EVALUATION OF CORN TORTILLAS SUPPLEMENTED
WITH COTTONSEED FLOUR

by

SUH-YUN L. OU, B.S.

A THESIS

IN

FOOD AND NUTRITION

Submitted to the Graduate Faculty
of Texas Tech University in
Partial Fulfillment of
the Requirements for
the Degree of

MASTER OF SCIENCE

IN

HOME ECONOMICS

Approved

August, 1975

AL
805
T3
1975
No. 80
Cop. 2

ACKNOWLEDGEMENTS

I am deeply indebted to Mrs. Clara M. McPherson for her helpful planning, guidance and direction of this thesis. I wish to thank the other members of my committee, Dr. S. P. Yang and Dr. J. S. Sevall, for their critical evaluations.

Special gratitude is extended to Mr. Dan Stickle of La Hacienda Mexican Food Products Company for the manufacture and donation of the food products which were used in the project. I would also like to thank the members of the sensory panel who so willingly contributed their valuable time to participate in product evaluation.

My special thanks and gratitude are extended to my husband for his understanding and cooperation which enabled me to complete this thesis.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
LIST OF TABLES	v
Chapter	
I. INTRODUCTION	1
Statement of the Problem	2
Purposes	3
Hypotheses	3
II. REVIEW OF LITERATURE	4
The Importance of Corn in Food Uses	4
Food Uses of Corn in Mexico, Central and South America	6
Nutritive Value of Corn Protein	8
Status of Development and Processing of Cottonseed Flour	11
The Potential of Cottonseed Flour As a Protein Source	13
Antioxidant Property of Cottonseed Flour	14
Evaluation of Protein Quality in Food	15
Sensory Evaluation	16
III. MATERIALS AND METHODS	18
Development of the Samples	18
Chemical Analyses	18
Bioassay	22
The Acceptability Study	25
Statistical Analysis of Data	25

IV. RESULTS AND DISCUSSION	27
Chemical Analyses	27
Bioassay	38
The Acceptance Study	53
V. SUMMARY AND CONCLUSIONS	55
LIST OF REFERENCES	58

LIST OF TABLES

Table	Page
1. Major U. S. Cereal Grains, 1970	5
2. Per Capita Consumption of Corn Foods, 1964-1966 Annual Average	6
3. Amino Acids in Normal and Opaque-2 Endosperms for the Same Ear of Corn (Expressed As gm/100 gm of Protein)	10
4. Approximate Composition of Corn	11
5. Composition of Experimental Diets	23
6. Moisture and Crude Protein Content of Samples	27
7. The Amino Acid Composition (g/100 g Protein) of Each Sample	29
8. Three-way Analysis of Variance of Fat Rancidity	31
9. Results of Duncan's New Multiple Range Test for Determining Dif- ferences Between Time Periods and Rancidity	32
10. Results of Duncan's New Multiple Range Test for Determining Dif- ferences Between Treatments and Rancidity	33
11. Results of Two-way Analysis of Variance of Fat Rancidity Between Time and Treatment Factors At -20°C	34
12. Results of Duncan's New Multiple Range Test for Determining Fat Rancidity Between Time Periods At -20°C	35
13. Results of Duncan's New Multiple Range Test for Determining Fat Rancidity Among Treatments At -20°C	35

Table	Page
14. Two-way Analysis of Variance of Fat Rancidity Between Time and Treatment Factors At 5°C	37
15. Results of Duncan's New Multiple Range Test for Determining Fat Rancidity Between Time Periods At 5°C	37
16. Results of Duncan's New Multiple Range Test for Determining Fat Rancidity Among treatments At 5°C	38
17. Results of Duncan's New Multiple Range Test on Mean Fat Rancidity Between Time and Treatment At Refrigerator Temperature	39
18. Growth Data of Young Rats Fed Various Experimental Diets for 28 Days	40
19. One-way Analysis of Variance of Weight Gain of Young Rats	42
20. Results of Duncan's New Multiple Range Test for Determining Differences Among Weight Gains of Young Rats	42
21. One-way Analysis of Variance of Food Intake of Young Rats	44
22. Results of Duncan's New Multiple Range Test for Determining Differences Among Food Intake of Young Rats	44
23. Analysis of Covariance for Final Weights of Young Rats	45
24. One-way Analysis of Variance of Protein Efficiency Ratios for Rats Fed Various Sources of Protein	48
25. Results of Duncan's New Multiple Range Test for Determining Differences Among Protein Efficiency Ratios for Various Protein Sources	48
26. One-way Analysis of Variance of Net Protein Ratio for Rats Fed Various Sources of Protein	50

Table	Page
27. Results of Duncan's New Multiple Range Test for Determining Differences Among Net Protein Ratios for Various Protein Sources	50
28. Analysis of Covariance for NPR and PER of Protein Quality	51
29. One-way Analysis of Variance of Nitrogen Digestibility Means for Rats Fed Diets with Various Sources of Protein	52
30. Results of Duncan's New Multiple Range Test for Determining Differences Among Nitrogen Digestibility Means of Young Rats Fed Various Diets	52
31. Two-way Analysis of Variance of Sensory Evaluation Between Blocks and Various Sources of Products	54
32. Results of Duncan's New Multiple Range Test for Determining Sensory Evaluation Among Treatments	54

CHAPTER I

INTRODUCTION

In most countries, especially in the developing ones, it appears that the most urgent problems are those related to insufficient quality of protein resulting in protein malnutrition. The quantity and quality of protein in the diet have received widespread attention during the last decade. A major portion of the protein in developed countries is derived from animal sources. However, relatively little animal protein appears in the diets of people in developing countries. Cereals or starchy foods are the chief dietary component for these people. Several authors (1, 2) have shown the benefits which may be derived from the fortification of cereals in order to improve overall protein quality.

LCP cottonseed flour is one of the potential resources to aid in combating protein malnutrition, especially in some developing countries where cottonseed flour is more available than most other protein sources and where processing costs are potentially less. Therefore, cottonseed flour would have an economic advantage in human nutrition. The flour prepared from glandless cottonseed has been studied for incorporation into human foods (3, 4). Also, extensive work has been done on the Liquid Cyclone Process (LCP) for producing high quality edible cottonseed flour by

investigators at Southern Regional Research Center, Agricultural Research Service, U. S. Department of Agriculture, New Orleans, Louisiana. The mass production of this flour would benefit low-income families because its addition to commonly consumed food items would increase the protein content and add amino acids to enhance the quality of the existing proteins (4).

Statement of the Problem

Protein malnutrition is closely associated with socioeconomic conditions in the undeveloped countries. The problems involved in seeking a solution to protein malnutrition are many and varied. The Institute of Nutrition of Central America and Panama (INCAP) has developed and promoted cottonseed-based protein supplements for human feeding for several years (5). Corn is an important plant source of food for the native American Indians and the Mexican Americans. The process of treating corn with lime is a long used one. The resulting corn "masa" and tortillas made from it are commonly consumed in Southwestern United States, Mexico, and Central and South America. Lysine and tryptophan are the first and second limiting amino acids in corn under normal conditions for maintenance of adequate protein nutrition of the human adult (6). It seems feasible that corn tortillas can be supplemented with cottonseed flour to provide a nutritious and economical supplement.

Purposes

The purposes of this study were to: 1) determine protein quality of tortillas when supplemented with 10%, 15%, 20%, and 25% cottonseed flour; 2) investigate acceptability of corn tortillas with various levels of cottonseed flour; 3) determine fat rancidity and shelf life of the above products; 4) evaluate biological effects of supplementing the tortillas with various levels of cottonseed flour.

Hypotheses

In determining the acceptability, shelf life, and protein quality of the fortified tortillas the following hypotheses were tested:

1. All levels of cottonseed flour when used to fortify tortillas will improve the protein quality.
2. The development of rancidity in the supplemented tortillas will differ according to level of supplementation.
3. Digestibility of the various tortillas will be similar.
4. Acceptability of products will be affected by different levels of cottonseed flour.

CHAPTER II

REVIEW OF LITERATURE

Cereal grains and their derivatives are the major components of the diets of low-income families (7, 8). Economic conditions make it impossible to effect any sudden increase in consumption of high quality animal protein, especially in the areas of the world where people suffer from hunger and severe protein deficiency exists. It has not been possible to obtain a mixture of indigenous plant foods having both high protein content and a protein quality equal to that of milk or eggs. However, glanded cottonseed flour (CSF) has received little consideration in the past as a protein source for human consumption. Supplementation of cereal grains with CSF has become increasingly important and of potential benefit to low-income families. It has also awakened people to the need that protein quality is a more significant factor than quantity.

The Importance of Corn in Food Uses

The native American Indian found many uses for corn which was the most important plant to them as a source of food. In 1492, the earliest European explorers returned to the Old World with corn from the Western Hemisphere. Its use spread rapidly throughout Europe, Asia, and Africa (9). Today, corn which is also called maize or Indian corn, is

the leading cereal grain in U.S. agriculture. Because corn has many advantages such as its grain size, high yield, ease of cultivation, various food uses and good storage characteristics it has become a staple food in many parts of the world. In the United States corn provides for most of the cereal and feed grains as indicated in Table 1 (10, 11). Corn has become the chief component in food items, industrial products, animal feeds and alcoholic beverages from both the dry and the wet-milling industries in U.S. (12). The relative importance of corn as a dietary component for the people of several countries is shown in Table 2 (13).

