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FINAL REPORT

A PRACTICAL APPROACH TO THE LYSINE AND TRYPTOPHAN SUPPLEMENTATION OF LIME-TREATED CORN

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PHASE I (Report No. 1)

STABILITY OF ADDED LYSINE AND TRYPTOPHAN I CRYSTALLINE FORM AND IN PROTEIN DURING TORTILLA PREPARATION FROM TORTILLA FLOUR.

INTRODUCTION

In the final stage of tortilla preparation the lime-treated corn dough is patted into round flat cakes which are placed on a plate of baked clay in which the temperature averages 212° C in the middle area and 170° C on the edges. Cooking is carried out for about 3 minutes for each side. Moisture content drops from about 61% to approximately 48% (1, 2). It is also well known that heating at high temperature causes reactions to take place between amino acids and carbohydrates, which results in a decrease in the physiological availability of amino acids, of which lysine is most affected (6).

In view of this and before proceeding with other studies, it was essential to determine if the process described above would decrease added lysine and tryptophan in crystalline form or as protein.

MATERIALS AND METHODS

For the study, 40 Kg of white corn dough was divided into four portions, of 10 Kg each, A, B, C and D. Portion A was used as control, Portion B, was supplemented with 3% Torula Yeast, on the basis of dried dough, Portion C, was supplemented with 3% Torula Yeast and 0.15% L-lysine HCl,

was also added on the basis of dried corn dough and Portion D was fortified with 0.3% L-lysine HCl and 0.1% DL-tryptophan on the basis of dried dough. The levels of Torula Yeast and amino acids added were those found most efficient to improve protein quality in other studies (3, 4).

Each preparation was mixed for about an hour and they were then divided into two portions of equal weight. One of the halves from each was then used for tortilla preparation, made by a person with experience in such operation.

After the tortillas were made, all samples were dried by lyophilization, ground and stored until used for chemical and biological assays. The samples were analyzed for moisture and nitrogen using AOAC official methods (5).

For the biological assays, the samples were incorporated into the basal diet described in TABLE 1, using 90% of each preparation. Casein, to provide 10% in the diet, was used as a control.

A total of 72 rats, of 21 days of age, of the Wistar strain, from the INCAP colony were distributed by weight among 9 experimental groups, so that initial weight per group was the same. The animals were placed in individual wire screen cages with raised screen bottoms and were fed ad libitum for

28 days, having water available at all times. Data on weight changes and food consumed were received every 7 days.

RESULTS AND DISCUSSION

Data collected during the process of cooking the uncooked corn masa is shown in TABLE 2. Temperature of the plate varied from 150 to 200° C depending on the spot used to make the measurement. The cooking time per tortilla averaged 4 minutes. These results are in agreement with previous reported findings (1, 2). The corn masa and the tortilla contained 54.6 and 34.6% moisture, with a 20% water loss due to cooking. These values are also similar to values previously reported (1, 2).

The values for moisture, lysine and tryptophan for the basal preparation and the moisture and nitrogen for the other preparations are shown in TABLE 3. The data shows slightly higher values for nitrogen content of tortillas, which in most cases can be accounted for on the basis of the lower moisture of tortillas and because Torula Yeast provided 1.5 g of protein in Portions B and C.

The results of biological tests are shown in TABLE 4. The effect of the supplements in weight gained and PER are shown in the four dough and tortilla preparations. When each dough preparation and its respective tortilla samples are

compared, no major change is observed, except in the preparations with 3% added Torula Yeast. In this case, a slightly lower weight gain and PER was noticed for the tortilla in comparison with the dough but the results are, however, better than those obtained with the unsupplemented dough and tortilla.

Best results were obtained when only amino acids were added. It is probable that 3% yeast did not provide the same relative amounts of equivalent amino acids as compared to that added in crystalline form.

None of the supplements improved the quality of the protein to the value obtained with casein.

CONCLUSIONS

1. The materials used as supplements improved the protein quality of corn protein, corroborating previous published results.

2. The conversion of dough into tortilla does not decrease the quality of the protein as determined by biological assay, indicating that the added amino acids were not inactivated by the type of processing involved.

CURRENT ACTIVITY

Various methods for the addition of crystalline amino acids at the scale of the typical village mill are being

investigated. Addition of amino acids into the water used to mill the corn, as compared to the addition of amino acids in dry form either in the pill form or as a powder has been undertaken. The relative efficacy of these procedures in terms of operating procedures and distribution of the amino acids in the masa is being studied.

TABLE 1

DIET COMPOSITION - grams/100g

INGREDIENTS		SUPPLEMENTS	1	2	3	4	5	6	7	8	9
Corn masa	A	none	90.0	----	----	----	----	----	----	----	----
tortilla		none	----	90.0	----	----	----	----	----	----	----
Corn masa	B	3% Torula Yeast	----	----	90.0	----	----	----	----	----	----
tortilla		3% Torula Yeast	----	----	----	90.0	----	----	----	----	----
Corn masa	C	3% Torula Yeast+ 0.15% L-lys HCl	----	----	----	----	90.0	----	----	----	----
tortilla		" " "	----	----	----	----	----	90.0	----	----	----
Corn masa	D	0.3% L-lys HCl+ 0.1% DL-Tryp	----	----	----	----	----	----	90.0	----	----
tortilla		" " "	----	----	----	----	----	----	----	90.0	----
Casein		----------	----	----	----	----	----	----	----	----	11.20
Mineral mixture		----------	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Cottonseed oil		----------	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Cod liver oil		----------	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Corn starch		----------	----	----	----	----	----	----	----	----	78.80
Total			100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Vit. Soln. ml			5	5	5	5	5	5	5	5	5

A: Control
B: 3% Torula Yeast
C: 3% Torula Yeast Plus 0.15% L-Lysine HCl
D: 0.3% L-Lysine HCl and 0.1% DL-Tryptophan

TABLE 2

DATA COLLECTED DURING COOKING OF CORN DOUGH TO MAKE TORTILLAS

MEASUREMENTS	
Temperature of plate:	150 - 200 C
Baking time, av.:	4 minutes, total
Weight of uncooked tortilla,av.:	55 grams
Weight of cooked tortilla, av.:	44 grams
Moisture content of uncooked tortilla:	54.6%
Moisture content of cooked tortilla:	34.6%
Water loss during cooking of tortilla:	20%

