

**THE EFFECTS OF ALKALI-COOKING OF CORN AND
SUPPLEMENTATION WITH AMARANTH SEED
ON ITS DEFICIENCIES IN LYSINE AND TRYPTOPHAN**

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SUMMARY

Corn, made into tortillas (flat cakes baked from lime-treated corn) is the staple food of Mexico. The amino acid deficiencies of tortillas (TT) and boiled corn (BC), and the supplementary value of amaranth seed (PA), another traditional Mexican food, roasted to the point of "popping", were studied. The feeding tests were 14-day PER trials using weanling rats; each diet contained 8.95% crude protein, all from corn, or with PA providing 3.6% protein and corn the remainder, with vitamin and mineral supplements. In addition each diet was supplemented with lysine (lys) so that tryptophan (trp) would be the first limiting amino acid or vice versa.

In no comparison did TT give a significantly different value from BC. With trp limiting, the mean PER for the two corn preparations alone was

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1.55, and with PA, 2.22. The amaranth had shown a high trp value (1.55 g/16 g N). With lys limiting, the mean PER for corn was 1.15; adding PA failed to improve this. Popping amaranth also reduced its reactive lysine value (by dye-binding) from 5.9 to 4.0 g/16g N. It appears that making tortillas has no adverse effect on protein value, but that 'popping' can reduce the value of amaranth seeds.

The higher PER for corn with trp as the limiting amino acid (rather than lys) was due to lower *ad libitum* food intake with the same weight gain.

INTRODUCTION

Corn foods, and particularly tortillas, contribute a very important portion of the protein and energy intake of the Mexican and Central American populations. According to Miranda, in the 1940's an average Mexican had a daily intake of 216 g of tortillas (1); 30 years later the daily intake was estimated to be about 315 g (2). Most of the work done on the lime-heat treatment effects on corn to make tortillas, versus either raw or boiled corn, has dealt with the pellagragenic activity of maize, and the niacin bound to the cereal prior to the lime treatment (3-6). Comparison of the protein quality of corn before and after lime-heat treatment in the presence of adequate niacin has received less attention. Some amino acid analyses of the corn proteins have indicated that the lime treatment causes significant losses of several amino acids (7-9); others found a fall only of arginine and cystine (10).

Since the lime-heat treatment of corn is carried out at about pH 11.6 and a temperature of $85 \pm 5^{\circ}\text{C}$, Massieu *et al.* suggested that some racemization of the corn protein could possibly take place (7). In a more recent report, sunflower proteins were treated with alkali at various concentrations under different temperatures, and isomerization of lysine as well as losses of arginine, threonine and serine were observed (11). No corresponding data on corn proteins are available. Recent reports have shown evidence of lysinoalanine (N^{ϵ} [DL-2-amino-2-carboxyethyl]L-lysine) formation in tortillas (10, 12). Lysinoalanine (LAL) in corn foods was determined in these studies by ion-exchange chromatography and thin layer chromatography.

Recently, a greater interest has developed in maintaining and improving the variety of indigenous foods in underdeveloped countries rather than totally replacing them with Western crops (13). One such food is amaranth whose seeds as well as leaves have

been eaten in Mesoamerica since pre-Columbian times (14). By far, the most popular form of consuming the amaranth seeds in Mexico, even today, is in the form of "alegrías", i.e. popped like 'popped corn'. Since the seeds of *Amaranthus edulis* had shown a high lysine content, one of the research priorities recommended by the National Academy of Sciences (15) was to determine the composition and nutritive value of other varieties also.

The purposes of our study were twofold: firstly we wished to compare the quality of the proteins of boiled corn versus the proteins of traditional lime-heat treated corn for growing rats. Preliminary results on the possible formation of D-amino acid residues and LAL in tortillas are also presented; these determinations were carried out using gas chromatography (GC). Secondly, since the most limiting amino acids of corn are lysine and tryptophan, we measured the supplementing effect of the popped seeds of *A. hypochondriacus*, on both boiled corn and tortillas as a source of each of these two essential amino acids. Since roasting caused racemization of several amino acid residues in a study of other proteins (16), preliminary measurements of D-amino acid residues formed due to popping the amaranth seeds were determined by GLC.