TABLE 1
MAJOR U. S. CEREAL GRAINS, 1970^a

Grain crop	Acreage harvested mil. acres	Yield per acre bu.	Production mil. bu.	Farm value mil. \$
Corn	57	72	4,100	5,500
Wheat	44	31	1,400	1,900
Sorghum	14	51	700	800
Oats	19	49	900	600
Barley	10	43	400	400

^aPreliminary figures.

Source: USDA agricultural statistics (11).

TABLE 2
PER CAPITA CONSUMPTION OF CORN FOODS,
1964-1966 ANNUAL AVERAGE^a

Region or Country	Average Consumption kg per year
Central America	140
Southern Africa, Kenya, and Zambia	126
Mexico	113
Albania and Romania	66
Portugal and Yugoslavia, Northern and Central Africa, and Northern portion of South America	24
Southern and Eastern Asia ^b	14
All other countries ^c	3

^aSource: FAO (13).

^b54% of world population.

^c30% of world population.

Food Uses of Corn in Mexico,
Central and South America

In any of the developing countries where corn is served as a food, preparation methods are usually primitive. Mexico, Central and South America are the major countries where people eat corn as a main staple of their diet. There have been many methods of grinding corn such as raw and dry, or after parching or after soaking or

steeping, and with or without the lime process, to make meal or dough in the preparation of foods. Although many variations have occurred in the preparation methods during certain periods, the most widely used and most highly standardized single article of food made from maize today is the Mexican and Central American tortillas. The alkali-peeled corn gives to the product a special flavor and a physical texture that cannot be imitated by ordinary dry-milled products. The process of treating corn with lime and grinding it on the metate has been used for a long time by the Indians of Mexico and Central America (9). Water is added into masa harina or tamalina to form a dough known as masa. The dough is rolled almost paper thin and fried or baked on both sides on a hot griddle to form tortillas. Throughout Mexico and Central America, tortillas have become the major food item for every meal for the majority of the people.

Tortillas are used for the preparation of such food products as enchiladas and tacos. Corn is also consumed in the form of the unleavened cakes or patties, called apepa, and as tamales which are generally eaten in Colombia and Venezuela and other neighboring countries (14, 15). These products alone account for 25% to 80% of the corn consumed by human beings in these countries. In the Southwestern United States, there is sufficient Southern Hemisphere cultural influence that many traditional foods of Mexico and

Central and South America are widely prepared and distributed. Many people have traveled around the world carrying these foods to other countries. In addition, foods such as tortillas, tacos, enchiladas, and tamales are available in the American supermarket. Packaged flour ready for making tortillas was marketed several years ago as the result of two U.S. patents: Lloyd and Sotres (1952) (16) and Diez de Sallano and Berriozabal (1955) (17). It may or may not be enriched with vitamins and minerals.

Nutritive Value of Corn Protein

In the developing nations, e.g., Mexico, Central America, Columbia, Peru, Brazil and Venezeula, in Latin America, and Kenya and South Africa, a major part of the corn is consumed directly on the farms by people who are among the most impoverished and malnourished. These individuals have been found to have serious protein malnutrition and a niacin-tryptophan-deficiency. Protein quantity in corn is attributed to a genetic variance. The high protein strain of corn has 25.2% protein while the low protein strain has approximately 5.2% (18). The average protein quantity in most strains of corn is about 10%. The major problem in corn is the protein quality which also is the problem of other cereals which are usually the staple energy source. In addition cereals supply a large proportion of the protein which is seriously deficient in lysine.

A preliminary report in 1964 by Mertz, et al. (19) gave the first evidence that protein quality in ordinary dent corn is deficient in lysine, methionine and tryptophan and contains an overabundance of glutamic acid and leucine. One approach to making corn more nutritious is the development of a new variety, such as opaque-2, that has considerably higher lysine. Opaque-2 endosperm contains 69% more lysine than normal corn endosperm (19). These data are compared in Table 3. Table 4 shows differences in the composition of samples of normal corn and high lysine corn. Nelson, et al. reported that floury-2 is the same as opaque-2 since both have high lysine and tryptophan contents (20). However, Lambert, et al. (1969) (21) reported that opaque-2 was 8% lower in yield and 5% lower in weight per hundred kernels, 20% higher in grain moisture, 89% higher in percent cracked kernels and 13% higher in oil content than its normal hybrid counterparts. In addition it has been customary in developing countries for the farmer to habitually plant some of the kernels from the original crop to start the new crop. Since the farmer has a superstitious nature because of his low educational level, it would be difficult to convince him that he should do something which would result in loss or reduction of his crop. Therefore, he may reject the opportunity to make a significant improvement in his family's nutrition (22). It is still not very popular to plant higher protein quality hybrids of corn in the world.

This indicates that other appropriate procedures should be investigated to improve the protein quality of corn.

TABLE 3
AMINO ACIDS IN NORMAL AND OPAQUE-2 ENDOSPERMS
FOR THE SAME EAR OF CORN (EXPRESSED
AS gm/100 gm OF PROTEIN)^b

Amino Acid	Endosperm ^a	
	Opaque-2	Normal
Lysine	3.4	2.0
Histidine	3.4	2.8
Amide ammonia	3.4	3.3
Arginine	5.1	3.8
Aspartic acid	8.4	6.2
Glutamic acid	19.1	21.3
Threonine	3.9	3.5
Serine	5.0	5.2
Proline	9.4	9.7
Glycine	4.0	3.2
Alanine	7.0	8.1
Valine	5.0	4.7
Cystine	2.4	1.8
Methionine	2.0	2.8
Isoleucine	3.9	3.8
Leucine	11.6	14.3
Tyrosine	4.7	5.3
Phenylalanine	5.0	5.3

^aBoth endosperms contain 8.69% crude protein (N x 6.25) as calculated on a fat and moisture-free basis.

^bSource: Mertz, et al. (19).

TABLE 4
APPROXIMATE COMPOSITION OF CORN^a

Components	Normal corn, %	High lysine corn, %
Moisture	10.6	13.8
Protein	9.4	10.5
Fat	4.3	5.3
Carbohydrate	72.8	67.0
Fiber	1.6	2.2
Ash	1.2	1.4

^aSource: Frost, et al. (22).

Status of Development and Processing of Cottonseed Flour

In the early stages of the processing history of cottonseed it was recognized that cottonseed could be a valuable source of edible protein (23). However, the effects of gossypol and gossypol-like pigment glands have been an extensive deterrent to its use in human foods. These pigment glands are dispersed throughout the flake and give undesirable flavors and colors. The gossypol also can combine with the free amino groups of lysine to form toxic factors, and render the lysine less available. Thus, it is the consensus of investigators that the goal in processing should be to remove as much of the gossypol from cottonseed flakes as possible (24). Pons, et al. (25)

found that the gossypol content of cottonseed was influenced by both climatic environment and heredity. In 1963, Hoper (26) found evidence that genetic factors exerted a significant influence on the gossypol content of cottonseed independent of environmental factors. Varieties of cottonseed from gossypium herbaceum were the lowest in gossypol. Progress has continued in the development of glandless varieties and many breeding programs have been conducted by Miravalle and Hyer (27). Glandless varieties, however, have not become a major percentage of cotton production in the U. S., nor in the developing countries.

Glandless cottonseed flours have been incorporated into human food (3). Extensive work has been done on methods of processing, isolating and concentrating glandless and glanded cottonseed by investigators at the Southern Utilization Research and Development Division, New Orleans, Louisiana. The methods of processing are improved from the hydraulic press process, screw press process, to the liquid cyclone process (LCP). The LCP process has been utilized since 1966. This process, called AHW, first used a solvent mixture composed of acetone 39%, hexane 60%, and water 1%. The gossypol from regular glanded cottonseed is dissolved in the solvent and is separated from the meal along with the oil. Two years later, Gastrock (28) exchanged the solvent from the AHW to a straight hexane process which resulted in higher protein quantity. It

contained from 68% to 70% protein, less than 1% residual lipids, and 0.02-0.04% free gossypol which was acceptable for food grade material. Cottonseed flour produced from the LCP process has been utilizable from both the Mississippi Delta and the Texas high plains. A petition was filed with the Food and Drug Administration requesting approval of the LCP cottonseed flour as a food additive (29). This approval was granted and appears in the Federal Register of July 13, 1973 (30). A pilot plant using this process was erected in India in 1969, and the Plains Co-operative Oil Mill in Lubbock, Texas, should be in production sometime in 1975.

The Potential of Cottonseed Flour
As a Protein Source

Cottonseed flour prepared from glandless cottonseed for use in human foods has been studied for years (3). Glanded and glandless cottonseed proteins were reported to have essentially the same amino acid composition, with the exception of unbound or "free" lysine content which was greater for the glandless. Although glandless cottonseed protein was reported as significantly lower in methionine and isoleucine when compared with the Food and Agriculture Organization standard protein, it was reported to be safe and nutritious for humans (31). Altschul (4) reported that the low-cost glandless cottonseed flour was better than the soybean or fish protein as a dietary ingredient in meeting

human nutritional requirements. Several workers (32, 33, 34) stated that cottonseed flour had great potential as a source of protein in human feeding programs, in nutritional improvement of staple food items, and in domestic uses as flour. Enrichment of wheat flour bread with 25% LCP concentrate has been found to approximately double the protein content to 20-22% (3). Bressani, et al. (35) also developed INCAP vegetable mixtures with cottonseed flour for human feeding which had a relatively high amount of protein of acceptable quality. Investigations have shown that cottonseed flour has high quality protein and can be manufactured in forms suitable for human consumption. It has the potential to become an important protein source for low-income families to compensate for a deficiency of animal protein.

