THIAMIN CONTENT OF THREE SOURCES OF CORN AND AREPAS AS DETERMINED CHEMICALLY AND MICROBIOLOGICALLY

by

LAURA LEE KELLER

B.S., Kansas State University, 1979

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1985

Approved by:

Carole A. Z. Harbers

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

An arepa is an unleavened round flat bread made from corn flour, water, and salt. Arepas are known as the national bread of Venezuela, being consumed by 90% of the population; however, arepas also are eaten in other Latin American countries [1]. Arepas are nutritionally deficient, especially in lysine, thiamin, vitamin C, and iron [2]. Nuñez and Maga [2] cited a pamphlet published by Consejo Nacional de Investigaciones Cientificas y Technologicos de Venezuela (CONICIT) in 1976 stating the nutritional and food technological priorities for Venezuela. Included in those was the development and supplementation of cereal products and the development of new products to meet the Venezuelan nutritional needs. As a primary cereal dish, arepas are the main source of thiamin for the Venezuelan population. Therefore, improvement of the thiamin content of arepas would fulfill a priority as stated by CONICIT.

White dent is the most common type of corn used in making corn flour for arepa preparation [1]. The primary reason for arepas' thiamin deficiency is the method of preparing the corn flour. In various methods of arepa production the pericarp, and often the germ, are lost; and since these are the principal thiamin sources of the corn kernel, the thiamin content is reduced. Sometimes the corn is soaked in an alkaline solution, thus the thiamin content is reduced in these arepas since thiamin is destroyed by alkali. Alter-

ing the method of preparation could decrease thiamin loss, but the resulting arepa likely would not be as acceptable to people accustomed to arepas prepared in traditional ways. Comparison of research on arepas shows that thiamin content of corns vary somewhat depending on the source, but a comparison of thiamin content of various corns could not be found in research literature.

Most thiamin determination relating to corn products is done by chemical assay. In their thiamin determination of arepas, Suãrez [1] and Jaffe [3] used chemical assays; and Bressani et al. [4] chemically determined the amount of thiamin lost from corn to tortilla. None of these researchers determined the amount of thiamin lost from corn to arepas made from homemade masa. Gregory and Kirk [5] stress that accurate chemical determination of the vitamin content of foods is not very significant if the correlation between the chemical value and biologically available amount of the vitamin in the food is not known. Determining the bioavailable amount of vitamins in food is important in determining the adequacy of dietary intakes. Gregory and Kirk [5] used rat bioassay to determine bioavailability of thiamin in foods. Bioavailability of vitamins usually is determined microbiologically. No research on microbiologically measuring the thiamin content of corn or arepas could be found.

The purpose of this research was to prepare arepas by a traditional method using white dent corn from three different

geographical regions: Kansas, Mexico, and Venezuela. The amount of thiamin in the whole corn was determined chemically, and the amount of thiamin in the arepa was determined by chemical and microbiological assays.

The data obtained were analyzed to determine

1) if there was a significant difference in thiamin content of the different corns used,

2) how much thiamin was lost during arepa preparation as measured chemically,

3) how much thiamin was bioavailable in the arepa as measured by microbiological assay, and

4) if there was a significant difference in the thiamin levels measured by the two different methods of analysis.

REVIEW OF LITERATURE

Corn: History, Use, and Types

Corn is a plant with several common names. Maize is the most widely used and international term; but in the U.S., maize's equivalent term is corn, thus that is the the term used with this research project. Zea mays L. was given to corn in the 18th century as it's botanical name. Zea is Greek for cereal, being derived from a verb meaning "to live", and mays is of Indian origin. The importance of corn in many populations' diets is shown by the fact that many Indian forms of the word corn meant "that which sustains us" [6]. Corn's probable origin is the Americas, the central and/or southern regions. The earliest ears of corn found were discovered in Mexico. They were small ears dating back to 2000 B.C. and were from a cultivated plant [7].

Although corn production has spread worldwide, countries differ in how much corn and corn products they consume. "Maize eating" populations are countries for whom maize is the staple food, and are usually in regions which are poor and less developed agriculturally. There are a number of these countries, but Tables 1 and 2 refer to four of the main ones; Romania, Mexico, Venezuela, and Guatemala [8]. Although this information is over 30 years old, it is useful in presenting a general idea of the importance of corn products in Venezuela and the other countries. Table 1

Table 1. Maize diets in selected countries
g/caput/day

	ROMANIA	MEXICO	VENEZUELA		GUATEMALA			
					Ladinos		Indians	
	Rural	Urban	Rural	Urban	Poor	Others	Poor	Others
Cereals and cereal products:								
Maize products	533	254	436	314	371	420	472	562
Wheat products	68	174	3	9	11	29	2	9
Other cereals: rice, millet, etc.	8	17	0	16	10	16	3	3
Starchy roots, tubers and analogous starchy foods	33	28	4	62	12	24	11	12
Pulses, nuts and seeds	48	42	80	80	44	57	51	58
Vegetables	74	100	1	4	92	103	87	114
Fruits	30	66	10	114	90	61	27	37
Meat, poultry, and fish	35	64	51	51	51	73	27	47
Eggs	4	6	6	2	6	18	3	6
Milk and cheese	45	316	93	186	59	147	12	29
Fats and oils	9	24	6	12	5	12	1	2
Sugars and jams	2	56	89	100	42	49	30	36

(from reference #8)

describes the diets of the various groups in the countries. Table 2 shows the calorie and nutrient intakes per nutrition unit per day of the countries compared to the RDA for an adult man. These figures reveal that the diets and nutrient intakes vary with the country and with the groups of people within the country. The poorer the people, the less nutritionally adequate their diet. In 1950, Venezuela was the only country whose daily nutrition unit intake for thiamin was lower than the RDA. The World Health Organization's 1967 nutrition intake statistics revealed that the total thiamin intake in Venezuela that year was 0.69 to 0.80 mg/person/day [9]. The Recommended Daily Allowance for an adult male is 0.5 mg/1000 kcal [10]. More recent information could not be found, but this information illustrates the need for increasing thiamin intakes for Venezuelans.

The main types of commercial corn are: dent, flint, floury, sweet, and popcorn. Dent is used in the preparation of arepas, and it can be white or yellow. White corn is almost always used for making arepas. Yellow corn is not as abundant as white in Venezuela and is more expensive; but yellow corn occasionally is used to make an arepa which is different in flavor and appearance, higher in niacin and carotene, and yet acceptable [1].