TABLE 3

NITROGEN CONTENT OF VARIOUS PREPARATIONS AND LYSINE AND TRYPTOPHAN CONTENT OF CONTROL MASA (Dough) AND TORTILLA

Preparation	Portion	Moisture %	Nitrogen %	Lysine %	Tryptophan %
Corn masa	A	8.65	1.59	0.278	0.058
tortilla	A	4.83	1.64	0.288	0.070
Corn masa	B	11.94	1.72	-----	-----
tortilla	B	5.34	1.79	-----	-----
Corn masa	C	11.41	1.69	-----	-----
tortilla	C	8.26	1.68	-----	-----
Corn masa	D	10.62	1.44	-----	-----
tortilla	D	5.50	1.51	-----	-----

A: Control
B: 3% Torula Yeast
C: 3% Torula Yeast Plus 0.15% L-Lysine HCl
D: 0.3% L-Lysine HCl and 0.1% DL-Tryptophan

TABLE 4

WEIGHT GAIN AND PER OF RATS FED THE VARIOUS PREPARATIONS

Diet	Treatment	Protein diet, %	Av. wt. gain, g[1]	PER[2]
Corn masa	none	8.87	25 ± 2.0[3]	1.28 ± 0.6[3]
tortilla	none	9.46	23 ± 3.5	1.25 ± 0.5
Corn masa	3% Torula Yeast	9.65	52 ± 3.6	1.96 ± 0.8
tortilla	3% Torula Yeast	10.46	47 ± 3.5	1.76 ± 0.6
Corn masa	3% TY[4] + 0.15% L-lys HCl	9.18	64 ± 3.0	2.29 ± 0.7
tortilla	3% TY + 0.15% L-lys HCl	9.62	64 ± 4.7	2.17 ± 0.5
Corn masa	0.3% L-lys HCl + 0.1% DL-tryp	8.09	59 ± 4.6	2.42 ± 1.0
tortilla	0.3% L-lys HCl + 0.1% DL-tryp	8.56	61 ± 7.3	2.53 ± 1.0
Casein	----------	9.88	101 ± 8.6	3.06 ± 0.8

1 Av. Initial Weight: 47 g.
2 Experimental Period: 28 Days
3 Average ± Std. Error
4 TY = Torula Yeast

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PHASE I (Report #2)

CHEMICAL STABILITY OF ADDED LYSINE AND TRYPTOPHAN IN CRYSTALLINE FORM AND IN PROTEIN DURING TORTILLA PREPARATION FROM TORTILLA FLOUR

And

IMPROVEMENT OF THE PROTEIN QUALITY OF LIME TREATED CORN MASA WITH SUPPLEMENTS RICH IN LYSINE AND TRYPTOPHAN

CHEMICAL STABILITY OF ADDED LYSINE AND TRYPTOPHAN IN CRYSTALLINE FORM AND IN PROTEIN DURING TORTILLA PREPARATION FROM TORTILLA FLOUR

INTRODUCTION

The results presented in the First Quarterly Progress Report indicated that the addition of lysine and tryptophan to lime-treated corn dough increased the quality of its protein. It was also shown that the process to convert enriched corn dough into tortilla did not decrease the quality of the product as measured by biological assays. This process involves the use of high temperature conditions for a short time, but which apparently do not decrease the amounts of added amino acids. In order to obtain additional information on this point, similar tests as those previously described were carried out, except that the evaluation was performed by chemical analysis for the added amino acids before and after processing.

MATERIALS AND METHODS

Common Guatemalan corn was cooked with lime as previously indicated. A total of four samples of 1200 grams each were then enriched with 3% Torula yeast, 3% Torula yeast + 0.15% L-Lysine HCl and 0.30% L-Lysine HCl + 0.10% DL-Tryptophan. Subsamples of 300 grams each were then made into tortillas, cooking them for 3, 4.5, and 6 minutes. After cooking, the samples were freeze dried, ground and stored in glass containers at 4°C until analyzed. All samples were analyzed for

moisture, fat, fiber, nitrogen and ash, following AOAC official methods of analysis (1). Total lysine and tryptophan was obtained by microbiological assay techniques (2), on acid or alkaline hydrolyzated and available lysine by the method of Conkerton and Frampton (3). From the results of the analysis it was possible to calculate the recoveries of added amino acids after processing.

RESULTS AND DISCUSSION

Table 1 summarizes the proximate composition of all freeze dried samples. Changes in composition within treatment are mainly attributed to moisture content. Differences in composition between treatments are due to the supplements added. It can be seen that the addition of Torula yeast increased protein content over the basal level as was expected. The addition of both amino acids, also increased nitrogen content slightly due to the nitrogen derived from them. Cooking time did not affect the results.

Table 2, summarizes the content of total lysine and tryptophan as well as the recoveries of the two amino acids in the various samples. No changes in lysine and tryptophan content due to baking time was observed when the tortilla contained 3% Torula yeast. It should be indicated that in this case the

amino acids are bound in the protein which reduces the chances of inactivation or destruction.

A substantial decrease in lysine content occurred when the corn masa was supplemented with 3% Torula yeast and 0.15% L-Lysine HCl and baked for 6 minutes. In this case, the calculations of lysine recovered took into consideration the moisture content of the samples, its nitrogen content and that only 80% of the lysine added is active. Tryptophan on the other hand remained essentially the same at all baking time intervals.

Good recovery results were obtained when corn masa was supplemented with synthetic amino acids only. The recovery value for lysine of 98.8% at 6 minutes of baking is being confirmed. As before, tryptophan did not change with baking time.

In conclusion, it appears that added lysine to corn masa decreased slightly with increases in baking time. However, a baking time of 6 minutes very seldom occurs in actual tortilla preparation.

Future studies will consist in getting information on the stability of added amino acids in samples prepared once a day, in the morning and warmed at least three times during the course of the day, before consumption. This is a custom usually

carried out by families with the tortilla.