Antioxidant Property of Cottonseed Flour

Antioxidants have been shown to be effective in increasing the rancid-free shelf-life of cereal products from various grains (36). Overman (37) reported that both cottonseed and soy flour added to wheat flour could delay the development of a rancid odor in an approximate proportion to the amount of cottonseed and soy flour used. When cottonseed flour was substituted for wheat flour at 3% and 10% levels in raw pastry mixes, the shelf-life of baked pastry was increased from two to ten times longer than

when only wheat flour was used. This investigation was conducted to show that supplementation with LCP cottonseed flour could increase the shelf-life of these products.

Evaluation of Protein Quality in Food

The protein quality of food is becoming an increasingly important aspect at the present time. The nutritive value of dietary protein is dependent upon the quality and quantity of amino acids available for biological uses. Thus, the efficiency of protein utilization is affected by the ingredients in the food. Inadequate methods of assessing the nutritive value of protein can lead to false conclusions. Therefore, protein quality of food must be measured using adequate methods.

Several researchers have reported on the various methods which have been devised for evaluation of protein quality. The chemical methods for evaluating protein quality such as the available lysine test (38) can determine when lysine is the limiting amino acid. This method has certain limitations and is only an approximation of biological response. The traditional biological method measures growth response and is still needed for a definitive evaluation of protein quality. Derse (39) and Chapman, et al. (40) have established the procedure for measurement of growth response and for determination of the protein efficiency ratio (PER) of foods. The ratio of body

weight gained in a specified period of time in relation to protein intake is defined as protein efficiency ratio. However, there are several factors influencing the PER of foods which must be considered, such as sex and age of rats, strains, length of experimental period, level of dietary protein, and the use of standard casein (41). This method is very widely used and correlates well with other methods of evaluating protein quality, however, it is still a criterion method. The greatest criticism is that it makes no allowance for maintenance.

Net protein ratio (NPR) assay is the other method selected for evaluation of protein quality and was proposed by Bender and Doell (42). This method is very similar to PER, but can overcome some of the criticisms of the PER assay. A group of rats is fed a nonprotein diet and the weight loss of this group is added to the weight gain of the test group.

Sensory Evaluation

Every food processor is involved in food research and the development of good quality food is related to consumer acceptability. The quality is dependent on the pleasantness of food judged by human senses of color, taste and odor. Without satisfactory flavor quality, the food will not be acceptable to the consumer. Therefore, it is necessary that sensory evaluation be done before products are marketed.

There need be no formal training procedure for the judges. However, the selection of judges for the sensory evaluation of food and related items is very important. Sandra, et al. (43) have conducted small-scale consumer testing in which emphasis was placed on selection of panelists rather than training, because by the nature of the test, no training should be done. The test was designed for the evaluation of the product by naive, untrained tasters who were already consumers of this type of product.

TEXAS TECH

CHAPTER III

MATERIALS AND METHODS

Development of the Samples

The manufacture of the tortillas supplemented with the various levels of cottonseed flour was done by La Hacienda Mexican Food Products in Lubbock. The 90% yellow and 10% white dent corn had been treated with lime to prepare masa harina. The different percentages of cottonseed flour mixed with the masa harina were 10%, 15%, 20%, and 25%. As the cottonseed flour level was increased the water was decreased due to the fact that the cottonseed flour had less absorptive quality than the masa harina. Given amounts of each sample were ground in a Waring blender, freeze-dried, reground in a Sorval Omni Mixer and refrigerated for later use in the bioassay and chemical analyses.

Chemical Analyses

Determination of Moisture Content

For determination of percentage moisture, approximately 2.0 gm of each sample were weighed and placed in a 50.0 ml beaker. The beakers were weighed before and after adding the sample and were then placed in an air drying oven at 110°C for 48 hours. The weight loss was used to calculate the percentage of moisture content in each sample.

The formula used to calculate was as follows: Moisture content % =
$$\frac{\text{wt. of sample (wet)} - \text{wt. of sample (dry)}}{\text{wt. of sample}} \times 100$$

Determination of Protein Content

Crude protein content of each sample was determined by a macro-Kjeldahl method (44). Nitrogen content was multiplied by 6.25. The macro-Kjeldahl procedure used for nitrogen analysis was a modification of the method described by the Association of Official Agricultural Chemists (44). Triplicate representative samples of the various tortillas were analyzed for crude protein content.

Determination of Total Lipid Content

Total lipid content was determined by a modification of the ether Soxhlet method. The procedure used was described by the Association of Official Agricultural Chemists (44). Approximately 2.0 g of each sample were dried overnight in a vacuum drying oven.

Determination of Fat Acidity

The degree of fat acidity or rancidity of samples was measured by a method using a titration extractive solution with 0.0178 N potassium hydroxide (45). Samples were stored at refrigerator (5°C) and freezer (-20°C) for periods of one, two, and four weeks. At the end of the selected

storage period, samples were ground and freeze-dried. Samples were reground by Sorval Omni Mixer into fine powder. 20.0 gm of well-mixed sample were transferred to an extractive flask. Fifty ml of benzene were used to extract fat from the samples. This benzene mixture was shaken in a flask for 30 minutes using a mechanical shaking device, after which 25 ml were filtered from the extraction solution. A 25 ml TC pipette was used to transfer 25 ml alcohophenolphthalein solution to the flask containing the benzene extraction. The extractive solution was titrated with 0.0178 N potassium hydroxide to an end point matching the color of the standard. Duplicate samples were used to determine fat rancidity which was calculated as follows:

$$\text{Fat acidity value} = \frac{(T - B) \times 10 \times 100}{100 - W}$$

Where T = ml of 0.0178 N KOH required to titrate sample extracted.

B = ml of 0.0178 N KOH required to titrate blank

W = g water in 100 g of sample

Determination of Amino Acid Composition

The amino acid composition of each sample was determined by a column chromatographic method (46). Samples containing 7.0 ± 0.5 mg protein were treated in a special Pyrex tube with 2.0 ml of 6 N hydrochloric acid. The sample was placed in a dry ice trichloroethylene cooling bath

under an oil vacuum pump. Subsequently, the sample was hydrolyzed at $110^{\circ} \pm 1^{\circ}\text{C}$ for 22 hours in an air drying oven. The hydrolysate solution was filtered with 2 ml deionized water through a disc funnel fitted into a 50 ml filtering flask and evaporated to dryness with a Buehler Rotary Evaporator. Deionized water was added and evaporated to dryness several times. The dried hydrolysate was made up to 5.0 ml with a pH 2.2 sodium citrate buffer in a 5.0 ml volumetric flask and stored at 4°C refrigerator. Amino acid composition of aliquots was determined with a Beckman 116 amino acid analyzer.

The tryptophan content of each sample was determined after $\text{Ba}(\text{OH})_2$ hydrolysis by a Beckman 121 HP amino acid analyzer (47). A 10.0 mg sample of protein was mixed with 5.0 g of $\text{Ba}(\text{OH})_2$, 400 mg of purified starch and 8 ml of deionized water in a nalgene bottle. The bottle was placed in an air oven at 110°C for 16 hours. Pressure was released immediately from the bottle with subsequent cooling at room temperature. The mixture was transferred to a beaker with deionized water and neutralized to pH 7.0 with 1 N H_2SO_4 and then made to volume with 100 ml deionized water. The solution was mixed completely and 40 ml were poured into a bottle and centrifuged for 2 hours at 9400 rpm. A 25 ml aliquot was transferred to a test tube and dried with a Buehler rotary vacuum evaporator. One ml of pH 2.2 buffer solution was added to the dry residue and a

0.25 ml aliquot was used to determine the tryptophan content by using a Beckman 121 HP amino acid analyzer (47).

Bioassay

Care of Rats

Eighty male albino rats of the Sprague-Dawley strain, 22 days old and of an average weight of 55 to 60 g were placed in four large raised screen-bottomed cages. The rats were given laboratory stock chow and distilled water ad libitum for 48 hours. After the 48 hour adjustment period, the animals were weighed and randomly assigned to individual cages and to one of eight dietary treatments. Water and food were offered ad libitum. During the assay period, all environmental conditions were maintained as uniformly as possible. Body weight and food intake were recorded for each rat at regular 7 day intervals. The experiment was conducted for 28 days.

Composition of the Diets

The composition of the eight experimental diets is given in Table 5. All the experimental diets contained 8.5% crude protein which was supplied either by casein or by cottonseed flour and tortillas except Diet 8 which was a negative control containing no protein. The crude protein content of each experimental diet was determined by the macro-Kjeldahl method (44). The total lipid content

TABLE 5

COMPOSITION OF EXPERIMENTAL DIETS

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
	gms	gms	gms	gms	gms	gms	gms	gms
Casein	9.09	-	-	-	-	-	-	-
Tortillas	-	93.20	-	-	-	-	-	-
Tortillas + 10% CSF	-	-	56.59	-	-	-	-	-
Tortillas + 15% CSF	-	-	-	47.48	-	-	-	-
Tortillas + 20% CSF	-	-	-	-	43.06	-	-	-
Tortillas + 25% CSF	-	-	-	-	-	37.38	-	-
100% CSF	-	-	-	-	-	-	-	-
Corn Oil	3.00	1.64	2.07	2.09	2.01	-	13.08	-
Vitamin Mixture ¹	1.00	1.00	1.00	1.00	1.00	2.14	2.88	3.00
Fiber	1.00	-	-	-	-	1.00	1.00	1.00
Salt Mixture ²	4.00	4.00	4.00	4.00	4.00	-	-	1.00
Corn Starch	81.91	0.16	35.94	45.02	49.93	55.48	79.04	91.00
Total (g)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
% Crude Protein	8.56	8.65	8.44	8.63	8.77	8.61	8.81	0.00

¹USPXVII salt mixture has essentially the same proportions as USPXVII salt mixture which contains 13.93 g NaCl, KH_2PO_4 38.90 g, CaCO_3 38.14 g, MgSO_4 5.73 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 2.70 g, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.0005 g, $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$ 0.0045 g, KI 0.008 g, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.0002 g, and ZnCl_2 0.0003 g.

