred at each stage of the process (raw maize to nixtamal to masa to tortillas), whereas in the QPM sample the increase peaked at the masa stage. The amount of nitrogen finally solubilized by these solvents resulted in part from the true glutelins already present in each of the two maize samples. The steady increase of nitrogen extracted in the true glutelin fraction from raw grain to tortillas has not been reported before and cannot be compared with the very different results obtained by using another protein fraction scheme for total glutelins (Trejo-Gonzalez et al 1982).

The fourth point concerns residual nitrogen not soluble in any of the extractants used in the fractionation scheme. This fraction increased throughout the tortilla-making process, by 79% Tuxpeño-1 tortillas and more than 100% in the QPM product. As indicated in Table III, the greater part of this increase in both materials occurred during production of tortillas from masa, probably as a result of protein denaturation.

In general, proteins underwent similar changes in solubility during nixtamalization and tortilla making in both low- and high-lysine maize samples. The solubility of fractions I, II, and IV components in tortillas decreased. These fractions were recovered either as soluble nitrogen in fraction V or in the residual nitrogen that simultaneously increased during maize processing. Hydrophobic interactions, protein denaturation, and cross-linking of proteins by uncommon amino acids such as lysinoalanine (Sanderson et al 1978) were probably responsible for changes in the solubility of these components during processing.

In both types of maize, processing had a similar effect on pepsin in vitro digestibility, it dropped after nixtamal preparation and after cooking masa to make tortillas (Table IV). The in vitro digestibility figures for masa were close to those for raw grain. QPM showed lower in vitro digestibility with pepsin than did normal maize. This was especially true in the tortillas. Differences in pepsin digestibility between the two samples of original, uncooked, whole kernels could have been the result of differences in the distribution of their protein fractions. As shown in Table IV and Figure 1, the increase in fraction V, consisting of the true glutelin components, correlates reasonably well ($r = -0.66$) with the reduction in pepsin digestibility during processing. Only in the masa made from both materials does the correlation not hold. Plotting the residual nitrogen against in vitro digestibility results in a better correlation ($r = -0.85$). The amount of residual nitrogen after protein fractionation is inversely correlated with pepsin in vitro digestibility. One possible explanation for improved digestibility at the masa stage is that certain components extracted as true glutelins by alkaline pH and the addition of 2-ME and SDS are probably zein proteins that were made insoluble in the process and more easily digestible by pepsin. Similar results have been reported by Sproule (1985), who observed a decrease of in vivo and in vitro crude protein digestibility when both food-grade maize and QPM maize were made into tortillas. Axtell et al (1981) found that in vitro pepsin digestibility drops drastically when sorghum is cooked to form gruel. Other researchers (Hamaker et al 1984) reported that the low digestibility of sorghum proteins is mainly attributable to the undigestibility of glutelinlike and true glutelin fractions. These findings support the conclusion that indigestible components present in the true glutelin and residual nitrogen fractions may be responsible for the decrease in digestibility. Previous reports by Paredes-Lopez and Mora (1983) indicated that the percentage of residue nitrogen increases markedly during the first 60 days of storage of nixtamalized maize (35° C, at relative humidities of 55 and 75%). Decreased in vitro digestibility was also

TABLE IV
In Vitro Protein Digestibility[a] Changes in Tuxpeño-1 and Blanco Dentado-1 QPM[b] During Tortilla Making

Maize Form	Tuxpeño-1	Blanco Dentado-1 QPM
Whole kernel	88 ab	82 a
Nixtamal	82 bc	73 b
Masa	91 a	80 a
Tortilla	79 c	68 b

[a] Mean values for duplicate samples are corrected figures assuming casein digestibility to be 100% with pepsin. Mean values in a column followed by the same letters are not significantly different at the 5% level (LSD 0.05).
[b] QPM = quality protein maize.

