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CHEMISTRY, TECHNOLOGY, AND NUTRITIVE VALUE OF MAIZE TORTILLAS

RICARDO BRESSANI

Institute of Nutrition of Central America and Panama (INCAP) Carretera Roosevelt, Zona 11, Guatemala Guatemala 01011, Central America

ABSTRACT

This document reviews the available literature dating from 1943 up to the present time, on the chemistry, technology, and nutritive value of tortillas, as produced by the calcium hydroxide-cooking of maize first used by the Maya and Aztec civilizations. In 45 years significant amounts of information have been derived from basic and applied research on the different steps of the food chain, including characterization of the raw materials; the description of the process, as conducted in rural areas and at the industrial level; the significance of the processing parameters; the chemical and nutritional changes which take place; the advantages and disadvantages of the process; the improvement of the nutritional value of the product, and the improvement of the shelf-life of the processed flour and product, and forms of consumption and other applications. Most of these studies have been conducted with common maize, but the document includes data on QPM, which gives an equally satisfactory product that has a significantly higher nutritional value. Important findings include: the loss of a number of nutrients, but improvements in the bioavailability of others, such as calcium, nicotinic acid and overall protein

quality. Likewise, small amounts of undesirable amino acid derivatives are formed, but the process helps in reducing fungi, antinutritional and toxic factors. Quality improvement can be achieved through addition of small amounts of lysine- and tryptophan-rich proteins, such as soybeans, without altering functional and organoleptic characteristics. If further developed, it could play a more important role in food security in developing countries.

INTRODUCTION

Cereal grains—particularly wheat, rice, and maize—have been closely associated with the origins and evolution of great civilizations. These three cereal grains were first domesticated in the Middle East, in Western Asia, and in the Western Hemisphere, respectively. Today, these three cereal grains provide important intakes of nutrients for the world population, and they will continue to do so in the future. Archaeological evidence indicates that maize existed as a wild precursor some 7,000 years ago in Central Mexico (1). Around 2,000 years later, maize was under cultivation and was very much used in the development of the great Middle American civilization. Its cultivation and transformation into edible forms has been part of the culture of those civilizations, and has also been the subject of paintings, books, poetry, and songs, by many different authors. Maize domestication by the ancient civilizations in Middle America was to a great extent responsible for the evolution of those societies, but as important as it was in terms of providing food energy, a wiser dietetic choice was the use of alkaline substances to convert the grain into edible forms. How and why such a processing choice was selected by Mayan and Aztec civilizations has no answer, although study of the process and of the physical, chemical, and nutritional changes which take place has offered a number of possible explanations which are discussed in later sections of this review. It may be possible that such a process was selected by trial and error. For example, fire was obviously available and possibly used to soften the grain. However, there was difficulty in the removal of the seed coat. The ash left over may have been used at first because it was found to help in removal of the coat and to provide better texture of the dough. It is also of interest to speculate on the use of lime rather than ashes. The dough from the boiled maize is made into tortillas and baked over a clay plate called "comal." This plate is held over the fire by stones, which upon continuous exposure to heat, slowly produce calcium hydroxide. The strength of the ashes possibly increased, which eventually led to the use of lime. On the other hand, the selection of the process may have been based on appearance and disappearance of physiological disorders, for example, pellagra. Katz and co-workers (2) analyzed the significance of alkali-cooking techniques, under the hypothesis that all societies that depend on maize as a major dietary staple practice alkaline cooking techniques.

For the purpose, they investigated 51 societies, seven of which were high-maize consumers and large cultivators, and used alkali in preparing the grain for consumption. Alkalinity was achieved through the use of ashes, lime, lye, or soda. Another group of 12 societies also used alkali-cooking and although they were not as high in consumption as the group of seven, they were as high as far as growing maize is concerned. The point made was that acceptance and production of maize by societies was highly associated with the alkaline process used for transforming it into food, while those societies which produced maize but consumed it in other forms, had other food sources to complement maize.