²The vitamin mixture contained the following vitamins in mg/100 gm diet: vitamin A, 0.002 (2000 I.U.); vitamin D, .000235 (200 I.U.); vitamin E, 10.0 (10 I.U.); menadione, 0.5; choline, 200; p-aminobenzoic acid, 10.0; inositol, 10.0; niacin, 4.0; calcium D-panthenate, 4.0; riboflavin, 0.8; thiamine-HCL, 0.5; pyridoxine-HCL, 0.5; folic acid, 0.2; biotin, 0.04; vitamin B₁₂, 0.003.

of each sample was determined by the ether Soxhlet method (44). The fat content of each diet was made up to 3% with corn oil.

Digestibility Determination

Feces from the entire experimental period were collected for each rat in an Erlenmeyer flask which contained 50 ml of 6N HCl. The feces were then placed in an autoclave for 2 hours at 121°C, 20 lb pressure. Subsequently, these mixtures were cooled to room temperature and then poured through a sieve and each made to a volume of 250 ml with distilled water. Aliquots of 10 ml were used for the nitrogen determination by a macro-Kjeldahl procedure. Duplicate tests were run on each fecal sample.

Protein Efficiency Ratio (PER)

The protein efficiency ratio (PER) is widely used and has been tested in collaborative assays. PER was measured as the ratio of grams body weight gained in a specified period of time in relation to the grams of protein consumed:

$$\text{PER} = \frac{\text{Weight gains (gm)}}{\text{Protein intake (gm)}}$$

Net Protein Ratio (NPR)

The net protein ratio determination was proposed by Bender and Doell (42). NPR is a modification and is similar

to PER. A control group of rats is fed on a protein-free diet and the weight loss of this group is added to the weight gain of the test group:

$$\text{NPR} = \frac{\text{weight gain (gm)} + \text{weight loss of protein free group (gm)}}{\text{protein intake (gm)}}$$

This procedure allows for maintenance requirement which is the assumed weight loss. It also permits the evaluation of poor quality proteins which do not promote growth (48). The results obtained are independent of food consumption.

The Acceptability Study

A sensory evaluation method (hedonic scale) was used for measuring organoleptic qualities of the tortillas. Three replications were used on a judgment basis. In this study, most of the members of the sensory panel were of Mexican American background as they are most familiar with tortillas. Since these individuals have difficulty reading English, a facial hedonic scale was used for judging the products. The scale contained five faces and each was assigned a numerical value. Twenty-five people were asked to judge the products.

Statistical Analysis of Data

One way analysis of variance (49) was used to test the differences of protein quality when rats were fed the experimental diets. When significant differences were found

among the groups of animals in relation to weight gain, food intake, PER, NPR, and nitrogen digestibility, Duncan's New Multiple Range Test was applied to determine the differences. Data for the sensory evaluation and fat rancidity of products were analyzed using the factorial procedure as outlined by Li (49). A three-way analysis of variance (50) was used to test the differences of fat rancidity between time, temperature, and treatments. A two-way analysis of variance determine fat rancidity between time and treatment in relation to different temperatures and for the sensory evaluation. Duncan's New Multiple Range Test was applied to determine the differences in fat rancidity values and differences in sensory evaluation scores for the products.

CHAPTER IV

RESULTS AND DISCUSSION

I. Chemical Analyses

The moisture, dry matter, and crude protein contents of the samples are shown in Table 6. The moisture content of the freeze-dried samples varied from 0.97% to 3.91%. The crude protein content of samples ranged from 9.12% to 93.51% on a dry matter basis. When plain tortillas were supplemented with 10%, 15%, 20%, and 25% levels of LCP cottonseed flour, the crude protein content was increased to 15.02%, 17.75%, 19.74% and 22.74% respectively. The LCP cottonseed flour contained 63.02% crude protein which is much higher than 9.12% of the plain tortillas.

TABLE 6
MOISTURE AND CRUDE PROTEIN CONTENT OF SAMPLES

Sample ¹	Moisture (%)	Dry matter (%)	Crude protein ² (%)	Crude protein based on DM ³ (%)
Plain tortillas	0.97	99.03	9.02	9.12
Tortillas + 10% CSF	0.88	99.12	14.89	15.02
Tortillas + 15% CSF	1.07	98.93	17.57	17.75
Tortillas + 20% CSF	2.79	97.21	19.19	19.74
Tortillas + 25% CSF	3.91	96.09	21.85	22.74
100% CSF	3.10	96.90	61.07	63.02
Casein	5.55	94.55	88.42	93.51

¹Samples were dehydrated by freeze drying except 100% CSF and casein.

²N x 6.25.

³Dry matter.

The amino acid composition of the samples are compared with the FAO pattern in Table 7. All of the essential amino acids were increased in amount except valine when the tortillas were supplemented with LCP cottonseed flour. The plain tortillas contained 2.91 gm of lysine per 100 gm protein. Lysine was the first limiting amino acid, however, when supplemented with the various levels of cottonseed flour, the samples contained 3.85, 4.02, 4.16, and 4.47 gm of lysine per 100 gm of protein respectively. When tortillas were supplemented with 20% cottonseed flour, the lysine content was similar to the FAO pattern (4.2). However, each sample was lower in methionine and tryptophan than the FAO pattern (2.2 and 1.2 respectively). The samples supplemented with LCP cottonseed flour were increased in their content of the second and third limiting amino acids, tryptophan and methionine. The leucine content (12.48 gm) was much higher in the plain tortillas than for the FAO pattern (4.8 gm). Feeding excess leucine has been demonstrated to influence excretion of niacin-tryptophan metabolites in both humans and in adult rats (52, 53, 54). The 100% LCP cottonseed flour contained 6.11 gm of leucine / 100 gm of protein which was much lower than for the plain tortillas. Amino acid imbalance may cause a decrease in the rate of growth of mammals. Harper, et al. (55) concluded that the effect of leucine is very largely reversed when the level of isoleucine in the diet is increased, the

TABLE 7

THE AMINO ACID COMPOSITION (g/100 g PROTEIN)
OF EACH SAMPLE

Amino Acid	Plain Tortil-las	Tortil-las + 10% CSF	Tortil-las + 15% CSF	Tortil-las + 20% CSF	Tortil-las + 25% CSF	100% LCP CSF	FAO Reference
Lysine	2.91	3.85	4.02	4.16	4.47	5.23	4.2
Histidine	3.04	2.85	3.10	3.12	3.09	3.15	2.4
Arginine	4.54	8.03	9.95	10.01	10.40	13.50	2.0
Aspartic Acid	7.80	8.57	9.19	9.93	9.98	10.98	--
Threonine	3.98	3.73	3.68	3.53	3.53	3.62	2.8
Serine	5.21	5.00	4.89	4.90	5.15	5.20	--
Glutamic Acid	22.44	22.60	22.89	23.19	23.80	23.87	--
Proline	10.23	8.17	6.91	6.36	6.65	3.94	--
Glycine	3.75	3.70	3.77	3.79	3.80	3.60	--
Alanine	5.75	4.95	4.25	4.13	4.10	3.26	--
Valine	4.17	4.06	4.00	3.90	3.88	3.49	4.2
Methionine	1.69	1.73	1.81	1.80	1.81	1.84	2.2
Isoleucine	2.96	3.01	3.17	3.08	3.10	3.15	4.2
Leucine	12.48	10.89	9.66	9.20	8.00	6.11	4.8
Tyrosine	3.70	3.20	3.25	3.28	3.39	3.20	--
Phenylalanine	5.20	5.66	5.70	5.80	5.82	5.85	2.8
Tryptophan	0.56	0.61	0.64	0.67	0.73	1.17	1.2

excess of dietary leucine apparently inhibits the utilization of isoleucine and thus increases the requirement of the rat for this amino acid. Further evidences from the research of Harper, et al. (56) indicated that rats grew well on corn diets supplemented with various amino acids

only when isoleucine was added. Isoleucine was found to be limiting for the growth of rats fed corn diets supplemented with lysine, tryptophan, valine, and threonine. Bressani, et al. (57) reported that growth in children consuming corn proteins is also limited to a minor degree due to insufficient isoleucine. The leucine to isoleucine ratio in the FAO pattern and in 100% LCP cottonseed flour are 1.4 : 1 and 1.92 : 1, but the ratio is very high in plain tortillas which is 4.22 : 1. When tortillas were supplemented with 10%, 15%, 20% and 25% of LCP cottonseed flour, the ratios of leucine to isoleucine were 3.62 : 1, 3.05 : 1, 2.99 : 1, and 2.58 : 1, respectively. These results compared with the animal study that weight gains were increased when the leucine to isoleucine ratio decreased. These results indicated that the limiting amino acid in the corn tortillas, after lysine, tryptophan, and methionine, is isoleucine.