Table 2.
Calorie and nutrient intake levels of maize diets in selected countries
Intake/nutrition unit/day

	ROMANIA	MEXICO	VENEZUELA		GUATEMALA				N.R.C. Adult Male RDA
					Ladinos		Indians		
	Rural	Urban	Rural	Urban	Poor	Others	Poor	Others	
Calories	3828	2765	2764	2876	2731	3232	2721	3184	3000
Protein g	85	74	85	83	64	82	63	77	70
Calcium mg	300	1550	426	556	706	862	805	981	1000
Iron mg	23	15	19	20	19	23	21	26	12
Vitamin I.U.	---	4060	414	960	4842	6252	5573	5291	5000
Thiamin mg	---	1.8	.9	1	2.9	3.3	3.1	3.7	1.5
Riboflavin mg	---	1.5	.9	1	1	1.3	.8	1	1.8
Niacin mg	---	10.8	10	9.8	13.3	16.2	12.7	15.7	15
Ascorbic acid	---	43	10	41	71	62	47	60	75

(from reference # 8)

Origin and Use of the Arepa

Suãrez [1] and Cuevas et al. [11] discussed the following history and use of arepas. The arepa probably originated with the first known Indians in Venezuela, and other corn dishes followed. The original name "EREPA" was the generic name of ripe corn in the Indian language Cumanagoto. In other countries the term arepa is used for a corn cake slightly different from the type made in Venezuela. The Indians shaped the arepa round to resemble the sun. The arepa always has been the primary bread of Venezuela, and no promotion to eat arepas has been needed; it is popular on its own. Arepas often become popular with foreigners who are visiting or staying in Venezuela.

The arepa is eaten by poor and wealthy alike. Once arepas only were eaten at home; but now arepas are sold at restaurants (areperias), schools, and other public places. Arepas usually are consumed for breakfast, lunch, or snack, but can also be eaten for dinner. They are eaten plain or in combination with cheese, butter, eggs, red meats, poultry, beans, jams, or other ingredients. Venezuelans eat an average of 3 to 4 arepas/day, or 155 g/day [1,11].

Arepas vary in size, shape, and thickness. Arepas can weigh from 15 g to 150 g or larger, be flat or slightly spherical, and range from very thin to thick. They can be boiled, baked, fried, or a combination of these, or can be

toasted. Food may be eaten on top of them, or the arepa can be split and filled [1].

Types and Preparation of Arepas

When reading research articles concerning arepas, one should realize that there are different types of arepas based on corn flour preparation. The corn may be totally degermed, partially degermed, or the germ left intact, and may be soaked in water, calcium, ash, or other alkaline substances [1]. The method of preparation will affect the appearance, consistency, and nutritional value of the arepa.

Suãrez [1] and Cuevas et al. [11] described the preparation steps of a common traditional type of arepa in Venezuela. The corn is degermed mechanically and soaked in water. Following are the steps:

1) mechanical phase--the pericarp and germ are separated and discarded by pounding the corn with a mortar in a wooden bowl (pilon), or an electric machine. The resulting pieces of corn look polished and are called pilado. Though the pilado is supposedly degermed, it can retain up to 40% of the germ [12].

2) wash.

3) cook--pilado is boiled in water to soften the grain.

4) mill--the soaked pilado is ground with a metal or stone mill. As the pilado is ground, water is added to make a masa (dough) which will stick to the mill. The masa will be white in color and semihard.

5) knead--the masa is kneaded with water and salt to the proper consistency. The person preparing the

masa pats it, and knows it is ready when it has a characteristic sound or "ring".

6) shape--a portion of masa is shaped in the palm of the hand by patting a ball into a flat circle.

7) cook--the arepas are cooked on a hot plate or grill called a budare. The budare is clay or iron. The clay budare and wood oven give the best flavor. The arepa should be golden brown and the sides should be hard.

Arepas also can be prepared from non-degermed corn which is cooked and steeped in an alkali solution to facilitate removal of the pericarp. The alkaline soaked corn is called nixtamal, and it is ground to form masa [1]. This masa can be shaped as the masa previously described and cooked in the manner desired.

Nutritional Studies on Arepas and Tortillas

Tortillas usually are prepared from nixtamal [3], thus, nutritional research on these tortillas can be useful for comparison with data on nixtamal-prepared arepa research. These tortillas can differ from arepas in the type of corn used and how they are cooked, so these differences, if present, should be kept in mind when making nutritional comparisons.

Early arepa research was conducted on homemade arepas. Suárez [1] conducted nutritional studies on arepas made from pilado, from nixtamal, and from yellow corn. Protein, fat, carbohydrate, mineral, and vitamin content were determined. Protein content was found to be low (3.95 to 5.50%); and the

B-vitamin, including niacin, amounts were low. Suãrez supplemented arepas with amino acids to improve protein efficiency and prepared a supplemental mix of thiamin, riboflavin, niacin, and iron to increase the arepa's nutritive value. Jaffe [3] determined protein, fat, ash, fiber, carbohydrate, mineral, and B-vitamin content of whole corn, pilado, nixtamal, tortilla, and commercial arepa. Compared to the nixtamal, the pilado had much more loss of fat, potassium, thiamin, riboflavin, and niacin. The nixtamal was considerably higher in calcium since it was soaked in a calcium solution.

Chavez and Pellet [13] studied protein quality of 12 Latin American food mixtures using rat bioassay. They tested five different arepa preparations including arepa with margarine, arepa with white cheese, arepa with sardines, arepa with black beans, and arepa with meat. The dry weight percent protein of the arepas ranged from 8.2 to 22.0, and the protein efficiency ratio (PER) ranged from 0.5 to 4.6.

Bressani et al. [4] studied chemical changes in white and yellow corn during tortilla preparation. For the white corn, the combined chemical and physical loss from corn to masa averaged 60% thiamin, 52% riboflavin, and 32% niacin.

More recent research has been conducted on arepas made from commercial corn flour. Nuñez and Maga [2] compared sensory properties of arepas made from packaged corn flour and extruded corn flour. Seventeen panelists assessed the

arepas for acceptability, texture, and flavor; and significant differences were revealed between packaged flour and extruded flour. By comparing water absorption, water solubility, and consistency of a water suspension, the researchers concluded that the extruded flour had better functional characteristics than did the packaged flour.