It is recognized that the method used to estimate recoveries of added amino acids is subject to various errors, which are those occurring in the determinations of moisture, protein and the amino acid itself. However, the errors are not large enough to alter the conclusions since when tryptophan recoveries were calculated from experimental results a value of 0.086 grams of tryptophan per gram N was found which compares favourably with the theoretical value of 0.084 grams tryptophan per gram N for the sample of unbaked corn masa supplemented with 0.30% L-Lysine HCl and 0.10% DL-Tryptophan.

In connection with the present study, rapid amino acid assay techniques are being developed, which consist in extracting the added amino acid, separating them from others using low-voltage electrophoresis, followed by their quantitative analysis. Methods for lysine are already working satisfactorily and some encouraging results have been obtained with tryptophan analysis.

The samples prepared for the experiment were analyzed for (a) proximate chemical composition (Table 1 of Progress Report No. 2); (b) lysine and tryptophan content, using microbiological techniques (Table 2 of Progress Report No. 2); and (c) for available lysine.

Available lysine analysis becomes of importance in foods which are subjected to high temperatures during processing. The values differ from microbiologically determined values in that, available lysine represents the amount of lysine which is biologically available, that is the amount of lysine that has not reacted with sugars during the preparation process and therefore is available for absorption because it has not been complexed.

On the other hand microbiologically determined figures represent total lysine since the method includes complete hydrolysis of protein including any carbohydrate bound lysine.

The available lysine results are given in Table 2B and indications are that usually there is a small decrease in lysine as baking time increases. The decrease, however, is not consistent since it was found in some preparations (tortilla alone, tortilla + 3% Torula yeast + 0.15% L-Lysine HCl), but not others. In any case the decrease is small and the observed variability could very well be due to sampling errors.

The determination of available lysine is important and additional studies will be carried out not only on tortillas baked for different time intervals, but, more importantly, on tortillas rewarmed during the day. It is a common practice

in Guatemala to prepare tortillas once a day, usually for the noon meal and then to rewarm them for the evening meal of the same day and for breakfast on the following day.

IMPROVEMENT OF THE PROTEIN QUALITY OF LIME TREATED CORN MASA WITH SUPPLEMENTS RICH IN LYSINE AND TRYPTOPHAN

INTRODUCTION

The results of previous investigations suggested that the highest improvement in the protein quality of lime treated corn was obtained when it was supplemented with 0.30% L-Lysine HCl and 0.15% DL-Tryptophan.

Since tryptophan is still an expensive amino acid increasing significantly the cost of the final product, its addition to corn masa could be carried out as well by adding it in the form of protein. Although lysine is now more economical, still the addition of 0.30% could increase the price of tortillas beyond the purchasing capacity of maize eating people.

In view of this, tests were performed to learn if it was possible to reduce the levels of the two amino acids by adding them as protein. These would have the added advantage that total nitrogen in the final product would also be increased.

MATERIALS AND METHODS

The studies to be described were carried out with common Guatemalan corn. As protein supplements, skim milk powder and soybean flour were used. These were added to corn in amounts which would provide sufficient tryptophan. To learn

if further increases in quality could be obtained by lysine addition, the difference of the lysine provided by the supplement to 0.30% was added as crystalline lysine.

All materials were added to the cooked corn at the time it was being milled to produce corn masa. A description of the various preparations is given in Table 3. Opaque-2 corn was included for comparative purposes. A subsample of the masa and of the tortilla made from it was freeze dried for chemical and amino acid analysis (the chemical results are not included in this report). The effect of the supplement in the form of dried masa only was measured biologically.

The composition of the rat test diets is given in Table 4. The diets were fed to groups of 8 rats each, for a 28 day experimental period. The animals were distributed by weight and sex among treatments, so that initial weight and number of male and female rats was the same in all groups. The rats were placed in individual cages with raised screen bottoms, receiving water and the diet ad libitum.

Two controls were included: lime treated Opaque-2 corn masa and casein.

RESULTS AND DISCUSSION

The growth performance of the rats and the protein efficiency of the various preparations are shown in Table 5.

The addition of synthetic amino acids, increased weight gain almost 3-fold and more than doubled the PER of corn masa. These results confirmed again the improvement to be obtained from the addition of the two amino acids to corn masa protein. It is of interest to point out that the corn masa made from Opaque-2 corn, induced better gains in weight although not in PER than the treatment with both amino acids added.

The addition of skim milk and of soybean flour increased weight gain and PER over the basal level; however, PER was not as high as that obtained from the addition of the two amino acids which was interpreted as the effect of the higher protein content in the diet, which is known to reduce PER. The finding of interest was that 0.10% Lysine HCl added with 8% skim milk increased both, weight gain and PER, significantly. This was not the case when corn masa was supplemented with 8% soybean flour and 0.06% Lysine HCl. A response was expected which did not occur probably because the level of lysine added was low. It is also possible that soybean protein already provided lysine in enough quantity making further addition unnecessary. If this is the case, methionine was probably the amino acid which was limiting.

This possibility will be the subject of future studies. The results show therefore, that the addition of 8% skim

milk and 0.10% lysine is a good supplement as well as 8% soybean flour, to corn masa. The levels used apparently supply the levels of tryptophan needed in a mixture with lysine to improve the quality of corn masa proteins.

These two sources of whole protein can be molded into specific forms, which could conceivably be used as supplements to cooked corn kernel, and added at the milling stage of tortilla preparation.