Information available today may provide the reasons for the adoption and adaptation of the alkaline process to convert maize into an edible form by these civilizations. Some of the effects are mainly physical in nature, since the seed coat is easily removed after cooking. Nevertheless, some hydrolysis of the cellulose of the seed coat probably takes place, since the seed coat is almost completely destroyed. Then, there is the softening of the grain, which can, of course, be achieved by simple water cooking. The effects are of a chemical nature as well; present information indicates that alkaline cooking destroys or inactivates toxins produced by fungi growing on the grain. Likewise, there is an increase in niacin availability, which together with a better isoleucine-to-leucine ratio, contributed to the absence of pellagra. Furthermore, with alkali processing, there is a significant increase in calcium and as a result rickets was eliminated as a nutritional problem—otherwise it would have been described by anthropologists, or it would have been mentioned in the writing of the Mayas and Aztecs. Since impure lime was used, the product was enriched with other minerals, and finally, a small improvement in the quality of the protein was obtained.

The physical and nutritional benefits derived from the alkaline-cooking process are numerous enough to suggest that these were the reasons for its use. The civilizations probably did not understand the mechanisms responsible for those effects; however, they were able to observe the adverse effects, if alkaline cooking was not used prior to converting maize into tortillas and other forms for consumption.

BACKGROUND

Consumption of Processed Maize

Maize is widely consumed throughout the world in very many forms as derived from a large number of different processes. This document reviews the processing of maize by lime-cooking, as originally practiced by Mayan and Aztec civilizations. When appropriate, other forms of processed maize are discussed, particularly those consumed in developing countries.

Recent data on the amounts of maize consumed by people are not readily available; however, amounts per person on a daily basis probably have not changed

significantly during the last 20-25 years, particularly in rural areas of Mexico and in some of the Central American countries. According to Deschamps (3), in 1982 13,050 MT of maize were consumed by the Mexican population, of which 23.6% was maize produced and consumed by farmers, 55.3% came from the tortilla industry, and 8.9% was derived from tortilla flour. Other industries and other sources provide maize products representing 6.5% and 5.7% of the total. He further stated that maize intake per person was around 170 kg/year; and tortilla consumption, about 10 per person/day. Chávez (4) reported that in Mexico, maize intake in rural areas provides up to 70% of the calorie intake, while in urban areas it supplies only 25%. Furthermore, the intrafamily distribution is related to the calorie needs of its members. Adult men consume 600 g/day, pregnant and lactating women over 400 g, and children below 5 years of age, between 100 and 200 g. It has been pointed out that in Mexico, at least in 1979, tortilla consumption was around 660 million/day (5). With respect to Central America, the results of surveys conducted in 1969 showed maize intakes to vary among countries, with El Salvador consuming around 350 g per person daily; Guatemala, 318 g; and Honduras and Nicaragua, 225 and 131 g per person/day, respectively (6). These intakes represent up to 59% and up to 45% of the daily intake of calories and protein, respectively. In Costa Rica and Panama, consumption is significantly lower. These studies also revealed maize intakes to be lower among urban as compared to rural population. Nevertheless, the intakes in Guatemala, El Salvador and Honduras were 102, 166 and 135 g of maize per person per day.

Other studies (6) have shown that intake of maize as tortillas increases with age. For example, for children 1-2 years of age, maize contributes 27% and 33% of the daily intake of proteins and calories respectively; while for 4- to 5-year old children, protein contribution from maize is 33% of the daily intake and 39% of the calorie intake.

In more recent studies, Garcia and Urrutia (7) reported that the consumption of tortillas for 3-year-olds averaged 226 g/day (around 104 g of maize); for pregnant women, 595 g of tortillas (247 g of maize); and for lactating women, 666 g/day, equivalent to around 306 g of maize. These levels provide 47% of the calories for preschool children and 60% for pregnant and lactating women. Maize, consumed as tortillas, is also used in the weaning process according to Urrutia and García (8). They found intakes of tortillas to be around 4 g/day at 6 months of age, and 23 g/day at 11 months. Although the emphasis has been on the high intake of calories and of protein derived from calcium hydroxide-processed maize, this food also provides significant amounts of other nutrients. The results of nutritional surveys conducted in the various Central American countries and reported in 1969 (9) show that lime-treated maize products contribute 26-68% of the daily intake of calcium, 22-51% of the iron, 32-62% of the thiamine, 19-36% of the riboflavin, and 39-56% of the niacin intake. The same study