Smith et al. [14] also produced corn flour using an extrusion cooker, and compared arepas made from the extruded flour to arepas made from flours from the conventional column-roller process. Based on sensory and instrumental tests they concluded that at appropriate extrusion conditions, the extruded flour produced arepas equal to or better than the conventional flour, thus extrusion could be a good alternative to column-roller pre-cooking.

Alvarez [15] analyzed the proximate and mineral composition of baked and fried arepas from packaged corn flour. The baked and fried arepas contained 54.81 and 26.82% moisture, 0.74 and 13.26% fat, 4.26 and 6.28% protein, 1.09 and 2.03% ash, 0.83 and 0.85% fiber, 38.27 and 48.96% carbohydrate, and 168.5 and 340.3 kcal/100 g, respectively. Zinc, Mg, K, Cu, and Ca increased during processing, but no minerals were present in sufficient quantity to make the common arepa a good source of any specific mineral. Water activity and pH did not change significantly. In other research Alvarez [16] studied the microbiological safety of baked and fried arepas. One hundred and thirty-six bacterial and 65

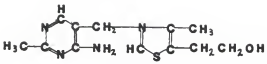
fungal isolates were identified from the flour dough and arepas; and although no pathogenic organisms were isolated in the arepas, strict sanitary conditions were recommended to achieve a safe product.

Harbers et al. [17] compared the quality of arepas made from American midwestern grown corn to arepas made from packaged corn flours. When compared to the doughs and arepas prepared from commercial flours, the homemade masa was more yellow; and arepas prepared from the homemade masa had a more intense corn flavor and darker crust color, as determined by a sensory panel and by instrument.

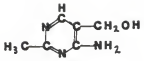
Cuevas et al. [11] reviewed traditional arepa preparation, development of precooked corn flour, and quality assurance. Important factors in quality control included hard endosperm corn, cooking and rolling conditions, and particle size of flour. Optimum particle size for arepa flour was listed as corresponding to screens between 35 and 48 mesh.

Thiamin

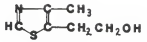
Chemical structure and properties. Thiamin is a water soluble B-vitamin. Its structure is a simple compound composed of a pyrimidine moiety and a thiazole moiety joined by a methylene bridge [18].



Thiamin

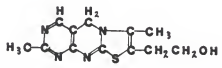


Pyrimidine



Thiazole

Thiamin is found in natural material in the free form, as mono-, di-, and triphosphoric esters, also as mono- and disulfides. Free thiamin is the most abundant form found in plant extracts. Below pH 5, thiamin is very stable. At pH 5 to 7, thiamin is destroyed by autoclaving, and above pH 7, thiamin is destroyed by boiling or storage at room temperature [19]. It is thought that the thiazole ring opens and is oxidized, especially upon heating. In a highly alkaline solution thiamine is oxidized by ferricyanide to thiochrome.



Thiochrome

Food sources Good sources of thiamin include dried beans and peas, nuts, whole grain cereals and breads, pork, and organ meats. Dairy products, fruits and vegetables, and other meats, poultry, and fish also contribute thiamin in the diet [1]. The recommended thiamin allowance is related to energy intake; 0.5 mg/1000 kcal is the daily recommendation for adults [10].

Functions Several of the major functions of thiamin include the body's use of thiamin to form the co-enzyme thiamin pyrophosphate, which is important in energy metabolism, and thiamin's action in nerve transmissions [20].

Deficiency symptoms When less than the minimum amount of recommended thiamin is consumed over a period of time, deficiency symptoms affecting the gastrointestinal, cardiovascular, and peripheral nervous systems appear [21]. Symptoms include loss of appetite, depression, and as deficiency continues, constipation and neurological changes. Beriberi is the severe form of thiamin deficiency, and characteristics include an enlarged heart, severe edema, and muscle wasting [20,21].

Effects of cooking Thiamin loss from foods is caused by cooking in water, heat, oxidation, and cooking in an alkaline solution [20]. The time of heating, processing, and storage also are important factors contributing to foods' thiamin loss [22].

Thiamin assays

Thiamin in foods can be determined by the following assays: animal, chemical, microbiological, high pressure liquid chromotography, colorimetric measurement, and measurement of absorption spectrum [23]. The most frequently used assays are the first three.

Animal assays The first methods used to determine thiamin in food were animal assays. The advantages of animal assays are that they are specific, measure the physiologically available thiamin amount, and do not require extraction procedures. They are not commonly used today, however, because of cost, time required, and lack of precision [23].

Chemical assay The thiochrome technique is the assay most often used for determining thiamin in natural materials. Thiamin is extracted from the sample by dilute acid hydrolysis and enzymatic digestion. The pH of the acid extract ensures that the thiamin is very stable, even when heated. The enzyme solution used contains phosphatase which hydrolyzes any phosphate esters of thiamin present, converting bound thiamin to its free form. The enzymes hydrolyze the starches in plant samples, aiding extract filtration. The thiamin present in the extract is oxidized to thiochrome by alkaline ferricyanide, and extracted by isobutanal. Thiochrome has an intense blue fluorescence under ultraviolet light, thus a photoflourometer is used to determine amount of flourescence; and thiamin amount is calculated. Several disadvantages of the thiochrome method include its lack of sensitivity and possible interference by other fluorescent substances [19].

Microbiological assay Accurate chemical determination of vitamin content of foods is of little value unless the chemically assayed value can be correlated to the biologi-

cally available amount of vitamin in the food. Bioavailability of vitamin data is important in evaluating the adequacy of dietary intakes [5]. Compared with chemical methods, microbiological methods for thiamin require less equipment and material for assay, can be more sensitive and specific, and many samples can be assayed inexpensively in a short time. These methods are quite sensitive to thiamin (0.001 mμg to 1 μg) and generally are reproducible to better than ±10%. However, microbiological methods can suffer from poor reproducibility with slight variations in procedure or if nonchemically defined media are used [18,23].

Microbiological methods for vitamin analysis are based on the observation that certain microorganisms require specific vitamins for growth. When the samples containing the vitamin are added to a nutrient medium and then inoculated with the specific bacteria, growth over a specified incubation time will be directly proportional to the amount of vitamin present. Growth is measured photometrically, and the sample solution and reference solution can be compared accurately [24].