TABLE V
Amino Acid Analyses of Tuxpeño-1, and Blanco Dentado-1 QPM[a] Samples Through Tortilla Making

Amino Acid (g/16 g of N)	Tuxpeño-1			Blanco Dentado-1 QPM		
	Whole Grain	Masa	Tortilla	Whole Grain	Masa	Tortilla
Alanine	8.2	8.6	7.9	6.8	6.4	6.6
Arginine	4.2	5.4	4.4	7.5	6.7	7.2
Aspartic acid	6.2	6.9	6.2	7.8	7.1	7.4
Cisteine[b]	1.4	1.7	1.5	2.3	2.2	1.8
Glutamic acid	19.4	19.4	18.3	17.7	16.2	16.4
Glycine	3.7	4.2	3.7	5.5	5.1	5.3
Histidine	3.3	3.7	3.2	4.7	4.4	4.3
Isoleucine	3.6	3.9	3.5	3.6	3.4	3.5
Leucine	13.4	13.9	12.9	9.6	9.4	9.6
Lysine	2.7	2.9	2.5	4.3	4.0	4.2
Methionine[b]	2.2	2.5	2.1	2.1	2.1	2.1
Phenylalanine	5.4	5.5	5.3	4.7	4.4	4.5
Proline	7.8	8.3	7.3	8.3	7.8	8.1
Serine	5.3	5.7	5.2	5.5	5.1	5.5
Threonine	3.8	4.1	3.7	4.5	4.1	4.2
Tryptophan[c]	0.56	0.54	0.50	1.01	0.89	0.86
Tyrosine	3.3	4.6	3.5	4.1	4.1	4.0
Valine	5.0	5.3	4.7	5.7	5.4	5.5

[a] QPM = quality protein maize.
[b] Partially destroyed during acid hydrolysis.
[c] Colorimetrically determined.

observed in these stored samples by using a multienzyme analysis system.

Complete aminoacid analyses for the raw grain, masa, and tortillas of both maize genotypes are given in Table V. Differences in the amount of lysine, glutamic acid, glycine, leucine, and arginine between Tuxpeño-1 and the QPM whole grains and tortillas are similar to those reported previously (Sanderson 1978) for normal and *opaque*-2 maize samples. As mentioned before, processing caused no significant loss of lysine in either maize type. The ratio of leucine to isoleucine was lower for the QPM sample than for normal maize across all stages of processing, a finding with important nutritional implications. The ratio of these amino acids was not improved by nixtamalization but remained constant for both materials. The amino acid scores for tortillas made from the two genotypes showed the nutritional superiority of the QPM product (Table VI).

Lysine data for the whole grains and tortillas from both maize samples are given in Table VII. The QPM samples had higher lysine availability than normal maize. In both maize types, available lysine dropped during tortilla making. In contrast, Trejo-Gonzalez et al (1982), using a different methodology, reported an increase of available lysine in lime-treated maize samples. Up to 75.3% of total lysine content of QPM tortillas remained available. The superiority of this product in this regard is plainly manifested by its higher absolute available lysine content (2.95 g/100 g of protein).

TABLE VI
Amino Acid Score[a] of Tuxpeño-1 and Blanco Dentado-1 QPM[b] Tortillas

Amino Acid	Tuxpeño-1	Blanco Dentado-1 QPM
Histidine	188	253
Isoleucine	83	83
Leucine	184	137
Lysine	49[c]	80[c]
Total aromatic amino acids	120	116
Total sulfur amino acids	138	150
Threonine	106	120
Tryptophan	45[c]	82
Valine	101	115

[a] Calculated using as reference pattern the essential amino acid requirements for humans from the Recommended Dietary Allowances (NRC/NAS 1980).
[b] QPM = quality protein maize.
[c] First limiting amino acids.

CONCLUSIONS

The protein content of Tuxpeño-1 and QPM increased slightly (on a dry basis) during tortilla processing. The limiting amino acids in maize, lysine, and tryptophan were reduced only by small amounts during preparation of tortillas from the two maize materials. The very small loss of lysine observed in the product suggests that minimal amounts of lysinoalanine must exist in both types of tortillas. Similar changes in the solubility of the different