Table No. 1 PROXIMATE COMPOSITION OF SAMPLES (g/100 g)

Samples	Baking time, min.	Water	Fat	Crude Fiber	Nitrogen	Protein	Ash
Tortilla (T)	0	13.6	4.3	2.3	1.448	9.0	1.4
Tortilla	3	7.2	3.6	3.1	1.552	9.7	1.5
Tortilla	4.5	9.3	3.5	2.7	1.511	9.4	1.4
Tortilla	6	7.3	3.5	2.7	1.576	9.8	1.5
T + 3% Torula Yeast	0	12.8	4.1	2.2	1.682	10.5	1.5
T + 3% Torula Yeast	3	6.2	3.7	2.6	1.801	11.2	1.6
T + 3% Torula Yeast	4.5	6.1	3.6	2.5	1.807	11.2	1.6
T + 3% Torula Yeast	6	6.3	3.7	2.8	1.802	11.2	1.6
T + 3% Ty + 0.15% L-Lys HCl	0	12.6	4.1	2.5	1.678	10.5	1.5
T + 3% Ty + 0.15% L-Lys HCl	3	7.3	3.7	2.8	1.800	11.2	1.6
T + 3% Ty + 0.15% L-Lys HCl	4.5	7.0	3.7	3.1	1.807	11.2	1.6
T + 3% Ty + 0.15% L-Lys HCl	6	6.5	3.7	3.0	1.801	11.3	1.6
T + 0.30% L-Lys HCl + 0.10% DL-Try	0	12.3	4.4	2.5	1.544	9.6	1.3
T + 0.30% L-Lys HCl + 0.10% DL-Try	3	5.4	3.9	3.0	1.644	10.2	1.5
T + 0.30% L-Lys HCl + 0.10% DL-Try	4.5	3.9	4.0	2.8	1.668	10.4	1.5
T + 0.30% L-Lys HCl +).10% DL-Try	6	3.9	4.0	2.8	1.676	10.4	1.5

Table No. 2 LYSINE AND TRYPTOPHAN CONTENT OF SAMPLES

Sample	Baking Time Min.	Lysine %	Lysine g/gN	Lysine Recovery %	Tryptophan %	Tryptophan g/gN	Tryptophan Recovery %
Tortilla	0	0.294	0.198	-----	0.079	0.053	-----
	3	0.318	0.205	-----	0.088	0.057	-----
	4.5	0.304	0.201	-----	0.081	0.054	-----
	6	0.314	0.199	-----	0.086	0.055	-----
Tortilla + 3% Torula Yeast (1)	0	0.429	0.255	105.8	0.095	0.056	94.9
	3	0.467	0.259	107.5	0.099	0.055	93.2
	4.5	0.465	0.257	106.6	0.113	0.062	105.1
	6	0.446	0.247	102.5	0.101	0.056	94.9
Tortilla + 3% TY* + 0.15% L-Lys HCl (2)	0	0.507	0.302	96.5	0.096	0.057	96.6
	3	0.575	0.319	101.9	0.105	0.058	98.3
	4.5	0.521	0.288	92.0	0.112	0.062	105.1
	6	0.412	0.228	72.8	0.116	0.064	108.5
Tortilla + 0.30% L-Lys HCl + 0.10% DL-Try (3)	0	0.572	0.370	106.3	0.124	0.080	95.2
	3	0.591	0.359	103.2	0.130	0.079	94.0
	4.5	0.519	0.311	89.4	0.165	0.099	117.8
	6	0.578	0.344	98.8	0.166	0.099	117.8

Theoretical values for Lysine:

	Lysine		Tryptophan	
(1)	0.241	g/gN	0.059	g/gN
(2)	0.313	g/gN	0.059	g/gN
(3)	0.348	g/gN	0.084	g/gN

*TY = Torula yeast

Table No. 2B

AVAILABLE LYSINE CONTENT OF SAMPLES

Samples	Baking Time Minutes	Available Lysine g/gN
Tortilla	0	0.211
	3	0.205
	4.5	0.232
	6	0.187
Tortilla + 3% Torula Yeast	0	0.239
	3	0.223
	4.5	0.217
	6	0.247
Tortilla + 3% Torula Yeast + 0.15% L-Lysine HCl	0	0.279
	3	0.268
	4.5	0.241
	6	0.226
Tortilla + 0.30% L-Lysine HCl + 0.10% DL-Tryptophan	0	0.289
	3	0.271
	4.5	0.271
	6	0.265

Table No. 3 DESCRIPTION OF VARIOUS PREPARATIONS

Preparation No. 1	10 lbs of corn were cooked with lime. No supplements added.
Preparation No. 2	10 lbs of corn were cooked with lime. Upon grinding 14g L-Lysine HCl (0.30%) and 4.5g DL-Tryptophan (0.10%) were added.
Preparation No. 3	10 lbs of corn were cooked with lime. Upon grinding 395g of skim milk (8%) were added.
Preparation No. 4	10 lbs of corn were cooked with lime. Upon grinding 395g of skim milk (8%) amd 4.5g L-Lysine HCl (0.10%) were added.
Preparation No. 5	10 lbs of corn were cooked with lime. Upon grinding 395g of soyflour (8%) were added.
Preparation No. 6	10 lbs of corn were cooked with lime. Upon grinding 395g of soyflour (8%) and 3g of L-Lysine HCl (0.06%) were added.
Preparation No. 7	10 lbs of Opaque-2 corn were cooked with lime as for Preparation No. 1.

Table No. 4 COMPOSITION OF RAT TEST DIETS (%)

Ingredients	1	2	3	4	5	6	7	8
Corn Masa	90.0	----	----	----	----	----	----	----
Corn Masa + Lys + Try	----	90.0	----	----	----	----	----	----
Corn Masa + Skimmilk	----	----	90.0	----	----	----	----	----
Corn Masa + Skimmilk + Lysine	----	----	----	90.0	----	----	----	----
Corn Masa + Soyflour	----	----	----	----	90.0	----	----	----
Corn Masa + Soyflour + Lysine	----	----	----	----	----	90.0	----	----
Corn Masa Opaque-2	----	----	----	----	----	----	90.0	----
Casein	----	----	----	----	----	----	----	11.2
Minerals	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Cottonseed Oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Cod Liver Oil	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Corn Starch	----	----	----	----	----	----	----	78.8
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Vit. Sol. ml	5	5	5	5	5	5	5	5

Table No. 5 SUPPLEMENTATION OF LIME-TREATED CORN WITH AMINO ACIDS AND PROTEINS

Supplement	Protein Diet, %	Av. Wt. Gain, g	P.E.R.
None	7.9	25	1.26
+ 0.30% L-Lys HCl + 0.10% DL-Try	8.0	72	2.78
+ 8% Skimmilk	9.8	81	2.23
+ 8% Skimmilk + 0.10% L-Lys HCl	9.7	114	2.99
+ 8% Soyflour	10.0	96	2.34
+ 8% Soyflour + 0.06% L-Lys HCl	9.7	98	2.43
Opaque-2 Corn	10.1	109	2.66
Casein	9.0	103	2.50