MATERIALS AND METHODS

Sample Preparation

Traditional tortillas were prepared from white corn by a published procedure (17). Whole corn boiled in water for 1 hr was prepared from the same batch of white corn; each of these prepared foods was then freeze-dried. Amaranth seeds (*A. hypochondriacus*) were obtained commercially in Tulyehualco, Mexico, and popped in the laboratory by heating in a metal saucepan at about 170°C for about 15 min. All samples were ground to pass 40 mesh and stored at 4°C.

L-lysine-HCl, L-tryptophan, and L-methionine were purchased from Sigma Chemical Co. (St. Louis, MO) and portions of each were treated separately with 2% w/v CaO in aqueous solution and kept at 90°C during 2 hr, simulating the first stage of the tortilla-making process.

Chemical Analyses

Proximate analyses of the materials were determined using

standard procedures. Reactive lysine of the several materials was determined using a dye-binding procedure (18). Tryptophan was first determined by alkaline hydrolysis of the materials followed by ion-exchange chromatography, which was performed on the short column of an amino acid analyzer (Beckman, Model 120B) (19). DL-5-methyl-tryptophan (Aldrich Chemical Co., Milwaukee, WI) was used as internal standard (20). Tryptophan was further determined by a specific colorimetric procedure (21). Measurements of D-amino-acid residues of the tortillas and the popped amaranth were determined by derivatization of the amino acids in acid hydrolysates and their separation by gas chromatography (22, 23) with some modification (24). Standards of D- and L-amino acids were run concurrently. Chromatography was performed using a Hewlett-Packard (Avondale, PA) Model 5840 gas chromatograph equipped with a flame ionization detector, fitted with a 25 m x 0.3 mm internal diameter, wall-coated open tubular (WCOT) Chirasil-Val glass capillary column. LAL was determined using the same derivatization technique but the gas chromatograph was Hewlett-Packard Model 5830 GC, with a glass capillary column 25 m x 0.2 mm i.d. WCOT, OV-17.

Animal Assays

Male weanling (21-day-old) Wistar rats averaging 50 g were obtained from a commercial supplier (Charles River Breeding Lab., Wilmington, MA). On arrival at the laboratory they were weighed individually and housed in screen-bottom stainless steel cages measuring 25 x 18 x 18 cm. The room was maintained at 22°C and lit for 14 hr out of every 24 hr. For the first two days they were fed on a stock diet; then they were allocated by a randomization procedure, two per cage, to 48 cages. Seven cages were then allotted to each of diets A-D and four to each of diets E-I. Food and water were provided *ad libitum*. Food consumption and body weight were recorded every four days until termination of the experiment at 14 days.

Protein efficiency ratio (PER) was determined for each cage and the results subjected to analysis of variance to obtain a pooled estimate of standard deviation, and to check the overall significance of treatment differences. Duncan's multiple range test was used to assess the significance of differences between particular treatment means (25).

The formulation of the diets is shown in Table 1. These were

TABLE 1

COMPOSITION OF THE TEST DIETS AND THE MEAN WEIGHT GAINS
AND FOOD CONSUMPTION OF THE RATS

	A	B	C	D	E %	F	G	H	I
Boiled corn	81.82	81.82	—	—	48.41	48.41	—	—	—
Tortilla	—	—	87.83	87.83	—	—	51.92	51.92	87.83
Popped amaranth	—	—	—	—	25.00	25.00	25.00	25.00	—
L-lysine-HCl*	0.95	—	0.95	—	0.86	—	0.86	—	0.95
L-tryptophan	—	0.102	—	0.102	—	0.09	—	0.09	0.102
Basal mix**	6.02	6.02	6.02	6.02	6.02	6.02	6.02	6.02	6.02
CaCO ₃	0.70	0.70	—	—	0.70	0.70	0.23	0.23	—
Cornstarch	10.51	11.36	5.20	6.05	19.00	19.78	15.97	16.74	5.10
Weight gain per cage, g	22.00	21.00	25.00	19.00	49.00	25.00	54.00	25.00	65.00
Food eaten per cage, g	172.00	212.00	168.00	183.00	257.00	234.00	251.00	230.00	261.00

* L-lysine-HCl contains 80% lysine.