The following characteristics are required for a test organism: require the vitamin, be genetically constant during prolonged subculture, have a growth response that is easily measured, have a rapid growth cycle, possess nutritional requirements similar to man, and be non-pathogenic. Lactobacilli are the microorganisms most often used. Yeasts,

molds, and protozoa are not used as frequently because their growth characteristics usually are less suitable [19].

There are five types of thiamin requiring microorganisms, differentiated by the type of thiamin they require. They can require intact thiamin, the pyrimidine moiety, the thiazole moiety, either the pyrimidine or thiazole moieties, or both the pyrimidine and thiazole moieties. Mammals require intact thiamin, thus the microorganism used for thiamin assay should also have this requirement [18].

Lactobacillus fermentum is a bacterium which requires intact thiamin for growth. This bacterium is rod shaped, variable in size, non-motile, and heterofermentative. There is no growth at 15°C; optimum growth occurs at 41-42°C; growth can occur at 45°C. Niacin also is required for growth, but riboflavin and folic acid are not [25]. L. fermentum can be affected by pentoses, reducing agents, fructose, maltose, calcium, and glucose heat degradation products. If the incubation period is limited to 18 hrs, the pyrimidine and thiazole moieties do not permit growth [23]. In 1944 Sarett and Cheldelin introduced the use of L. fermentum for thiamin determination. It is extremely sensitive for thiamin and is not affected by thiamin moieties. It has been considered the best bacteriological method for thiamin and has undergone improvement over the years [23]. Lactobacillus viridescens is another bacterium frequently used for thiamin assay. L. viridescens is said to compare favorably with the thiochrome

method but is more specific and convenient [23].

Microbiological vitamin assay studies

Voight et al. [26] compared protozoan and conventional methods of vitamin analysis. Thiamin content of tomato juice, orange juice, blood, yogurt, round steak, and spinach was determined with the bacteria Lactobacillus viridescens, the protozoa Ochromonas danica, and the thiochrome method. Except for tomato juice, all samples indicated higher ($P < 0.05$) amounts of thiamin as measured by L. viridescens than by thiochrome measurements. Generally, all three methods indicated increased amounts of vitamins in samples when the food extracts received acid hydrolysis and enzymatic treatments.

Voight et al. [24] used L. viridescens and O. danica for thiamin determination in another study. Standard vitamin calibration curves were prepared to determine minimum and maximum vitamin concentrations that could be determined by microorganisms and protozoa, and to determine the incubation times needed for the growth responses to stabilize. From 0.2 to 10 ng per ml, the two assay methods for thiamin were found to be equally sensitive. The L. viridescens assay was limited to 16 to 18 hrs since longer incubation time allows enzymatic digestion of the bacterial cells, resulting in decreased absorbance. Although O. danica was found to have a

stabilized growth response, it required four more days for incubation than was needed for L. viridescens.

To determine the thiamin content of triticale, wheat, and rye, Michela and Lorenz [27] used L. fermentum for microbiological assay. The bacteria were received and rehydrated just before the grains were analyzed so that the culture did not need to be maintained over a period of time. The researchers did this because occasionally the bacteria can develop the ability to synthesize thiamin when the recommended culture maintenance procedure is followed. The whole-grain wheat thiamin values were higher than previous thiochrome-determined values reported in the literature. Thiamin amount of triticale was equal to wheat, and rye's thiamin amount was significantly lower than that of wheat or triticale. The microbiologically-determined thiamin amounts for the grain samples were believed to be accurate. Therefore, those higher thiamin values were attributed to environmental and agronomic conditions under which the grains were grown, and not to method differences.

No studies determining the thiamin amount in corn or arepas by microbiological assay could be found; however, Wang and Fields [28] used Pediococcus cerevisiae for lysine assay, and Lactobacillus plantarum for tryptophan assay in home-prepared tortillas enriched with germinated corn. Germination increases the lysine and tryptophan amounts in corn, which is deficient in these two amino acids. As mea-

sured microbiologically, the lysine and tryptophan values increased from 23 mg/g N and 3 mg/g N for nongerminated corn to 68 mg/g N and 26 mg/g N after germination. However, taste panels found the tortillas made from lime-treated corn to be preferred over the germinated corn-enriched tortillas.

MATERIALS AND METHODS

Source of materials

Preliminary research Preliminary work included chemical thiamin determination of six types of Kansas white hybrid corns. Their thiamin values (μg/g, dry weight) were: 4.34, 4.63, 5.03, 5.49, 5.74, and 6.05, showing differences among varieties. Small hybrid sample amounts did not allow using any of these corns for arepa production.

Selection of corn Three different corns were selected to compare the amount of thiamin yielded in the arepas made from each corn. A mixture of white hybrid dent seed harvested in Kansas (White Hybrid Performance 1984) was obtained from the Kansas State University Department of Agronomy; a white dent corn was obtained from The International Center for Improvement of Maize and Wheat (CIMMIT), Mexico City, Mexico; and a third white dent corn was obtained from Venezuela through International Multifoods, Minneapolis, Minnesota. These three corns were stored at 0°C until prepara-

tion of the arepas. Cracked and broken kernels were sorted out by hand and discarded.

Preparation of arepas

For making arepas, 100 g of each corn were rinsed in distilled water. A modification of a procedure developed by Hendershot [29] for corn tortillas was used to prepare home-made masa. Two g of calcium hydroxide were mixed with 280 ml of distilled water and placed in a two-quart stainless steel pan. The corn was added, the pan covered, and the mixture heated to 90°C on a Roper gas range and held at a temperature of between 85°C and 90°C for 40 min. The pan was removed from the heat, and the corn mixture was steeped for 11 hrs.

After steeping, the liquid was drained. The corn was placed in a metal sieve and rinsed with running tap water for 5 min to remove pericarp, then frozen for 24 hrs at 0°C. The frozen corn was freeze-dried for 24 hrs. The freeze-dried corn was ground using a KitchenAid mixer (Hobart Corp, Model K5-A) with a grain mill attachment (Model GM-A) to produce approximately 90 g of corn flour. Sixty g of the Venezuelan flour was combined with approximately 70 ml of distilled water, and 60 g of each of the Mexican and Kansan flours were combined with approximately 65 ml of distilled water. Each flour was mixed in a stainless steel bowl to produce approximately 125-130 g of cohesive dough.