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PHASE I (Report #3)

STUDIES ON PRACTICAL METHOD OF FORTIFICATION OF CORN WITH PROTEINS OR AMINO ACIDS

STUDIES ON PRACTICAL METHOD OF FORTIFICATION OF CORN WITH PROTEINS OR AMINO ACIDS

INTRODUCTION

Evidence was presented in the previous quarterly progress reports (No. 1 + No. 2) that the quality of corn proteins in the form of tortilla could be significantly improved through the addition of 0.30% L-Lysine HCl and 0.10% DL-Tryptophan, or 8% Skimmilk and 0.15% L-Lysine HCl, 3% Torula yeast, and 0.15% L-Lysine HCl or other combinations. Likewise, it was shown that the conditions used for preparing tortillas does not significantly destroy or inactivate the added supplements, although additional evidence is still needed. The question of the most appropriate method by which to add such supplements to corn is important since the corn consumed is either processed in the home, or carried out in small mills almost always present in villages or localities where people live. The present report deals with attempts to study the methodology which could be used to implement the findings reported in previous reports.

MATERIALS AND METHODS

To study the distribution of amino acids during milling the following experiments were carried out.

1. Four pounds of cooked corn were divided into four equal parts before milling. Part A, was milled and used as

control, while part B, C, and D, were milled at which time carmine or charcoal present in the concentration of the two amino acids (0.30% L-Lysine HCl and 0.10% DL-Tryptophan) was added as: (a) powder, (b) synthetic corn kernel or pill, and (c) aqueous solution. The mill was thoroughly washed after finishing the milling of each part with the specific form studied. After milling the dough was picked up, pressed together without mixing and cut in half with a knife. The distribution of the red or black color in the cut halves was used as evidence of the efficiency of distribution of the substances added.

2. Four pounds of white corn were cooked in lime and after cooking the corn was divided into four equal parts. Part A served as control and no amino acids were added to it. Parts B, C, and D, were supplemented with 0.8625 grams of lysine HCl and 0.230 grams of DL-tryptophan added as powder, synthetic kernels or pills, and as a solution. The supplements were added at the time of milling and collected as before. Tortillas were made from each part, taking at random 8 per group. These were warm air dried for 24 hours and then ground. The

content of lysine present in each tortilla was then assayed by microbiological techniques. No attempts were made to measure tryptophan because previous results suggested it to be stable and because of the lack of appropriate extraction and analytical techniques. This aspect however, is being examined in current studies, the results of which will be reported in a later report.

RESULTS AND DISCUSSION

A. Distribution of Carmine and Charcoal

The color photographs show that the synthetic kernels or the powder were the most efficient ways to fortify the corn at the mill (Figures 1 and 2).

B. Distribution of Lysine

Table 1, shows the results of lysine recoveries with respect to method of fortification. It is of interest to see that the addition of lysine in solution was not very efficient, since recoveries of lysine ranged from 60.9 to 75.3% with an average of 65.9% being the lowest among the three methods of fortification. On the other hand, when the amino acids were added as powder or as a pill or synthetic kernel recoveries averaged 94.0 and 97.1% respectively but the range in recoveries was not substantially different when tortilla

sample no. six is considered. It appears to be a significant outlier, in the case of both the powder and pill form results.

The results indicate, therefore, that the addition of the amino acids is efficient when added as powder or as a synthetic kernel, but not when added as a solution. These results corroborate the distribution of colors and it may be explained on the basis of loss of liquid when the corn is being milled, either by the splashing of the liquid, on the sides and that which is lost by not being absorbed by the dough.

Considering all the information thus far available it appears that the method which at the present time would have the greatest chance of practical success may be the use of synthetic kernels made from soybean flour and lysine, to which riboflavin, vitamin A or other nutrients could also be added. These synthetic corn kernels have already been produced at our request by the Institute of Technology of Colombia, using a regular pasta extruder with the appropriate dye. No information is available as yet on the use of these synthetic kernels. The experiments previously described were made with kernels prepared at the INCAP laboratories. Similarly, no information is available on their cost. It is hoped

that answers to these questions can be provided prior to the completion of the present AID grant, as well as answers to questions raised in previous reports.

TABLE NO. 1 LYSINE RECOVERIES (%)

Tortilla No.	Lysine added as:		
	Solution	Powder	Pill (synt. kernel)
1	60.94	95.04	98.24
2	64.73	90.41	98.64
3	66.38	97.50	96.46
4	61.60	94.83	98.04
5	63.75	100.70	96.67
6	68.83	84.85	87.17
7	66.50	95.04	98.00
8	75.29	97.50	103.25
Average	65.92	93.96	97.06
Range	16.08	15.85	14.35