for Guatemala showed that milk intake provided 16% of the daily calcium intake, food legumes around 25% of the iron, and meat and food legumes provided 13% and 19% of the daily intake of protein, respectively. Similar results have been published from other maize-consuming countries in Central America (9). These data, then, clearly indicate the great nutritional importance maize tortillas have as a source of nutrients for these populations. Chávez (4), paraphrasing Zubirán, has indicated that maize is the cause of good fortune and of misfortune for the Mexican population: good fortune because maize has been the basic staple which allowed the development of the Mexican culture in all areas of the country; misfortune because the nutritional deficiencies in maize have influenced the nutritional status and the development of the individual and of the population as a whole. But the problem is really not due to maize itself, which, in fact, does not differ in nutritive value from other cereal grains. The problem is in the quality of the foods which are consumed along with maize. If these do not provide the nutrients deficient in maize, then dietary deficiencies are observed. In spite of this, the situation could have been worse, if not for the alkaline process used to convert maize into tortilla—a process that research has shown to induce nutritionally interesting changes. These are described in subsequent sections of this review.

Forms of Consumption

The introductory section has indicated that there are many forms of maize consumed in various parts of the world. These range from the use of maize grits for polenta and corn bread to popped grain, and to products such as maize flakes (10). The grain undergoes special processing and fermentation to give ogi in Nigeria (11), and a decorticated, degerminated precooked flour is made into arepas in Colombia and Venezuela (12, 13). The lime-cooking process described later, however, is particular to Mexico and Central America, although today the technology has been exported to countries such as the U.S. From lime-cooked maize, a dough is prepared which is the main ingredient for many widely consumed dishes such as atole, a beverage prepared in a great variety of flavors; tamalitos, which are also highly accepted and which are made by subjecting the dough wrapped in maize husks to steam cooking for 20-30 min; this gelatinizes the starch, providing a different structure. This form is usually prepared with young leaves of amaranth or chipilin (Crotalaria longirostrata), or the flowers of Loroco (Fernaldia pandurata), within the gelatinized dough (14), thus improving the nutritional quality of the product and its flavor. Tamalitos—which resemble arepas are consumed as prepared, or else they are baked. The dough is also used for tamales, a more complex preparation because of the number of ingredients. Tamales in most cases are made with chicken or pork added to the gelatinized

dough. The dough is also used to provide support for enchiladas, tacos, and pupusas, the latter made with fresh cheese placed between two layers of dough and baked as tortillas. When the dough is fried and flavored, it yields foods such as chips and chilaquilas. If the dough is allowed to ferment for 2 days, wrapped in banana or plantain leaves, it provides a food named pozol (15). From this, a number of drinks are made. It has been claimed this preparation is of high nutritional quality. Therefore, there are many ways to convert the lime-cooked dough into interesting and highly acceptable forms which, if presented in attractive and safe products, could balance to some extent the trend toward a greater consumption of wheat-derived foods in tortilla-eating countries, as well as in others where corn is a major staple.

THE TECHNOLOGY

As Practiced in Rural Areas: The Original Technology

The maize conversion process as practiced in rural areas of countries where it is consumed as tortillas, has been described and reviewed by a number of workers (16-19). The process follows the flow diagram shown in Figure 1, and the differences reported are more associated with the levels of water and lime used and cooking time than with the specific steps followed.