Each arepa was made by pressing 40 g of dough into a 6.3 cm (2 1/2 ") x 8.2 cm (3 1/4") round mold sprayed with vegetable spray. The arepas were cooked 10 min on each side on an iron griddle which had been preheated for 10 min to 204.4°C (400°F) on a Roper gas range.

Flour particle size

To separate and discard flour particles larger than 0.425 mm, each corn flour was sieved with a 42-mesh screen using a Ro-Tap Testing Sieve Shaker (Model B) for 15 min [11].

pH

pH was measured on duplicate samples of corn and arepas following AOAC procedure [10]. Ten g of each sample were mixed with 100 ml distilled water and allowed to set for 30 min. The supernatant was poured off, and after 10 minutes, the pH of the supernatants was taken.

Moisture

Percentages of total moisture of the raw corn, freeze-dried corn, and the baked arepa were determined in a C.W. Brabender Semi-Automatic Rapid Moisture Tester (Model SAS 577). Duplicate 10 g samples were held at 120°C for 60 min before percentages were determined [17].

Chemical analysis

Amounts of thiamin in the raw corn and cooked arepas were determined using the thiochrome method [31]. The raw corn was ground with grain mill attachment, and 3.5 g samples were analyzed. The arepas were blended in a Waring blender (Model 8), and 5 g samples were used for analysis. Preliminary work indicated that the Decalso purification step was not needed. Flourescence was determined using an electric photoflourometer (PH Coleman, Model 12-C). Thiamin content was determined on a dry weight basis from four replications of the thiochrome procedure.

Microbiolgical analysis

Amounts of thiamin in arepas were determined using the procedure for thiamin assay described in the Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures [32]. The filtrate obtained during chemical analysis of cooked arepas was inoculated with Lactobacillus fermentum, incubated at 37°C for 17-18 hrs, and turbidity was determined using a spectrophotometer (Bausch and Lomb Spectronic 20) at a wavelength of 540 nu. Four replications of the procedure were made for each type of arepa.

Statistical Analyses

Data for thiamin amounts of corns and arepas (both methods), pH values of corns and arepas, % moisture of are-

pas, thiamin loss during arepa preparation, pH changes, and microbiological and chemical values comparisons were analyzed by analysis of variance (ANOVA) for a split-plot design. When the ANOVA procedure indicated differences in means, Duncan's Multiple-Range Test was used to determine significant differences [33].

RESULTS AND DISCUSSION

Percentage moisture

Moisture content was not significantly different among arepas (Table 3). The actual moisture content of arepas is presented in Table 4. These % moistures are within the range of 44.48% to 60.50% which Suãrez [1] listed for 16 types of arepas.

Table 3. F-values for variables among sources.

Variable	F-values
Corn pH	1.08
Arepa pH	134.94***
Thiamin content	
Corn	134.5***
Arepa	
chemical	1227.56***
microbiological	764.91***
Percentage moisture	0.56

*** P<0.001

Table 4. Mean* percentage moisture, thiamin loss, and pH change values among sources.

	Corn Source		
	MEX	KAN	VEN
Percentage moisture	45.01	44.68	45.89
Percentage thiamin loss from corn to arepa	24.1	30.4	47.5
Change in pH (corn to arepa)	+1.00	+1.43	+2.12

MEX = Mexico KAN = Kansas VEN = Venezuela

*Mean of four replications

pH

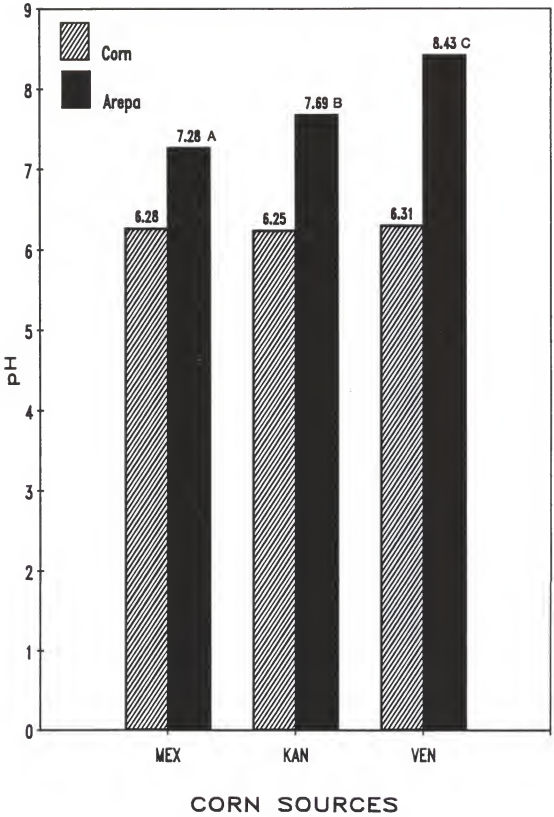
The F-values for differences in pH among corns and among arepas revealed no significant differences for corns, but did show differences ($P<0.001$) among arepas (Table 3). The Venezuelan arepa had the highest pH, while the Mexican arepa had the lowest pH (Figure 1). Harbers et al. [17] found mean pH of arepas from homemade masa to be 8.16, which is in the range of pH values found in this study. For each corn, there was an increase in pH from corn to arepa ($P<0.05$) (Figure 1), and these increases differed among sources ($P<0.001$). As illustrated in Table 4, the Mexican corn had the smallest pH increase, while the Venezuelan corn was affected most by the alkaline steeping.

Figure 1. Mean* pH values of corns and arepas.

*Mean of four replications

MEX = Mexico KAN = Kansas VEN = Venezuela

ABC-values with different letters among sources differ significantly ($P<0.05$) as determined by Duncan's Multiple-Range Test.


Corn
Arepa
pH
9
8
7
6
5
4
3
2
1
0
6.28
7.28 A
6.25
7.69 B
6.31
8.43 C
MEX
KAN
VEN
CORN SOURCES

Thiamin in corn

Table 5 shows that when data for thiamin content in corn were analyzed, the only variable to show significant difference was source. Values among replications and between samples did not vary. Thiamin content of raw corns differed ($P<0.001$) (Table 3). The Mexican corn had the most thiamin, and the corn from Venezuela had the least (Figure 2). With the exception of Venezuelan corn, which contained much less thiamin than the other two sources, these amounts are similar to the findings of Bressani et al. [4] and Jaffe [3], who reported 4.57 and 5.0 μg/g thiamin, respectively.