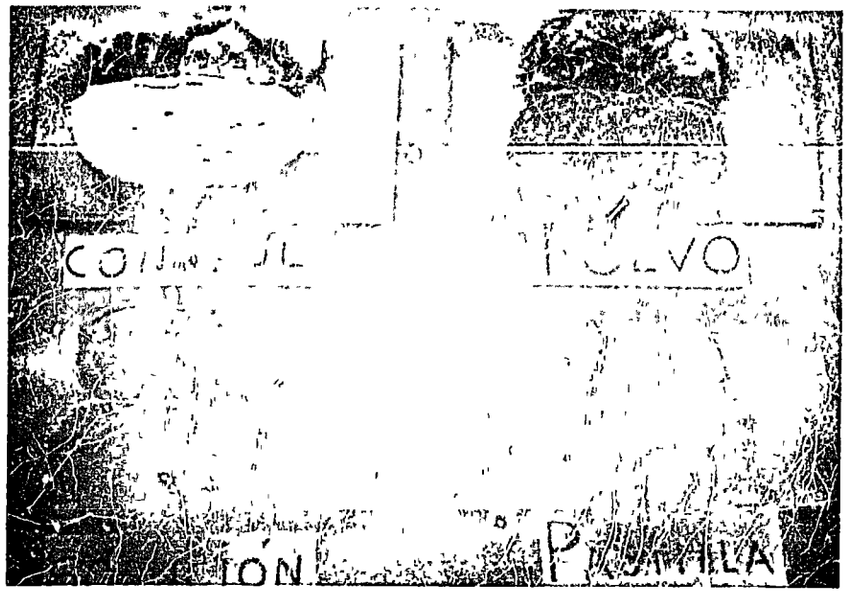

Figure No. 1

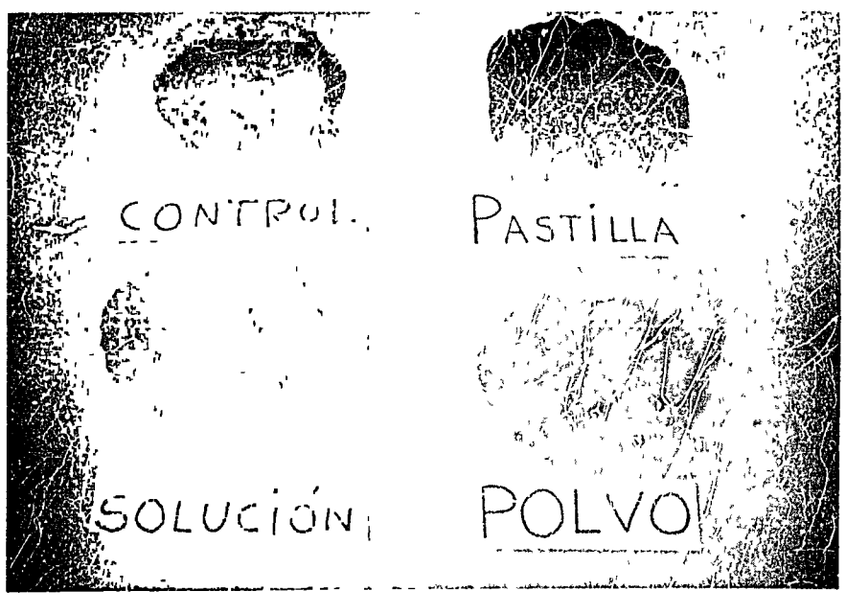

Figure No. 2

PHASE I (Report #4)

FORMULATION AND COST COMPARISON OF CORN FORTIFICATION MIXTURES

FORMULATION OF SYNTHETIC CORN KERNEL (OR PELLETS)

From the evidence presented in Progress Report No. 3 (January 1 - March 31, 1970), it was concluded that the fortification method for lime-treated corn at the milling stage that technically has the best chance of practical success is that involving the use of synthetic kernels. Results of biological tests in rats as presented previously in Progress Reports No. 1, 2, and 3 indicated that three types of supplements were effective in improving significantly the quality of lime-treated corn. These are: (1) the addition of 0.30% L-Lysine HCl and 0.10% DL-Tryptophan; (2) the addition of 8% skimmilk powder (as a source of tryptophan) and 0.10% L-Lysine HCl; and (3) the addition of 8% soybean flour (as a source of tryptophan) and 0.10% L-Lysine HCl. Furthermore, it was also found that supplements of riboflavin, thiamin and niacin, should be added in the following amounts, 3 mg of B_1 and B_2 per 100 g, and 5 mg of niacin per 100 g of corn. These supplements permit maximum efficiency in the utilization of the added amino acids. Since nutritional surveys carried out in the Central American area have found wide spread vitamin A deficiency, this vitamin should be included in the formulation to be used for fortification.

Taking the above information into consideration, three synthetic corn kernel formulations were developed. These

are shown in Table I.

COST OF FORMULATIONS

Based on the prices of the ingredients as shown in Table 2, the estimated cost per 100 lbs of each formulation as well as the cost of fortification per pound of corn is shown in Table 3.

Formula No. 1 costs $30 per 100 lbs not including manufacturing of the synthetic kernel if this was the form in which it is to be added. On the same basis formulation No. 2 has an ingredients cost of $35 per 100 lbs while formulation No. 3 has a cost of $9.15 per 100 lbs. Using the rates of addition shown in Table 1, the cost of fortification of 100 lbs of corn would be $1.50, $2.80 and $0.732 with formulations 1, 2, and 3 respectively. That is, fortification of one lb of corn would cost 1.5¢, 2.8¢ or 0.732¢ respectively. It should be indicated that 0.732¢ per pound of formulation No. 3, will provide the amino acids and vitamins needed to increase the nutritional quality of corn protein and will increase the total protein of the fortified product by 4%. Further, this additional protein will increase the efficiency of utilization of the added nutrients.

This cost analysis reveals the most economical fortifying

mixture to be formulation No. 3. It is the most economical and provides the most additional protein (which without doubt increases the efficiency of the other nutrients added).

There are three possible ways at present to introduce formulation No. 3 into corn. One, as a flour, it could be added to industrial corn-masa flour; two, as synthetic corn kernels to raw whole corn as sold or provided in wages on many fincas (plantations); and three, as synthetic corn kernels to be added to cooked corn at small wet corn mills typical in the rural Indian villages. There is no question that fortification approach No. 1 would work, since it only requires the blending of ingredients in the correct proportions and there is considerable precedence and experience with this type of enrichment at commercial levels. The addition would not be detected, however, a major limitation may be the economic feasibility of the supplement. In this respect, it should be realized that the cost of the additional quantity of protein in formulation No. 3 is significantly less than the cost of the equivalent amount of unsupplemented corn protein, even without considering the improvement in protein quality which results from the addition of the supplement. Furthermore, this approach at the present time is more economical than that from the use of Opaque-2 corn, which currently sells at a 30%

premium over conventional corn in Columbia.

Evaluation of fortification approaches No. 2 and 3, will be the subject of the second phase of this research program.

One of the problems foreseen was the need to determine the adequacy of the corn fortifying mixture, therefore, analytical methods for control of the supplements were developed.

ANALYTICAL METHODS FOR LYSINE IN FORTIFIED CORN

The method which was developed is based on the electrophoretic separation of free amino acids on paper at low voltage.

Samples of fortified tortillas weighing 1.5 g (with lysine alone, or with both lysine and tryptophan) are extracted with 8 ml of 0.1 N hydrocholoric acid solution, repeating the extraction three times. The extract is then diluted to 25 cc with distilled water.