Fat Rancidity

Results of the fat rancidity study is shown in Table 8. A three-way analysis of variance for the factorial design was applied to test the hypothesis that (1) there was no significant difference in rancidity of samples stored at refrigerator and freezer temperatures; (2) there was no significant difference which could be attributed to storage time; (3) there was no significant difference due to the treatment of samples; and (4) there was no significant

TABLE 8

THREE-WAY ANALYSIS OF VARIANCE OF FAT RANCIDITY

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Value	Critical F-Value*
Temperatures	1	38.80	38.80	838.58	7.56
Times	2	28.76	14.38	310.83	5.39
Treatment	4	27.39	6.85	147.96	4.02
Temperatures x Times	2	4.90	2.45	52.98	5.39
Temperatures x Treatments	4	5.43	1.36	29.35	4.02
Times x Treatments	8	3.77	0.47	10.19	3.17
Temperatures x Times x Treatments	8	1.37	0.17	3.71	3.17
Within Replicates	30	1.39	0.05		
Total	59	111.82			

*P < 0.01.

interaction of these three factors, in relation to their effect on rancidity during the storage of samples. The study consisted of an equal number of observations.

Table 8 shows that all of the hypotheses were rejected at the 0.01 level of significance. These results suggested that all three factors were significant in the development of fat rancidity in relation to storage of the samples. The samples stored at refrigerator temperature developed greater rancidity than those stored at freezer temperature. Fat rancidity was increased with prolonged storage time. Duncan's New Multiple Range Test (Table 9) showed that fat rancidity was significantly different at one, two, and four weeks. Table 10 shows that rancidity values were greater in the plain tortillas than for all other treatments ($P < 0.01$). When the LCP cottonseed flour was increased rancidity was decreased.

TABLE 9

RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST
FOR DETERMINING DIFFERENCES BETWEEN
TIME PERIODS AND RANCIDITY

Time	Mean ¹
One week	3.14 ^a
Two weeks	3.99 ^b
Four weeks	4.84 ^c

¹Means with the same superscript indicate no significant difference ($P < 0.01$).

TABLE 10

RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST
FOR DETERMINING DIFFERENCES BETWEEN
TREATMENTS AND RANCIDITY

Treatment	Means ¹
Plain Tortillas	5.13 ^a
Tortillas + 10% CSF	4.27 ^b
Tortillas + 15% CSF	3.86 ^{b,c}
Tortillas + 20% CSF	3.47 ^{c,d}
Tortillas + 25% CSF	3.21 ^d

¹Means with the same superscript indicate no significant difference ($P < 0.01$).

A two-way analysis was used to test the relationships of fat rancidity between treatments and storage time in refrigerator and freezer temperature. The hypotheses were (1) there was no significant difference between time of storage; (2) there was no significant difference attributable to the treatment of samples; and (3) there was no significant difference in interaction of times and treatments.

A two-way analysis of variance was used to test the hypothesis for samples stored at freezer temperature.

The results (Table 11) indicated that (1) there was a significant difference attributable to the varying storage times; (2) there was a significant difference

attributable to sample treatments; and (3) there was no significant interaction between times and treatments.

Duncan's New Multiple Range Test (Table 13) showed that fat rancidity was significantly higher in the plain tortillas and in tortillas containing 10% CSF than in all others.

The tortillas containing 15% and 20% CSF were similar in the development of rancidity. Table 12 shows that samples stored in the freezer for two and for four weeks showed no significant difference but had higher rancidity values than when stored for only one week.

TABLE 11

RESULTS OF TWO-WAY ANALYSIS OF VARIANCE OF
FAT RANCIDITY BETWEEN TIME AND
TREATMENT FACTORS AT -20°C

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F-Value	Critical F-Value*
Time	2	5.92	2.96	194.02	6.36
Treatment	4	4.25	1.06	69.62	4.89
Time x Treatment	8	0.41	0.05	3.32	4.00
Within Replicates	15	0.23	0.015		
Total	29	10.80			

* ($P < 0.01$).

TABLE 12
RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST
FOR DETERMINING FAT RANCIDITY BETWEEN
TIME PERIODS AT -20°C

Time	Mean ¹
One week	2.56 ^a
Two weeks	3.35 ^{b,c}
Four weeks	3.63 ^c

¹Means with the same superscript indicate no significant difference ($P < 0.01$).

TABLE 13
RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST
FOR DETERMINING FAT RANCIDITY AMONG
TREATMENTS AT -20°C

Treatment	Mean ¹
Plain Tortillas	3.84 ^a
Tortillas + 10% CSF	3.33 ^b
Tortillas + 15% CSF	3.09 ^c
Tortillas + 20% CSF	2.89 ^{c,d}
Tortillas + 25% CSF	2.79 ^d

¹Means with the same superscript indicate no significant difference ($P < 0.01$).

A two-way analysis of variance was applied to test the hypotheses for samples stored at refrigerator temperature.

The results in Table 14 show that (1) there was a significant difference in the development of rancidity with the various storage times; (2) there was a significant difference attributable to sample treatments; and (3) there was a significant difference attributable to interaction of times and treatments, in their effect on the development of rancidity of the samples. The results of Duncan's New Multiple Range Test (Table 15) showed that fat rancidity values were greater in tortillas after 4 weeks of storage than after 2 weeks and one week of storage. Tortillas stored for one week had lower fat rancidity values than when stored for two weeks. The results of Duncan's New Multiple Range Test (Table 16) showed that tortillas supplemented with 20% and 25% CSF were similar and had lower fat rancidity values than other tortillas. Plain tortillas had the highest fat rancidity values than any supplemented with LCP cottonseed flour. Table 17 indicates that fat rancidity values were significantly different when interaction between time and treatment was tested. The plain tortillas had a significantly ($P < 0.01$) higher fat rancidity value at one month of storage, while tortillas with 25% CSF had the lowest fat rancidity value at one week of storage. When tortillas with 25% CSF were stored for two weeks, the fat rancidity value

was similar to tortillas supplemented with 15% and 20% CSF stored for one week. The results indicate that LCP cottonseed flour has some antioxidant property at both refrigerator and freezer temperatures.

TABLE 14
TWO-WAY ANALYSIS OF VARIANCE OF FAT RANCIDITY
BETWEEN TIME AND TREATMENT
FACTORS AT 5°C

Source of Variation	Degree of Freedom	Sums of Squares	Mean Squares	F-Value	Critical F-Value*
Time	2	27.75	13.88	179.51	6.36
Treatment	4	28.57	7.14	92.41	4.89
Time x Treatment	8	4.74	0.59	7.66	4.00
Within Replicates	15	1.16	0.08		
Total	29	62.22			

* (P < 0.01) .

TABLE 15
RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST
FOR DETERMINING FAT RANCIDITY BETWEEN
TIME PERIODS AT 5°C

Time	Mean ¹
One week	3.70 ^a
Two weeks	4.64 ^b
Four weeks	6.04 ^c

¹Means with the same superscript indicate no significant difference (P < 0.01) .

TABLE 16
RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST
FOR DETERMINING FAT RANCIDITY
AMONG TREATMENTS AT 5°C

Treatment	Mean ¹
Plain Tortillas	6.42 ^a
Tortillas + 10% CSF	5.22 ^b
Tortillas + 15% CSF	4.64 ^c
Tortillas + 20% CSF	4.05 ^d
Tortillas + 25% CSF	3.64 ^d

¹Means with the same superscript indicate no significant difference ($P < 0.01$).

II. Bioassay

The growth data of the young rats are shown in Table 18. During the 28 days of the experimental period, each group of rats, except the protein-free group was fed an isocaloric diet with 8.5% of crude protein. The average weight gain ranged from 98.8 gm for the rats consuming Diet 7 to 36.8 gm for those consuming Diet 2. The protein quality was determined by protein efficiency ratio (PER) and net protein ratio (NPR). The PER values for the diets containing 8.5% protein from Diets 1, 7, 6, 5, 4, 3, and 2 were 2.73, 2.62, 2.61, 2.52, 2.32, 2.23, and 1.67 respectively. The NPR values for these same diets were 3.54,

TABLE 17

RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST ON MEAN
FAT RANCIDITY BETWEEN TIME AND TREATMENT
AT REFRIGERATOR TEMPERATURE

Time x Treatment	Mean ¹
One week x Plain Tortillas	4.56 ^a
One week x Tortillas + 10% CSF	4.15 ^{b,c}
One week x Tortillas + 15% CSF	3.46 ^{d,e}
One week x Tortillas + 20% CSF	3.33 ^{d,e}
One week x Tortillas + 25% CSF	3.01 ^f
Two weeks x Plain Tortillas	6.11 ^g
Two weeks x Tortillas + 10% CSF	5.10 ^h
Two weeks x Tortillas + 15% CSF	4.46 ^{a,c}
Two weeks x Tortillas + 20% CSF	3.92 ^b
Two weeks x Tortillas + 25% CSF	3.59 ^e
Four weeks x Plain Tortillas	8.60 ⁱ
Four weeks x Tortillas + 10% CSF	6.42 ^j
Four weeks x Tortillas + 15% CSF	5.99 ^g
Four weeks x Tortillas + 20% CSF	4.89 ^h
Four weeks x Tortillas + 25% CSF	4.31 ^{a,c}

¹Means with the same superscript indicate no significant difference ($P < 0.01$).