No information could be found on factors influencing the thiamin content of corn. However, for several other grains, researchers do not agree on whether thiamin is influenced genetically or by environment [33]. Both factors likely are important, and further research is needed.

Table 5. Analysis of variance for differences:
A. of thiamin amounts among corns
B. between two methods among sources

		mean square/significance	
source of variation	df	A	B
replication	3	0.00	0.16
source	2	2.38***	26.56***
rep x source	6	0.01	0.09
sample	1	0.00	0.00
source x sample	2	0.00	0.01

*** $P<0.001$

Thiamin in arepas - chemical measurement

The chemically assayed thiamin contents of the arepas were different ($P<0.001$) (Table 3). As in the raw corns, Mexican arepas showed the highest thiamin content, Venezuelan arepas showed the lowest, and Venezuelan arepas differed the most in thiamin content (Figure 2). Suárez [1] determined the amount of thiamin in 16 types of arepas; the values ranged from 0.27 μg/g to 2.79 μg/g. Jaffe [3] reported 0.34 μg/g thiamin for commercial arepas. Thus, the Mexican and Kansan arepa values were higher than previously reported values.

Percentage thiamin lost in arepa preparation

For each source, thiamin amounts decreased from corn to arepa ($P<0.05$) (Figure 2), and these decreases differed among sources ($P<0.001$). Table 4 shows that the Mexican corn had the smallest, and the Venezuelan corn had the largest amount of thiamin lost during arepa preparation. An increase in % thiamin loss during arepa preparation corresponds to an increase in pH of the arepa. This may be attributed to thiamin's decrease in stability as pH increases.

Using similar preparation techniques as the arepa, Bressani et al. [4] found a 60% loss of thiamin from corn to masa (tortilla dough), and Jaffe [3] showed a 34% loss of thiamin from corn to tortilla. Variations in reported %

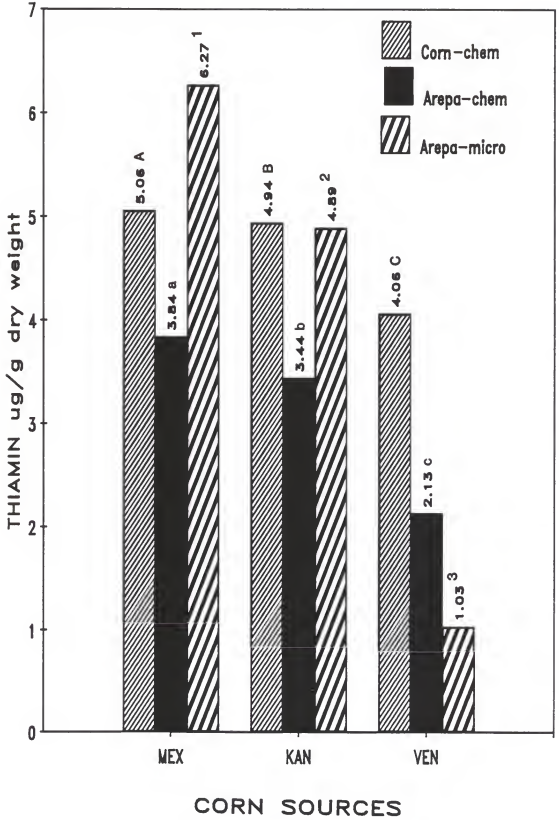
Figure 2. Mean* thiamin amounts of corns and arepas as measured chemically and microbiologically.

*Mean of four replications

Corn & Arepa-chem = thiochrome assay
Arepa-micro = L. fermentum assay

MEX = Mexico KAN = Kansas VEN = Venezuela

ABC-values with different letters/numbers among sources
abc differ significantly ($P<0.05$) as determined by
123 Duncan's Multiple-Range Test.


Corn-chem
Arepa-chem
Arepa-micro
THIAMIN ug/g dry weight
0
1
2
3
4
5
6
7
5.06 A
3.84 a
6.27 1
4.94 B
3.44 b
4.89 2
4.06 C
2.13 c
1.03 3
MEX
KAN
VEN
CORN SOURCES

thiamin loss during tortilla preparation could be due to different types of corn, and/or different methods of preparation which remove varying amounts of germ and bran, the thiamin rich parts of the corn kernel.

Thiamin in arepas - microbiological measurement

Arepa thiamin contents as determined microbiologically differed ($P<0.001$) (Table 3). The Mexican arepa had the highest value, and the Venezuelan arepa had a much lower thiamin value when compared to the other arepas (Figure 2). The three types of arepas had differing amounts of thiamin (Table 5). These results may reflect accurately the amount of thiamin that is bioavailable in the arepas; but due to variability of results possible with microbiological methods, further research would be needed to determine conclusively the amount of thiamin bioavailable in the arepas.

Microbiological determination of corn was not conducted because the bioavailability of thiamin in raw corn was not regarded as necessary information. However, with the high arepa values from the microbiological method, having those measurements would have been beneficial in interpreting the microbiological values.

Chemical and microbiological method differences

Within sources, the arepa's thiamin content differed ($P<0.05$) as measured by the two methods (Figure 2). When chemical and microbiological assays were compared, the Mex-

ican and Kansan arepas showed an increase in thiamin amount (63.3% and 42.4 %), while the Venezuelan arepa showed a decrease in thiamin amount (-51.5%). Among the corns, those differences between methods differed ($P<0.001$) (Table 5).

The higher microbiological values agree with Michela and Lorenz [27], who reported higher thiamin values for wheat, triticale, and rye using L. fermentum than for thiochrome-determined values. Voight et al. [26] claimed that when compared to thiochrome values, L. viridescens indicated higher thiamin values for all samples except one, which showed a lower value.