** The basal mix contains L-isoleucine, 0.25; L-methionine, 0.3; L-valine, 0.27; corn oil, 1.2; AIN-76 mineral mix, 3; and AIN-76 vitamin mix, 1 (29).

prepared five days before starting the experiment, and stored at 4°C. The vitamins were pre-mixed with an aliquot of cerelose. Since tortillas contain calcium from the lime treatment, variable additions of CaCO_3 were used so that the total calcium content of each diet was similar. Each diet was calculated to contain 8.95% crude protein (CP, $\text{N} \times 6.25$) from corn and popped amaranth. Where the latter was used, it contributed 3.65% CP in each diet. The levels of supplementary lysine, tryptophan, isoleucine, methionine and valine were calculated to be sufficient to meet the rats' requirements for all amino acids (26), assuming that the corn proteins were 90% digestible (27). Lysine, however, was omitted from four diets so as to make that the first limiting amino acid, and tryptophan was similarly omitted from another four, to become the first limiting. Only diet I, used as a positive control, had neither omitted.

RESULTS

Analytical Results

The proximate analysis and tryptophan and reactive lysine values for the test materials are detailed in Table 2.

In the popped amaranth we detected 5-15% racemization of alanine, aspartic acid, glutamic acid and phenylalanine. D-amino acids were not detected in the alkaline digests of either lysine, tryptophan or methionine. Very low levels (less than 5%) of the D-enantiomers of valine, aspartic acid and glutamic acid were detected in the hydrolysates of tortillas; however, with this test material some technical difficulties (possibly due to the high carbohydrate level in the corn) have so far made the GC procedure less sensitive. The level of LAL in the tortillas was approximately 450 $\mu\text{g/g}$ crude protein.

Response of the Rats

The mean weight gains and food consumption per cage are included in Table 1. The PER values are presented in Table 3. The analysis of variance of the PER results showed a significant F value for treatments and the differences between individual treatment means are indicated by the Duncan superscript letters in Table 3. The diet supplemented with both lysine and tryptophan gave the

TABLE 2
COMPOSITION OF THE TEST MATERIALS

Material	Proximate components, %				Amino acids (g/16g N)*		
	H ₂ O	Ether extract	Ash	Crude protein	Reactive lysine	Tryptophan	
						Ion-exchange	Colorimetric
Boiled corn	1.8	4.8	1.8	10.9	2.69(4)	0.73(4)	n.d.
Tortillas	6.8	5.0	2.1	10.2	2.88(4)	0.72(2)	0.56(2)
Raw amaranth	—	—	—	—	5.80(5)	1.55(3)	1.61(3)
Popped amaranth	3.6	6.0	2.4	14.6	4.04(2)	1.61(3)	1.49(3)

* The number of replicates on which each amino acid value is based is given in parentheses. The pooled estimates for the standard deviation of a single determination are 0.043 for lysine and for tryptophan, 0.051 by ion-exchange chromatography, and 0.108 by the colorimetric method.

TABLE 3

PER OF DIETS ACCORDING TO PROTEIN SOURCES
AND SUPPLEMENTARY AMINO ACIDS (WITH NUMBER
OF CAGES PER DIET IN PARENTHESES)*

Protein sources	Supplementary amino acids		
	Lys (i.e. Trp limiting)	Trp (i.e. Lys limiting)	Lys & Trp
Boiled corn (BC)	1.44 ^{cd} (7)	1.13 ^d (7)	—
Tortilla (TT)	1.65 ^c (7)	1.17 ^d (7)	2.77 ^a (4)
BC + popped amaranth	2.11 ^b (4)	1.16 ^d (4)	—
TT + popped amaranth	2.32 ^b (4)	1.19 ^d (4)	—

* Two treatment means are significantly different at the 5% probability level if they do not share a common superscript letter.

highest value. There was no difference between PER values for boiled corn and tortilla, regardless of how they were supplemented. Adding the popped amaranth gave a significant response to both materials in the presence of lysine (i.e., when tryptophan was limiting) but not when lysine was limiting.