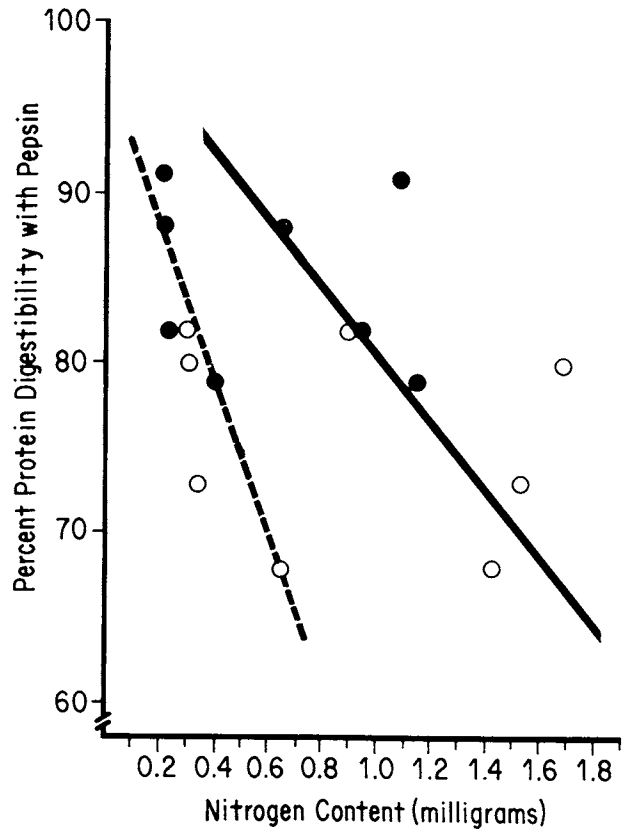

Fig. 1. The relationship between in vitro protein digestibility and nitrogen content in the true glutelin (——) and residue (– – –). These were obtained by protein fractionation of the different stages in tortilla making for Tuxpeño-1 (●) and Blanco Dentado-1 (○).

TABLE VII
Available Lysine Content[a] of Whole Grain and Tortilla Samples from Tuxpeño-1 and Blanco Dentado-1 QPM[b]

	Whole Grain			Tortilla		
	Total Lysine	Non-Available Lysine	Available Lysine	Total Lysine	Non-Available Lysine	Available Lysine
Tuxpeño-1	2.52 (100%)	0.68 (27%)	1.84 a (73%)	2.46 (100%)	0.76 (30.9%)	1.70 a (69.1%)
Blanco Dentado-1 QPM	4.17 (100%)	0.60 (14.4%)	3.57 b (85.6%)	3.92 (100%)	0.97 (24.7%)	2.95 b (75.3%)

[a] Average values of triplicate analyses. All lysine figures presented in grams per 100 g of protein. Numbers followed by the same letter in the same column are not significantly different at the 5% level (LSD 0.05).
[b] QPM = quality protein maize.

protein fractions of maize occurred during processing of tortillas from the normal maize type and from its QPM counterpart. The alkali-heat treatment (nixtamalization) and baking of the masa during tortilla making induced hydrophobic interactions, cross-linking, and denaturation of globulins, zeins, and glutelinlike polypeptides as noted in the first reviews. As a consequence, these entities were either recovered in soluble form with solvents containing a reducing agent and SDS at an alkaline pH, or they remained insoluble at the end of protein fractionation as part of the residual nitrogen. In vitro pepsin digestibility of Tuxpeño-1 and Blanco Dentado-1 QPM decreased as raw grain was processed to form nixtamal and then tortillas. This drop in pepsin digestibility correlates well with the increase in true glutelins (which were only partially digested by pepsin) and in the residual nitrogen that occurs in nixtamal and tortilla preparation.

Aminograms for the raw grain, masa, and tortillas of each maize genotype showed large differences in several amino acids between the maize samples and indicated that processing had no major effect on the content of any particular one. The amino acid score of tortillas produced with Tuxpeño-1 and Blanco Dentado-1 QPM confirmed the clear superiority of the QPM tortillas in this regard. The available lysine present in the original grain of both genotypes was reduced during tortilla making. Even though the percentage of available lysine in the QPM tortillas was only 9% greater than that in the normal maize product, the absolute values indicate that the QPM tortillas provide 73% more available lysine than those made from the normal maize counterpart. In developing countries where QPM would be used in tortilla preparation, the nutritional benefits of the *opaque*-2 gene thus may be expected to be present in the final product.

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