There are several possible reasons for the much lower microbiological value of the Venezuelan arepa. As other studies have shown, the microbiological method can give varied results since it is a sensitive assay, thus the lower value could be attributed to variability within the microbiological method. The Venezuelan arepa may have contained an unknown factor which inhibited growth of L. fermentum. The treatment of corn to prevent germination can inhibit bacterial growth, but the Venezuelan corn was untreated. A third possible reason for the lower thiamin value would be an actual reflection of increased loss of thiamin in the Venezuelan arepa. The Venezuelan corn showed the largest increase in pH and the greatest % loss of thiamin in arepa preparation (Table 4). Because the microbiological method can be more sensitive than the thiochrome method, the L.

fermentum assay may be showing a more sensitive measurement of the amount of thiamin lost in the Venezuelan arepa. Additional research would be needed to draw further conclusions.

Personal Observations

During arepa preparation, the author noticed subjective differences among the corns and arepas. When thiamin is dissolved in a strong alkaline solution, it turns yellow and then fades [34]. All three corns became more yellow when soaked in the alkaline solution, but the Venezuelan corn's color became a deeper yellow, which resulted in the Venezuelan arepa being noticeably more yellow than the other two arepas.

In addition to color, the Venezuelan flour had other differing characteristics when compared to the Mexican and Kansan corn flours. The Venezuelan flour required less water to form a dough; the dough formed was less sticky and more closely resembled commercial arepa dough. The Mexican and Kansan corns produced whiter, more sticky doughs which were harder to shape than were the Venezuelan arepas. All three types of arepas required the same cooking time.

Recommendations

Little is known about how thiamin is determined in corn, and few studies have determined corn's thiamin content. Corns analyzed in preliminary research had varying thiamin amounts. The three sources of corn from this study had dif-

ferent thiamin contents and had different changes in alkalinity, resulting in varying thiamin loss during arepa preparation. Therefore, research seeking to understand factors affecting corn's thiamin content, and work on developing corns with higher thiamin amounts that lose less thiamin during alkaline soaking could help improve arepas' thaimin values.

SUMMARY

White dent corns from Kansas, Mexico, and Venezuela were used for arepa preparation from homemade masa. For each source of corn, thiamin content was determined by the thiochrome method. Thiamin content of each type of arepa was determined chemically and microbiologically. pH change and thiamin loss from corn to arepa were calculated.

Data were analyzed by ANOVA using a split plot design. When differences in means were indicated, Duncan's Multiple-Range Test was used to determine significant differences.

Thiamin content varied among sources of corn. Measured chemically, thiamin loss occurred from corn to arepa. When compared to chemically determined amounts, microbiological thiamin determinations resulted in higher values for Mexican and Kansan arepas, and a lower value for the Venezuelan arepas. The two methods gave differing amounts of thiamin for each type of arepa.

The pH values increased from corn to arepa due to alkaline steeping. Among sources during arepa preparation, an increase in % thiamin loss corresponded to a higher increase in pH value. Percentage moisture of arepas did not differ.

CONCLUSIONS

Under the conditions of this study,

1. Thiamin content among the three sources of corn varied significantly; with Mexican corn having the greatest amount, and Venezuelan corn having the least.
2. Thiamin loss during arepa preparation increased proportionally to increased alkalinity of the arepa.
3. For arepas, microbiological assays gave significantly different thiamin values from those of chemical assays.
4. Among sources, those having higher thiamin content for the corn resulted in higher arepa thiamin values.

REFERENCES

1. Suãrez AM (1954) La arepa criolla. Arch Venez Nutr 5:407-423

2. Nuñez MA, Maga JA (1979) The sensory and functional properties of arepas made from extruded, iron-fortified corn flour. FSTA 12:65-67

3. Jaffe WG (1950) Nota sobre el maiz como alimento humano. Acta Cient Venezolana 1:165

4. Bressani R, Paz Y Paz R, Scrimshaw NS (1958) Chemical changes in corn during preparation of tortillas. J Agric Food Chem 6:770-774

5. Gregory JF, Kirk JR (1978) Comparison of chemical and biological methods for determination of thiamin in foods. J Agric Food Chem 26:338-342

6. Weatherwax P (1923) The story of the maize plant. Chicago: The University of Chicago Press, p 5

7. Wellhausen EJ, Roberts LM, Hernandez E (1952) Races of maize in mexico. Harvard: The Bussey Institution of Harvard University, p 19

8. Food and Agriculture Organization (1953) Nutritional studies: maize and maize diets. Rome, pp 55-57

9. Hulse JH, Laing EM, Pearson DE (1980) Sorghum and the millets: their composition and nutritive value. New York: Academic Press, p 595

10. Food and Nutrition Board/National Research Council (1980) Recommended Dietary Allowances, 9th edn. Washington: National Academy of Sciences, p 84

11. Cuevas R, Figueira E, Racca E (1985) The technology for industrial production of precooked corn flour in Venezuela. Cereal Foods World 30:707-712

12. Suãrez AM (1955) Mejoras de la eficiencia proteica de la arepa con triptofano, lisina y treonina. Arch Venez Nutr 6:185-193

13. Chavez JF, Pellett PL (1973) Protein quality of some representative latin american diets by rat bioassay. J Nutr 106:792-801

14. Smith O, De Buckle TS, De Sandoval AM, Gonzalez GE (1979) Production of precooked corn flours for arepa making using an extrusion cooker. J Food Sci 44:816-819

15. Alvarez RJ (1981) Selected nutritional and biochemical changes during the production of baked and fried arepas. Ecol Food Nutr 11:201-205

16. Alvarez RJ (1982) Microbiological safety of baked and fried arepas. Ecol Food Nutr 11:207-210

17. Harbers CAZ, Stone M, Sabatka T (1985) Quality of arepas made from commercial flours or homemade masa. J Food Qual 7:191-200

18. Baker H, Frank O (1968) Thiamine. In: Clinical vitaminology. New York: Interscience Publishers, pp 7-21

19. Voight MN, Eitenmiller RR (1978) Comparative review of the thiochrome, microbial, and protozoan analysis of B-vitamins. J Food Prot 41:730-738

20. Guthrie HA (1975) Introductory nutrition, 3rd edn. Saint Louis: The CV Mosby Company, pp 256-262

21. Robinson CH, Lawler MR (1977) Normal and therapeutic nutrition, 15th edn. New York: Macmillan Publishing Co, p 175

22. Dwivedi BK, Arnold RG (1973) Chemistry of thiamine degradation in food products and model systems: a review. J Agric Food Chem 21:54-60