TABLE 18

GROWTH DATA OF YOUNG RATS FED VARIOUS EXPERIMENTAL DIETS FOR 28 DAYS

Diet No.	No. of Rats	Treatment	Weight Gain (g)	Food Intake (g)	Protein Intake (g)	PER ²	Corrected PER ³	NPR ⁴	Fecal Nitrogen (g)	Nitrogen Digestibility (g) ⁵
1	10	Casein	67.05	286.35	24.52	2.71	2.50	3.52	151.84	92.84
2	10	Tortillas (plain)	36.83	255.53	22.10	1.67	1.54	2.57	234.40	86.08
3	10	Tortillas + 10% CSF	64.38	342.75	28.93	2.23	2.05	2.90	211.20	90.56
4	10	Tortillas + 15% CSF	72.49	363.45	31.36	2.30	2.13	2.93	242.91	89.91
5	10	Tortillas + 20% CSF	76.91	364.66	30.40	2.52	2.32	3.14	223.88	90.10
6	10	Tortillas + 25% CSF	87.26	388.48	33.45	2.60	2.40	3.19	243.08	90.63
7	10	100% CSF	98.84	427.02	37.62	2.63	2.41	3.17	219.08	92.47
8	10	Protein-Free	-19.59	127.91	----	----	----	----	38.04	----

¹Diet 1-7 contain 8.5% protein²Protein Efficiency Ratio (PER) = $\frac{\text{Weight gain (g)}}{\text{Protein Intake (g)}}$ ³Adjusted PER with casein = 2.50⁴Net Protein Ratio = $\frac{\text{Wt. gain of protein containing diet} + \text{wt. loss of protein-free diet}}{\text{Protein Intake}}$ ⁵Nitrogen Digestibility = $\frac{\text{Nitrogen intake} - (\text{fecal nitrogen} - \text{fecal nitrogen of protein-free})}{\text{Nitrogen Intake}}$

3.19, 3.17, 3.15, 2.94, 2.90, and 2.55 respectively. In 1973, FAO proposed that "high quality protein" is defined as having a PER equal to or greater than that of casein (PER = 2.5). "Proteins in general" are those with a PER less than that of casein. Total protein with a PER less than 2.0 is considered an insignificant source of protein (51). Thus, the plain tortillas which had a PER of 1.54 (corrected, Table 18) would contain an insignificant amount of protein. However, the tortillas supplemented with 10%, 15%, 20%, and 25% levels of CSF would all be considered a significant source of protein.

A one-way analysis of variance for a randomized block design was used to test the hypothesis that (1) there was no significant difference among blocks and (2) the experimental treatments equally affected weight gain, food intake, PER, NPR, and nitrogen digestibility according to initial weights of the rats.

The first hypothesis was accepted at the 0.01 level of significance (Table 19) indicating that there was no difference among blocks due to initial weight of the animals. The second hypothesis was rejected at the 0.01 level of significance indicating that there were significant differences among weight gains attributable to the various treatments. The results of Duncan's New Multiple Range Test (Table 20) showed that rats fed Diets 5, 6, and 7 had similar weight gains, which were higher than those of rats

TABLE 19

ONE-WAY ANALYSIS OF VARIANCE OF WEIGHT
GAIN OF YOUNG RATS

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Value	Critical F-Value*
Block	9	787.78	87.53	0.65	2.72
Treatment	7	96317.14	13759.59	101.57	2.95
Residual	63	8534.19	135.46		
Total	79	105639.06			

*(P < 0.01) .

TABLE 20

RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST
FOR DETERMINING DIFFERENCES AMONG
WEIGHT GAINS OF YOUNG RATS

Diet No	Treatment	Mean ¹
1	Casein	67.05 ^a
2	Plain Tortillas	36.83 ^b
3	Tortillas + 10% CSF	64.38 ^a
4	Tortillas + 15% CSF	72.49 ^a
5	Tortillas + 20% CSF	76.91 ^{a,c}
6	Tortillas + 25% CSF	87.26 ^c
7	100% LCP, CSF	98.84 ^c
8	Protein Free	-19.59 ^d

¹Means with the same superscript indicate no significant difference (P < 0.01) .

consuming Diets 4, 3, 2 and 1. The rats fed Diets 3, 4, 5 and 7 had similar weight gains which were significantly higher ($P < 0.01$) than the weight gains attained by animals on Diets 1 and 2.

To determine if there were significant differences in weight gains attributable to differences in food intakes, a one-way analysis of variance for a randomized block design was used. The hypothesis was rejected at the 0.01 level of significance, indicating there were significant differences among food intakes of the various treatments (Table 21). The results of Duncan's New Multiple Range Test indicated that rats consuming Diet 8 had a significantly lower food intake than those on Diets 1, 2, 3, 4, 5, 6 and 7 (Table 22). The rats fed Diets 1 and 2 had a significantly lower food intake than those on Diets 3, 4, 5, 6 and 7. The rats fed Diets 6 and 7 had a significantly higher food intake than those given Diets 1, 2, 3, 4, 5 and 8. There was no significant difference ($P < 0.01$) in the food consumption of the rats given Diets 3, 4 and 5.

An analysis of co-variance for a randomized design was used with x being the initial weight (covariant) and y being the final weights (58) to determine the relationship between initial and final weights (Table 23). Results indicated that the final weights of the animals which varied from 182.8 g for the 25% CSF treatment to 91.2 g for the plain tortilla treatment were significantly different from

TABLE 21
ONE-WAY ANALYSIS OF VARIANCE OF FOOD
INTAKE OF YOUNG RATS

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Value	Critical F-Value*
Block	9	10852.33	1205.81	0.72	2.72
Treatment	7	613865.04	87695.00	52.65	2.95
Residual	63	104934.75	1665.63		
Total	79	729652.06			

* (P < 0.01).

TABLE 22
RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST
FOR DETERMINING DIFFERENCES AMONG
FOOD INTAKE OF YOUNG RATS

Diet No	Treatment	Mean ¹
1	Casein	286.35 ^a
2	Plain Tortillas	255.53 ^a
3	Tortillas + 10% CSF	342.75 ^{b, c}
4	Tortillas + 15% CSF	363.45 ^{b, c}
5	Tortillas + 20% CSF	346.66 ^{b, c}
6	Tortillas + 25% CSF	388.48 ^{c, d}
7	LCP, CSF	427.02 ^d
8	Protein Free	127.91 ^e

¹Means with the same superscript indicate no significant difference (P < 0.01).

TABLE 23

ANALYSIS OF COVARIANCE FOR FINAL WEIGHTS OF YOUNG RATS

Source of Variation	Degrees of Freedom	Sum of Squares			Deviation about Regression		
		ΣX^2	ΣXY	ΣY^2	$\frac{\Sigma Y^2 - (\Sigma XY)^2}{\Sigma X^2}$	Degrees of Freedom	Mean Square F-Value
Block	9	399.92	376.79	1252.21			
Treatment	6	41.37	378.81	23671.44			
Error	54	256.51	309.46	8773.87	8400.53	53	158.50 32.84*
Treatment + Error	60	297.88	688.27	32445.31	30855.02	59	
Difference for testing among treatment means					31253.41	6	5204.73

* $P < 0.01$.

each other ($P < 0.01$). However, the sum of squares due to the regression of Y on X was not significant as shown by the following:

1. Formula to test if the sum of squares due to regression of Y on X is significant

$$F_{1, (b-1) (t-1) - 1} = \frac{(\sum XY)^2 / \sum X^2}{S^2 E}$$

$$F_{1, 63} = \frac{(309.46)^2 / 256.51}{158.50} = 2.35$$

2. To test regression coefficient

$$t = \frac{b - \beta}{S_b} = \frac{1.2064}{0.7861} = 1.535 ;$$

$$b = \frac{\sum XY}{\sum X^2} = \frac{309.46}{256.51} = 1.2064 ;$$

$$S_b = \frac{S^2 E}{\sum X^2} = \frac{158.50}{256.51} = 0.7861 .$$

(Assume $\beta = 0$)

3. Proportion of variation due to regression

$$r^2 = \frac{(\sum XY)^2 / \sum X^2}{\sum Y^2} = 0.0425$$

The coefficient, $r^2 = 0.0425$, showed 4.25% variation due to the regression of Y on X. Thus, the difference in weight gains were not related to initial weights.

A one-way analysis of variance was used to test the hypothesis that the experimental treatments equally affected protein efficiency ratio (PER) (Table 24). The hypothesis was accepted that no difference existed among blocks, but was rejected for the various treatments ($P < 0.01$). The results of Duncan's New Multiple Range Test showed that PER values of 2.71 for casein, 2.63 for 100% LCP cottonseed flour, 2.60 for 25% CSF treatment, and 2.52 for 20% CSF treatment were similar to each other and higher than the PER value of other treatments ($P < 0.01$). The PER value for plain tortillas was lower than for all other treatments ($P < 0.01$). The PER value for 10% and 15% CSF treatment were similar to each other. The PER value for 15% CSF treatment was no different from the 20% CSF treatment (Table 25).

Net protein ratio (NPR) is used to overcome some of the criticisms of the PER assay. Mean values for NPR are shown in Table 16. An analysis of variance for a randomized block design was used to test the hypothesis that there was no significant difference in NPR values among treatments (Table 26). The hypothesis was accepted at 0.01 level of significance, indicating that no difference existed among blocks, however, the hypothesis was rejected

TABLE 24

ONE-WAY ANALYSIS OF VARIANCE OF PROTEIN EFFICIENCY
RATIOS FOR RATS FED VARIOUS SOURCES OF PROTEIN

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Value	Critical F-Value*
Block	9	0.23	0.03	0.67	2.77
Treatment	6	7.78	1.30	34.86	3.17
Error	54	2.01	0.04		
Total	69				

* ($P < 0.01$) .