Since tryptophan is a neutral amino acid whose isoelectric point is at pH 5.9 and lysine is a basic amino acid with an isoelectric point at pH 9.7, a good separation is obtained by electrophoretic separation at pH 5.3.

Tortilla samples without supplement are also processed by the same procedure to obtain a correction factor for the free

amino acids in such samples. The concentration of lysine and tryptophan are then calculated from standard curves previously obtained.

In order to test the method, samples fortified with lysine and tryptophan were analyzed by the electrophoretic technique and by microbiological methods. The results shown in Table 4 show good recoveries of the added amino acids.

Table I

SYNTHETIC CORN KERNEL FORMULATIONS

Ingredients	FORMULATIONS No. 1	No. 2	No. 3
		%	
L-Lysine HCl	6.000	1.25000	1.25000
DL-Tryptophan	2.000	-------	-------
Corn	91.844	-------	-------
Skimmilk Powder	------	98.65250	-------
Soybean Flour (Soyoflok 200)	------	-------	98.65250
Riboflavin	0.0060	0.00375	0.00375
Thiamin	0.0400	0.02500	0.02500
Niacin	0.0600	0.03750	0.03750
Vitamin A, g	0.0500*	0.03125*	0.03125*
	100.0000	100.00000	100.00000
Rate of Addition, %	5	8**	8***

*Equivalent to 25,000 I.U. of vitamin A or vitamin A acetate (500,000 I.U./g). This amount provides one-half the recommended level of 750 mcg of retinol per 100 g of tortilla.

**Amount of skimmilk providing 0.04% L-Tryptophan, and 2.8 g of protein.

***Amount of soybean flour providing 0.05% L-Tryptophan and 4.0 g of protein/100 g.

Table 2

PRICES OF INGREDIENTS USED IN FORTIFYING MIXTURES

Ingredients	$/100 lbs
L-Lysine HCl	100.00*
L-Tryptophan	2000.00*
Corn	4.00**
Soybean Flour	8.00**
Skimmilk Powder	34.00**
Vitamin Mix	Not Available

*Reference Altschul, A.M. Food: Proteins for Humans. Chem. & Eng. News, Nov. 24, 1969.

**Local price. Guatemala.

Table 3

COST OF FORMULATIONS AND OF FORTIFICATION*

	FORMULATION		
	No. 1	No. 2	No. 3
Cost/100 lbs	$30.00	$35.00	$9.15
Rate of Addition	5%	8%	8%
Cost of Fortification per 100 lbs	$ 1.50	$ 2.80	$0.732
Cost of Fortification per lb	1.5¢	2.8¢	0.732
Cost of Fortified Corn per lb**	5.5¢	6.8¢	4.732
% Increase in Price	37.5	70.0	18.3

*Considering only the cost of ingredients.

**Current (1970) cost of white corn in Guatemala: $4.00/100 lbs.

Table 4

RECOVERIES OF LYSINE AND TRYPTOPHAN SUPPLEMENTS ADDED TO CORN MASA AND TORTILLAS
COMPARISON OF MICROBIOLOGICAL AND ELECTROPHORETIC METHODS

Samples	% Recovery			
	Microbiological		Electrophoretic	
	Lysine	Tryptophan	Lysine	Tryptophan
Lime-Treated Corn + 0.3% Lysine + 0.1% Tryptophan	106.3	95.2	107.8	110.7
Tortilla 3 min + 0.3% Lysine + 0.1% Tryptophan	103.2	94.0	106.7	100.0
Tortilla 4.5 min + 0.3% Lysine + 0.1% Tryptophan	97.7	117.8	103.2	111.4
Tortilla 6 min + 0.3% Lysine + 0.1% Tryptophan	98.8	117.8	107.9	74.3*
Lime-Treated Corn + 3% Torula + 0.15% Lysine	96.5	-----	111.0	-----
Tortilla 3 min + 3% Torula + 0.15% Lysine	101.9	-----	111.7	-----
Tortilla 4.5 min + 3% Torula + 0.15% Lysine	92.0	-----	103.1	- ---
Tortilla 6 min + 3% Torula + 0.15% Lysine	104.1	-----	113.0	-----

*This value is correct and cannot be explained at this time.

PHASE II

ACCEPTABILITY TESTS ON TORTILLAS FORTIFIED WITH LYSINE AND SOY (FORMULATION No. 3)

ACCEPTABILITY TESTS ON SOYBEAN FLOUR FORTIFIED TORTILLAS

In Central America, corn is consumed in the form of round cakes, known as tortillas. Previous reports described the experiments which led to the formulation of a fortifying mixture based on soybean flour, lysine and vitamins. The addition of 8% of the fortifying mixture to lime-treated corn, improved its protein quality significantly. As part of the program it was necessary to carry out consumer's acceptance tests of the fortified corn in the form of tortillas. For this purpose two different population groups were chosen: one from the urban area representing the middle class, and the other from the rural area, representing the low income group. This group consume tortillas daily in amounts equivalent to about 600 grams of corn.

URBAN AREA

In the case of the urban area, the test was carried out in the cafeteria of an Institution serving lunch. The procedure used was as follows: the supplement was added to the cooked corn, mixed in the laboratory and taken to a mill to make the dough (common masa). The fortified corn masa was then given to the person who was in charge of preparing the tortillas. The tortillas were offered as part of the meal at lunch time, for a five-day period. The first day,

tortilla with no supplement was used in order to have an estimation of the average number of tortillas consumed per person. Those who used the cafeteria facilities were not aware of the test taking place. The consumer acceptance was evaluated by the average consumption of tortillas made with the fortified corn, as compared with those of unsupplemented corn. The results of this test are shown in Table 1. Average tortilla consumed per person, at lunch time, was 2.13 for the unfortified tortilla. With the exception of the second day, the consumption of tortillas made of fortified corn masa, was higher when compared to the consumption of tortillas made the first day. Consumption of fortified tortillas averaged 3.40 per person, per day. The lower fortified tortilla intake during the second day was due to the type of meal offered. The consumption of tortillas varied according to the type of dishes presented. This variable was eliminated in the following days of the consumer's acceptance test, by giving the type of dishes which are usually consumed with tortillas.