23. Lamden MP (1972) Thiamine estimation in foods. In: Sebrell WH, Harris, RS (eds) The vitamins, 2nd edn. New York: Academic Press, pp 110-114

24. Voight MN, Eitenmiller RR, Ware GO (1978) Vitamin assay by microbial and protozoan organisms: response to vitamin concentration, incubation time, and assay vessel size. J Food Sci 43:1418-1423

25. Buchanan RE, Gibbons NE (eds) (1974) Bergey's manual of determinative bacteriology, 8th edn. Baltimore: The Williams and Wilkins Co, pp 586-587

26. Voight MN, Eitenmiller RR, Ware GO (1979) Comparison of protozoan and conventional methods of vitamin analysis. J Food Sci 44:729-734

27. Michela PP, Lorenz K (1976) The vitamins of triticale, wheat, and rye. Cereal Chem 53:853-861

28. Wang Y, Fields ML (1978) Enrichment of home-prepared tortillas made from germinated corn. J Food Sci 43:1630-1631

29. Hendershot LR (1977) Tortilla production: study of variables affecting the processing of raw corn into tortillas. MS Thesis. Manhattan: Kansas State University

30. A.O.A.C. (1980) W Horowitz (ed) Official methods of analysis, 13th edn. Washington: Association of Official Analytical Chemists, p 213

31. Freed M (1966) Methods of vitamin assay, 3rd edn. New York: Interscience Publishers, pp 127-145

32. Difco (1977) Difco manual of dehydrated culture media reagents for microbioloical and clinical laboratory procedures, 9th edn. Detroit: Difco Laboratories, pp 212-213, 217-218

33. Snedecor GW, Cochran WG (1980) Statistical methods, 17th edn. Ames, Iowa: Iowa State University Press, pp 325-329

34. Hopmann RFW (1982) The alkali-induced transformations of thiamin. In: Sable HZ, Gubler CJ (eds) Thiamin: twenty years of progress. New York: The New York Academy of Sciences, p 32

ACKNOWLEDGEMENTS

The author expresses sincere appreciation to Dr. Carole A. Z. Harbers, Associate Professor of Foods and Nutrition and Major Professor, for encouragement, patience, and advice throughout the writer's graduate study; and for guidance in the preparation of this manuscript. The author wishes to thank the following who helped during the course of this study: Dr. Daniel Y. C. Fung, Professor of Animal Sciences and Industry, Dr. Meredith F. Smith, Assistant Professor of Foods and Nutrition, and Dr. Clyde E. Wassom, Professor of Agronomy, for technical advice and for serving on the writer's committee; Mr. Ronald Wasserstein, Instructor of Statistics, Washburn University, for the experimental design and analysis of data; Dr. Leniel H. Harbers, Professor of Animal Sciences and Industry, and Miss Kathlean J. Zeleznak, Research Assistant, Department of Grain Science and Industry, for technical assistance; and Mr. and Mrs. Jerry Williams, friends of the author, for assistance in typing the manuscript.

APPENDIX

Table 6. Raw data for corn: thiamin* and pH* measurements.

source	µg/g thiamin[a]	pH
Mexico	5.12	6.24
	5.00	6.25
	5.01	6.31
	5.06	6.33
mean	5.06	6.28
Kansas	4.95	6.29
	4.88	6.27
	4.98	6.23
	4.98	6.22
mean	4.94	6.25
Venezuela	4.13	6.24
	4.12	6.24
	4.03	6.36
	3.98	6.40
mean	4.06	6.31

*Mean of two samples/replication

[a] Thiochrome assay

Table 7. Raw data for arepas: chemical*, microbiological*, pH*, and moisture* measurements.

	µg/g thiamin			
source	chem[a]	micro[b]	pH	%moisture
Mexico	3.67	6.01	7.09	44.00
	3.93	5.91	7.41	46.15
	3.95	6.53	7.41	45.35
	3.81	6.64	7.23	44.55
mean	3.84	6.27	7.28	44.26
Kansas	3.30	4.88	7.62	45.01
	3.52	4.99	7.69	43.50
	3.56	4.97	7.78	46.70
	3.36	4.92	7.65	42.80
mean	3.44	4.94	7.69	44.68
Venezuela	1.99	0.93	8.38	46.80
	2.11	1.08	8.45	44.00
	2.35	1.03	8.47	45.35
	2.07	1.09	8.44	47.40
mean	2.13	1.03	8.43	45.89

*Mean of two samples/replication

[a] Thiochrome assay

[b] L. fermentum assay

THIAMIN CONTENT OF THREE SOURCES OF CORN AND AREPAS AS DETERMINED CHEMICALLY AND MICROBIOLOGICALLY

by

LAURA LEE KELLER

B.S., Kansas State University, 1979

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1985

ABSTRACT

The arepa, a popular corn bread in Venezuela, is a primary thiamin source for that country. Arepas are low in thiamin due both to low thiamin content in corn and to thiamin destruction during arepa preparation. Accurate determination of amount of thiamin present, including amount bioavailable, is important in assessing actual thiamin contribution of arepas to the Venezuelan diet. This study was designed to investigate thiamin values of corn and thiamin loss during arepa preparation, and to compare chemical and microbiological thiamin assays.

Thiamin amounts were chemically determined by the thiochrome method for corns from Mexico, Kansas, and Venezuela. Homemade arepas were prepared from each source of corn, then thiamin amounts were determined both chemically (using the thiochrome method) and microbiologically (using Lactobacillus fermentum). Changes in pH values during arepa preparation and total moisture were determined.

Data were analyzed using a split-plot design ANOVA. When F-values were significant, Duncan's Multiple-Range Test was used to determine differences between specific means.

Among sources, differences ($P<0.001$) were found for thiamin amounts in corn and arepas (both chemical and microbiological measurements). Both within and among sources, differences ($P<0.05$) were found for % thiamin loss during arepa preparation, increase in pH from corn to arepa, and

differences in arepa thiamin values determined chemically and microbiologically. The microbiological method gave higher thiamin values for Mexican and Kansan arepas, and lower thiamin values for Venezuelan arepas when compared to the chemical method.

Among sources during arepa preparation, the larger the increase in alkalinity, the greater the loss of thiamin. For thiamin measurements (both methods) of corn and arepas, the Mexican corn had the highest values, and the Venezuelan corn yielded the lowest values.