It was a consistent finding when corn was the sole protein that the diets supplemented with lysine resulted in lower food consumption than the corresponding diets supplemented with tryptophan, even though the former supported equal or better growth in every case (Table 1). Calculation of net protein retention (NPR) (28), assuming that the rats would have lost 1.4% of their body weight on a protein-free diet, did not change the ranking of the diets from that obtained by PER calculations.

DISCUSSION

The analyses in Table 2 demonstrate little difference between boiled corn and tortillas. Also the tortillas failed to show a detectable level of D-lysine under our rather insensitive conditions. This indicates that there would be little difference in the PER values of the two materials and that is precisely what we found in the rat experiment. It was interesting to note that the three pure amino

acids (including lysine) submitted to a similar alkali treatment also gave no evidence of racemization. It is possible, therefore, that the fall in solubility of $\text{Ca}(\text{OH})_2$ as water temperature increases reduces the severity of the treatment.

The LAL value for tortillas of 450 ppm of crude protein is of the same order as the values of 200 ppm (12) and 810 ppm (10) reported in the literature. It corresponds to approximately 1% of the lysine originally present in the corn. This level of LAL has apparently had no effect in our short-term rat experiment, and we do not know what effect it might have in a longer study.

As expected, the raw amaranth seeds have shown quite a high lysine content, but the analysis of the popped amaranth shows a significant fall of about 30% in reactive lysine as a result of the cooking process. However, in view of the failure of the popped amaranth to supplement the value of the corn proteins when lysine was made the first limiting amino acid by the addition of tryptophan, it appears that the level of "available" lysine was reduced even further than the 30% measured by the chemical test.

The tryptophan analyses reveal approximately the expected value for whole corn. The values for the two amaranth samples are considerably higher than for corn and higher than has hitherto been reported (29). We thought it possible that the results from the ion-exchange are slightly high due to interference from the LAL formed during the alkaline hydrolysis step in the analysis and/or to the presence of amino-sugars (19). The values, however, were confirmed using a specific colorimetric method (21). It also seems certain that the true tryptophan value is considerably higher than that of corn in view of the considerable supplementary value of the popped amaranth in the PER study when tryptophan was made the first limiting amino acid. It will be interesting to determine whether heat-labile amino acids such as cystine are more damaged during popping than tryptophan appears to be.

RESUMEN

EFFECTOS DEL TRATAMIENTO ALCALINO DEL MAIZ Y DE SU SUPLEMENTACION CON SEMILLAS DE AMARANTO, RESPECTO A SUS DEFICIENCIAS EN LISINA Y TRIPTOFANO

El maíz en forma de tortillas es el principal alimento del pueblo mexicano. El presente trabajo tuvo por objeto estudiar las deficiencias de amino-

ácidos en las tortillas (TT) y en maíz hervido (BC), así como el efecto suplementario de la semilla de amaranto (PA), otro alimento tradicional usado en México después de tostado, proceso mediante el cual revienta, convirtiéndose en "alegrías". La duración del experimento en ratas de 23 días de edad, para determinar su PER, fue de 14 días. Cada dieta contenía 8.95% de proteína cruda (CP) derivada totalmente del maíz, o de PA que aportaba solamente 3.6% de CP, proviniendo el resto del maíz, adicionado de suplemento mineral y vitamínico. Las dietas fueron suplementadas con lisina o triptofano, a modo de hacer limitante uno u otro aminoácido. En ningún caso dieron las TT un valor estadísticamente diferente al del BC. Cuando el triptofano era limitante, el PER promedio del BC y de las TT fue de 1.55, y con la PA, de 2.22. El contenido de triptofano en el amaranto fue relativamente alto (1.55 g/16g N). Al ser lisina el limitante, el PER promedio de las dietas con maíz fue de 1.15; la adición de PA no incrementó este valor. El tratamiento térmico al amaranto redujo el valor de la lisina reactiva (método de enlace con colorantes) de 5.9 a 4.0 g/16g N. Aparentemente, la preparación de tortillas no ejerce ningún efecto adverso en el valor nutricional de la proteína, pero el tratamiento térmico en el amaranto reventado, sí reduce dicho valor.

Los PER más altos en las dietas que contenían maíz con triptofano limitante vs. lisina como limitante, se debieron a un menor consumo de alimento *ad libitum* con el mismo aumento en peso.

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