From the results presented in Table 1, and from the absence of adverse comments of the people subjected to these tests, it was concluded that the addition of the fortifying mixture did not affect the taste of the tortillas. The person making the tortillas, did not find differences in

cooking time, texture or physical characteristics between the dough with or without the fortifying mixture, or between the respective tortillas.

RURAL AREA

The approach used in the rural area, was different from that used in the urban area, in the sense that people were aware of the addition of the fortifying mixture. They were told that a vitamin supplement would be added to the tortillas in order to improve their health. The technique for the addition of the fortifying mixture, as well as for the evaluation of the acceptance test, were the same as those used in the urban area. The place selected for the test was the INCAP Experimental Farm, and the subjects involved were the laborers working in the field. The results of this test are shown in Table 2. As in the previous case, it can be seen that consumption of tortilla was not affected by the addition of the supplement. The results also show a higher consumption of tortillas in the rural area, as compared to the urban area. As before, the person milling the corn and the one making the tortillas did not report differences when processing corn with or without the addition of the fortifying mixture.

It was concluded that the supplemented tortilla, is completely accepted by both, urban and rural population, with both groups not able to detect differences in the flavor and texture of the tortilla.

TABLE 1

CONSUMER ACCEPTANCE TEST

(Urban Area)

Treatment to corn masa	Number of subjects each day	Number of tortillas consumed	Average tortilla consumed/person per lunch
None	37	79	2.13
+ supplement	30	48	1.60
+ supplement	26	92	3.53
+ supplement	33	106	3.21
+ supplement	28	94	3.36

TABLE 2

CONSUMER ACCEPTANCE TEST

(Rural Area)

Treatment to corn masa	Number of subjects each day	Number of tortillas consumed	Average tortilla consumed/person per day
+ supplement	22	200	9.1
+ supplement	24	263	11.0
None	24	296	12.0
+ supplement	24	316	13.2
+ supplement	26	323	12.4
+ supplement	27	310	11.5
+ supplement	27	302	11.2
None	28	322	11.5
+ supplement	27	326	12.1
+ supplement	28	343	12.4

CORN FORTIFICATION - RECOMMENDED ACTIONS

In the total scheme of national development in any country there are a number of key areas which can affect human resource development. Given that considerable and very valid data exists indicating that this human resource is compromised by the existence of malnutrition, in particular protein calorie malnutrition, and is further stressed by public health problems, with a high incidence of mortality and morbidity from infections and a concomitant low educational performance (a low literacy rate) in spite of efforts to promote education at every level, a national development scheme has three areas which it may emphasize: education, public health and nutrition intervention.

Existing data demonstrates with very little reservation that efforts to promote education, i.e. increase the literacy of the developing people, can be severely constrained by apathy and disease and in particular, protein malnutrition while "in utero" or during infancy. Data also exists to show that efforts to increase the quality of the public health through sanitation and innoculation schemes as well as other preventive medicine procedures does not invariably increase the <u>quality</u> of the human resource even though it may occasionally increase the life span of these individuals and even decrease

the incidence of diseases. In other words, the public health procedures decrease the incidence of insults to the human resource in question, however, these procedures do very little for the biochemistry of the human resourse per se and thus his behavioral and educational performance. Further, we know that a nutrition intervention based on the provision of supplements which are not a part of the diet habits of the people are of limited usefulness in many respects. Further, nutritional interventions with supplements very often fail to reach the critical individuals i.e. the mothers during pregnancy and the child during lactation and immediately after weaning.

Now if one considers a corn eating country in which the diet is substantially corn, i.e. provides 70-80% of the daily calories and protein, it follows that a significant nutrition intervention could possibly occur through the manipulation of this important food in the diet, hopefully without incurring any change in the dietary habits of the people. There are two major approaches available for the nutritional improvement of corn:

(1) The use of a corn which would have a higher concentration of quality protein such as the high lysine strains of corn.

(2) Fortification of the existing corn by the addition of additional protein and the limiting amino acid(s).

Both of these nutrition interventions can increase the quality of the diet and the quality of the human resource. The use of improved hybrid corn requires that the breeding technology already exist in the country in question. that the proper quantity of seed be available and that an Extension Service be sufficient to promulgate the extensive use of this corn such that the economic value of the corn, in spite of its possible low yields or higher price, will be recognized as an improved commodity.

It is our contention that existing agricultural research points to the potential of improved strains of corn to meet the need in question, however, that the production, processing, marketing, Extension activity, and other considerations associated with nutritionally improved strains of corn require considerable time and extensive investments in terms of trained personnel and money which most developing countries simply do not have at this time. It, therefore, seems worthy to consider the possibilities of fortification of this staple which can be accomplished on a short time basis and with minimal investment.

The results of this research (AID/csd 2500) indicates that the technology for the addition of lysine and tryptophan to corn and/or the addition of lysine plus soy or milk as sources of tryptophan to corn is technically feasible and suitable. Moreover, this approach is compatible with the existing market distribution channels. The above information can be summarized as follows: (1) the quality of nutrition can make a most significant impact on national development; (2) the nutritional concept of corn fortification is valid; and (3) the technology of corn fortification is feasible.

The following recommendations for action can be stated: If we desire action having a maximum impact for a minimum cost and in the least time, it is proposed that the first priority should be: (1) to determine the significance of corn fortification as a nutrition intervention at the village level, and (2) to concurrently plan expansion of this nutrition intervention to other villages, fincas, plantations, suburban and urban markets in each sub-division in each country in all of Central America and in each of the corn eating areas of Latin America and Africa.

Further, if we also desire action having a maximum impact for a minimal cost on a continuing but non specific time

schedule it is proposed that the <u>second priority</u> should be to concurrently (1) develop and optimize agricultural research in corn agronomy and breeding; (2) establish education programs in food science that are integrated and capitalize on, as well as optimize, existing capabilities in agricultural science including the development and inauguration of a food technology technician program; (3) develop and implement a food and agricultural extension program; (4) develop and utilize a management plan to integrate and monitor the aforementioned three activities and finally (5) that procedures be set up to develop, test and implement reliable "parameters" of national development which reflect in a systematic manner the status and significance of the overall development intervention program.