TABLE 25

RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST FOR
DETERMINING DIFFERENCES AMONG PROTEIN
EFFICIENCY RATIOS FOR VARIOUS
PROTEIN SOURCES

Diet No.	Treatment	Mean ¹
1	Casein	2.71 ^a
2	Plain Tortillas	1.67 ^d
3	Tortillas + 10% CSF	2.23 ^b
4	Tortillas + 15% CSF	2.30 ^{b,c}
5	Tortillas + 20% CSF	2.52 ^{a,c}
6	Tortillas + 25% CSF	2.60 ^a
7	100% LCP, CSF	2.63 ^a

¹Means with the same superscript are not significantly different ($P < 0.01$) .

for the various treatments. Using Duncan's New Multiple Range Test (Table 27), it was shown that NPR values for casein and 100%, 25%, 20% CSF treatments were similar to each other. The NPR value for plain tortillas was lower than for all other treatments.

An analysis of covariance was applied to determine the relationship between PER of the protein quality (X) and NPR (Y) (Table 28). Results indicated that the NPR value was not significantly different from PER value. Thus, showing the correlation of PER and NPR to determine protein quality.

Values of nitrogen digestibility for the various treatments are shown in Table 16. A one-way analysis of variance for a randomized block design was used to test the hypothesis that there was no significant difference among blocks. The results show that the hypothesis was rejected at the 0.01 level of significance among the various treatments (Table 29). Results of Duncan's New Multiple Range Test (Table 30) showed that the digestibility values for the diets containing 8.5% protein from casein, and 100%, 25%, 20%, 15%, and 10% CSF treatments were similar but higher than for plain tortillas ($P < 0.01$). It may be assumed that rats consuming a diet with a PER value lower than 2.0 and with a high leucine content tend to have a lower percentage of digestability than those having a higher PER value and an adequate balance of amino acids.

TABLE 26

ONE-WAY ANALYSIS OF VARIANCE OF NET PROTEIN RATIO
FOR RATS FED VARIOUS SOURCES OF PROTEIN

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Value	Critical F-Value*
Block	9	0.14	0.02	0.43	2.77
Treatment	6	5.27	0.88	24.08	3.17
Error	54	1.97	0.04		
Total	69	7.38			

* (P < 0.01).

TABLE 27

RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST FOR
DETERMINING DIFFERENCES AMONG NET PROTEIN
RATIOS FOR VARIOUS PROTEIN SOURCES

Diet No.	Treatment	Mean ¹
1	Casein	3.52 ^a
2	Plain Tortillas	2.57 ^d
3	Tortillas + 10% CSF	2.90 ^b
4	Tortillas + 15% CSF	2.93 ^{b,c}
5	Tortillas + 20% CSF	3.14 ^{a,c}
6	Tortillas + 25% CSF	3.19 ^a
7	100% LCP, CSF	3.17 ^a

¹Means with the same superscript are not significantly different (P < 0.01).

TABLE 28

ANALYSIS OF COVARIANCE FOR NPR AND PER OF PROTEIN QUALITY

Source of Variation	Degrees of Freedom	Sum of Squares			Deviation about Regression		
		ΣX^2	ΣXY	ΣY^2	$\frac{\Sigma Y^2 - (\Sigma XY)^2}{\Sigma X^2}$	Degrees of Freedom	F-Square Value
Block	9	0.23	2.44	0.14			
Treatment	6	7.78	6.02	5.26			
Error	54	2.01	2.26	1.97	1.45	53	1.46*
Treatment + Error	60	9.79	8.27	7.23	0.24	59	
Difference for testing among treatment means					520.37	6	0.04

* P < 0.01.

TABLE 29

ONE-WAY ANALYSIS OF VARIANCE OF NITROGEN
DIGESTIBILITY MEANS FOR RATS FED DIETS
WITH VARIOUS SOURCES OF PROTEIN

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Value	Critical F-Value*
Block	9	127.42	14.16	1.95	2.77
Treatment	6	293.19	48.87	6.74	3.17
Error	54	391.55	7.25		
Total	69	812.16			

* (P < 0.01) .

TABLE 30

RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST FOR
DETERMINING DIFFERENCES AMONG NITROGEN
DIGESTIBILITY MEANS OF YOUNG
RATS FED VARIOUS DIETS

Diet No.	Treatment	Mean ¹
1	Casein	92.84 ^b
2	Plain Tortillas	86.08 ^a
3	Tortillas + 10% CSF	90.56 ^b
4	Tortillas + 15% CSF	89.91 ^b
5	Tortillas + 20% CSF	90.10 ^b
6	Tortillas + 25% CSF	90.63 ^b
7	100% LCP, CSF	92.47 ^b

¹Means with the same superscript are not significantly different (P < 0.01) .

III. The Acceptance Study

Twenty-five persons, 20 Mexican-Americans and 5 Americans, were asked to judge the tortilla products. A random selection record of 15 persons was applied to a two-way analysis of variance to test the hypothesis (1) that there was no significant difference among the blocks, (2) there was no significant difference among the treatments, (3) there was no significant interaction of these two factors, in their effect on sensory evaluation of samples. The first and third hypotheses were accepted at the 0.01 level of significance (Table 31). There was no significant difference among the blocks of person selection. There was no significant interaction between person selection and sample treatments. The second hypothesis was rejected at the 0.01 level of significance, indicating significant differences among sample treatments.

Results of Duncan's New Multiple Range Test showed (Table 32) that plain tortillas and tortillas supplemented with 10% and 15% CSF were similar and also had a higher degree of acceptability than tortillas supplemented with 20% and 25% CSF. Tortillas supplemented with each level of CSF were acceptable and no significant difference was noted. These results indicated that all the samples were acceptable by the judges. However, comments indicated that the tortillas with 20% and 25% CSF tasted bland and were thicker than the plain tortillas.

TABLE 31

TWO-WAY ANALYSIS OF VARIANCE OF SENSORY EVALUATION
BETWEEN BLOCKS AND VARIOUS SOURCES OF PRODUCTS

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Value	Critical F-Value*
Block	14	27.04	1.93	2.39	2.42
Treatment	4	30.11	7.53	9.31	3.69
Block x Treatment	56	45.29	0.81	1.04	1.65
Within Replicates	75	58.50	0.78		
Total	149	160.94			

* (P < 0.01) .

TABLE 32

RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST
FOR DETERMINING SENSORY EVALUATION
AMONG TREATMENTS

Treatment	Mean ¹
Plain tortillas	4.53 ^a
Tortillas + 10% CSF	4.27 ^{a,b}
Tortillas + 15% CSF	4.20 ^{a,b}
Tortillas + 20% CSF	3.40 ^b
Tortillas + 25% CSF	3.50 ^b

¹Means with the same superscript indicate no significant difference (P < 0.01) .

CHAPTER V

SUMMARY AND CONCLUSIONS

Corn tortillas were supplemented with 10%, 15%, 20% and 25% levels of cottonseed flour to determine the potential in improving the diets of individuals who consume tortillas as a major dietary component. This research was conducted to determine the value of LCP cottonseed flour when added to tortillas in relation to amino acid composition, crude protein content, PER, and NPR in young rats, antioxidant potential and acceptability for humans.

Data obtained from both crude protein content and amino acid analyses have shown that LCP cottonseed flour increased protein quality and quantity when used to supplement corn tortillas since lysine, methionine, and tryptophan are higher in LCP cottonseed flour than in tortillas. Lysine, especially, was increased when various levels of LCP cottonseed flour were added to the tortillas. It has been indicated that excessive amounts of the essential amino acid leucine has a negative effect on protein nutrition in rats. This suggests that when cereal products are evaluated for protein value one should consider amino acid balance in terms of those supplied in super-optimal amounts as well as those supplied at less than ideal levels (59). plain corn tortillas have a high content of leucine, therefore, when they were supplemented with LCP cottonseed flour,

the leucine was decreased, thus improving the nitrogen digestibility. This can be attributed to an improvement in the leucine and isoleucine ratio.

The results of the fat rancidity tests indicated that tortillas supplemented with LCP cottonseed flour had lower rancidity values. These values further demonstrated that as the levels of cottonseed flour were increased, the rancidity values decreased, thus improving the shelf life of the tortillas.

In the animal study, the rats showed greater weight gain from rations containing various levels of cottonseed flour than from the plain tortillas. The protein efficiency ratios and net protein ratios were significantly higher in tortillas supplemented with LCP cottonseed flour. This indicated that tortillas supplemented with cottonseed flour had an improved protein quality. Results of Duncan's New Multiple Range Test indicated that tortillas supplemented with 20% and 25% cottonseed flour compared with casein in that values for both PER and NPR showed no significant difference.

The acceptability study showed that corn tortillas can be supplemented with 10% and 15% cottonseed flour without a significant difference in acceptability from the plain corn tortillas. However, there was no significant difference in acceptability of any of the supplemented tortillas. Comments from sensory panel members indicated that

tortillas with 20% and 25% cottonseed flour would be more acceptable if fried, which would improve color and flavor. The higher level of cottonseed flour seemed to produce a more bland product with less lime odor and flavor. Apparently the flavor resulting from lime treatment is considered desirable by Mexican-Americans. The results of the acceptability study indicated that tortillas can be supplemented with cottonseed flour up to 25%.

LIST OF REFERENCES

1. Hegsted, D.M. Amino acid tortification and the protein problem. The American Journal of Clinical Nutrition 21: 688, 1968.
2. President's Science Advisory Committi. The World Food Problem. Washington, D.C.: U.S. Govt. Printing Office, Vol. II, 295, 1967.
3. Matthews, R.H. Principles for the use of oilseed flour in cereal products to ensure consumer acceptance. Presented to Conference on Protein Rich Food Products from Oilseeds, New Orleans, Louisiana. 1968.
4. Altschul, A.M. The agricultural scientific and economic basis for low-cost protein foods. Presented at International Conference on single-cell protein, MIT. Oct., 1967.
5. Behar, M. INCAP. Experiences with food products containing cottonseed protein concentrates. In: Conference on cottonseed processing clinic, p. 36, New Orleans, Louisiana. 1965.
6. Kies, C., and Fox, H.M. Effect of the level of total nitrogen intake on second limiting amino acid in corn for humans. J. Nutr. 100: 1275, 1970.
7. Matthews, R.H., Murphy, E.W., March, A.C., and Dawson, E.C. Baked products: Consumer Quality, Composition, yield and preparation time of various market forms. USDA, ARS, Home Econ. Res. Report No. 22, 1963.
8. Eagles, J.A. Diets of low-income families. USDA, ARS. February 19, 1969.
9. Weatherwax, P. Indian Corn in Old America. The Macmillan Co., New York. 1954.
10. Inglett, G.E. Corn: Culture, Processing, Products. The AVI Publishing Company, Inc., Westport, Connecticut. 1970.
11. U.S. Department of Agriculture Agricultural Statistics. 639 p. United States Government Printing Office: Washington, D.C. 1971.

12. Liebenow, R.C. What is ahead for the corn industry
proc. corn quality conf., Univ. Illinois, Urbana.
1967.
13. Food and Agriculture organization of the United
Nations. Food balance sheets, 1964- 1966 aver-
age. 766 p. Rome, 1971.
14. Senti, F.R. and Schaefer, W.C. Corn its importance
in food, feed, and industrial uses. Cereal
Science Today 17: 352, 1972.
15. Mottern, H.H. Protein enrichment of Colombian corn
cakes. Cereal Science Today 15: 108, 1970.
16. Floyd, W.R. and Sotres, R.M. Method of making a
tortilla flour. U.S. Pat. 2,584,893. Feb. 5,
1952.
17. Diez De Sallano, C.S.F. and Berriozabal, J.M. Method
of producing corn tortilla flour. U.S. Pat.
2,704,257. March 15, 1955.
18. Dudley, J.W. and Lamber, R.J. Genetic variability
after 65 generations of selection in Illinois
high oil, low oil, high protein, and low protein
strains of zea mays L. Crop. Sci. 9: 179, 1969.
19. Mertz, E.T., Bates, L.S. and Nelson, O.E. Mutant gene
that changes protein composition and increases
lysine content of maize endosperm. Science 145:
279, 1964.
20. Nelson, O.E., Mertz, E.T. and Bates, L.S. Second
mutant genes affecting the amino acid pattern
of maize endosperm proteins. Science 150:
1469, 1965.
21. Lambert, K.J., Alexander, D.E. and Dudley, J.W.
Relative performance of normal and modified
protein (opaque-2) maize hybrids. Crop Sci.
9: 242, 1969.
22. Frost, H.C. and Robinson D. High lysine corn-what
lies ahead. J. of American Oil. Chem. Soc.
48: 407, 1971.
23. Gastrock, E.A., DA quin, E.L., Eaves, P.H. and
Cross, D.E. Edible flour from cottonseed by
liquid classification using hexane. Cereal
Science Today 14: 8, 1969.

24. Lawhon, J.T. and Rao, H.S. Edible cottonseed protein concentrate. Cereal Science Today 12: 40, 1967.
25. Pons, W.A., Jr., Hoffpanir, C.L. and Hopper, T.H. Gossypol in cottonseed: Influence of variety of cottonseed and environment. J. Agr. Food Chem. 1: 1115, 1953.
26. Harper, G.A. Development of glandless cottonseed. Proceedings-24th American Cotton Congress. 79-96, 1963.
27. Miravalle, R.J. and Hyer, A.H. Identification of the G/2 g/2 G/3 g/3 Genotype in breeding for glandless cottonseed. Crop Sci. 2: 395, 1962.
28. Gastrock, E.A. USDA, Agricultural Research Service 72-71, "Conference on protein-rich food products from oilseeds." New Orleans, La., p. 26, May, 1968.
29. Ridlehuber, J.M. Production of food-grade cottonseed protein by the liquid cyclone process. J. of the Ame. Oil Chem. Soc. 51: 153, 1974.
30. U.S. Food and Drug Administration, Fed. Regis. 37: 13713, 1972.
31. Smith, K.J., Lyman, C.M. and Watts, A.B. Nutritional value of glandless cottonseed protein. In: Conference on protein-rich food products from oilseeds, p. 81. New Orleans, La., May, 1968, USDA, ARS. 72-71 (1968).
32. Graham, G.G. Value of oilseed proteins for child feeding. In conference on protein-rich food products.
33. Wilcke, H.L. and Brew, W. Use status of cottonseed and other plant proteins. In: Proc. of conference on cottonseed protein concentrates. p. 105, New Orleans, La., 1964.
34. Milner, M. General outlook for seed protein concentrates. In: World protein resources, ed. by R. Gould, p. 52, Amer. Chem. Soc. Publ.: Washington, D.C., 1966.

35. Bressani, R., Elias, L.G., Braham, J.E. and Erales, M. Vegetable protein mixtures for human consumption. The development and nutritive value of INCAP mixture 15, based on soybean and cottonseed concentrates. Arch. Latinoamer, Nutrition 17: 177, 1967.
36. Abbot, J.C. Protein supplies and prospects. The Problems, World protein resources. Advances in Chemistry Series 57. Washington, D.C., 1966.
37. Overman, A. Antioxidant effect of soy and cottonseed flour in raw pastry mixes and baked pastry. Food Research 16: 39, 1951.
38. Carpenter, K.J. The estimation of the available lysine in animal-protein foods. Biochemical J. 77: 604, 1960.
39. Derse, P.H. Evaluation of protein quality II Methods. J. Asso. Off. Agr. Chem. 41: 192, 1958.
40. Chapman, D.G., Castillo, R. and Campbell, J.A. Evaluation of protein in foods. I. A method for the determination of protein efficiency ratios. Canar. J. Biochem. Physiol. 37: 679, 1959.
41. Morrison, A.B. and Campbell, J.A. Evaluation of protein in foods. V. Factors influencing the protein efficiency ratio of foods. J. Nutrition 70: 112, 1960.
42. Bender, A.E. and Doell, B.H. Biological evaluation of proteins: A new aspect. Brit. J. Nutr. 11: 140, 1957.
43. Sandra, L.M. Selection and Training of Sensory Judges. Food Technol. 27: 22, November, 1973.
44. Official Methods of Analysis of the Association of Official Analytical Chemist. 1970 11th ed., Association of Official Analytical Chemists, Washington, D.C.
45. American Association of Cereal Chemists Approved Methods 1: 02-03, 1971.
46. Beckman Model 116 Amino Acid Analyzer Instruction Manual, 1969. Palo Alto, California, Spinco Division of Beckman Instruments, Inc.

47. Beckman Model 121 American Association Analysis Instruction Manual, 1970. Spinco Division of Beckman Instruments, Inc., Palo, Alto, California.
48. McLaughlan, J.M. Nutritional Evaluation of Proteins by Biological Methods. Cereal Science Today 17: 162, 1972.
49. Li, J.C. Statistical Inference I. Distributed by Edwards Brothers, Inc., Ann Arbor, Michigan, 1964.
50. Snedecor, G.W. and Cochran, W.G. Statistical Methods, Sixth Edition, The Iowa State University Press. Ames., Iowa, U.S.A.
51. Edwards, C.C. The Food and Drug Administration in the Federal Register. p. 2124, January 19, 1973. (38: 2124, 1973)
52. Gopalan, C. and Srikantia, S.G. Leucine and Pellagra. Lancet 1: 954, 1960.
53. Belavady, B., Srikantia, S.G. and Gopalan, C. The effect of oral administration of leucine on the metabolism of tryptophan. Biochem. J. 87: 652, 1963.
54. Raghuramulu, S.G., Narasingarao, B.S. and Copalan, C. Amino acid imbalance and tryptophan-niacin metabolism. I. Effect of excess leucine on the urinary excretion of tryptophan-niacin metabolites in rats. J. Nutr. 86: 100, 1965.
55. Harper, A.E., Benton, D.A. and Elvehjem, C.A. L-Leucine and isoleucine antagonist in the rat. Arch. Biochem. Biophys. 57: 1, 1955.
56. Benton, D.A., Harper, A.E. and Elvehjem, C.A. Effect of isoleucine supplementation on the growth of rats fed zein or corn diets. Arch. Biochem. Biophys. 57: 13, 1955.
57. Bressani, R., Scrimshaw, N.S., Behar, M. and Viteri, F. Supplementation of cereal proteins with amino acids. J. Nutr. 66: 501, 1958.
58. Dixon, W.J. and Massey, F.J., Jr. Introduction to Statistical Analysis. McGraw-Hill Book Co., 1969.

59. Kies, C. and Fox, H.M. Interrelationships of leucine with lysine, tryptophan, and niacin as they influence protein value of cereal grains for humans. Cereal Chem. 49: 223, 1972.