Illescas first described the process as carried out in Mexico (16). It involves the addition of one part whole maize to two parts of approximately 1% lime solution. The mixture is heated to 80 °C for 20 to 45 min, and then allowed to stand overnight. The following day the cooking liquor is decanted, and the cookedsoaked maize, now referred to as "nixtamal," is washed two or three times with water, removing the seed coat, the tip cap, excess lime, and any impurities in the grain. The addition of lime with the cooking and steeping steps helps the removal of the seed coat of the kernel. The by-products are either thrown away or fed to swine. The original conversion into dough was done with a flat grinding stone, repeating the process until the coarse particles were fine enough. Today the initial grinding is carried out with a meat grinder or a disk mill, followed by refining the dough with the stone. About 50 g of the fine dough are then patted flat and cooked on both sides on a hot iron or clay plate. The process as described by Bressani et al. (18) for Guatemala, using either white or yellow maize, is quite similar, except that lime concentration varies from 0.17% to 0.58% based on the weight of maize, with a grain-to-water ratio of 1:1.2, and cooking times range from 46 to 67 min at a temperature of 94 °C. The rest of the process is essentially the same as that described by Illescas (16), except that dough preparation is achieved using a disk mill. The 45 g of dough used are patted flat and cooked for about 3 min on each side on a clay or iron plate at a temperature of 170 °C on the edges, to 212 °C in the center. Often, the plate is sprayed with very dilute

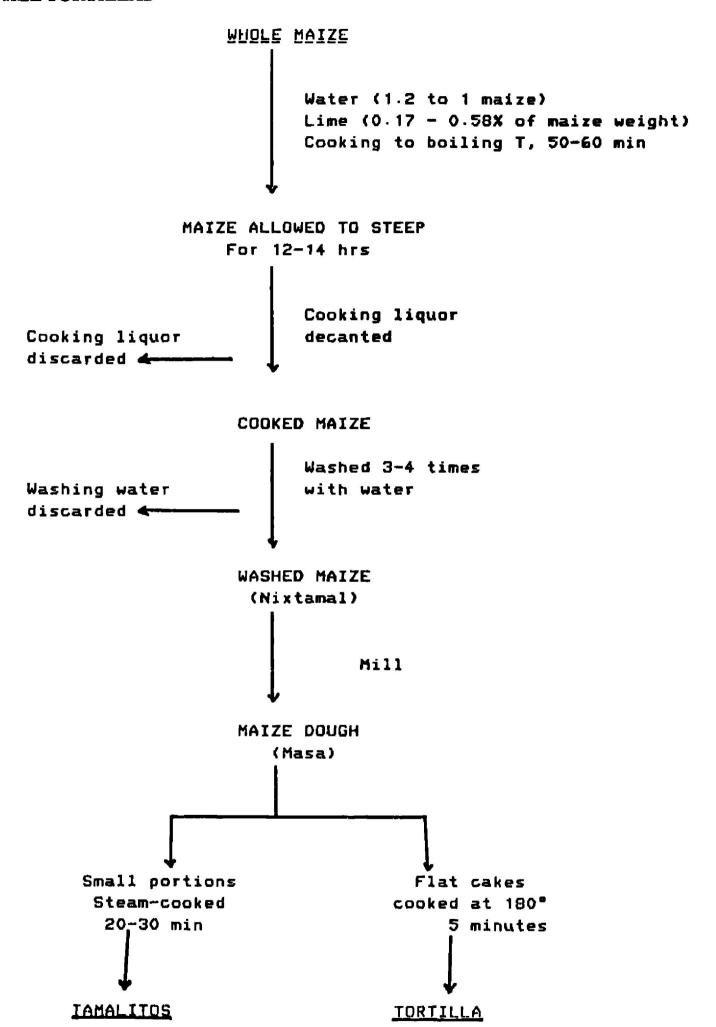


Figure 1. Flow diagram of the alkaline-cooking process used by rural people in Guatemala to convert maize into tortilla and tamalitos.

lime water to facilitate turning the tortillas. When this is done and water vapor forms, it causes a rise of the surface first cooked. Often the pressure breaks the surface, at which time the tortilla is removed from the hot plate, and placed in a small basket. The stacks are covered with a cloth to keep them warm and soft. Sometimes overheating of the surface cause the development of dark spots. The variations in the use of lime weight, cooking time, and temperature were attributed to family characteristics, although cooking time and temperature were also related to age of maize and altitude where the process was carried out. In a number of towns, the dough is wrapped in corn husks and placed in a clay or metal container to cook over steam for about 20-30 min. These are called tamalitos, which have the advantage over the flat tortillas of staying softer for longer periods of time. Small changes in procedure are carried out with maize recently harvested, such as adding less lime and decreasing cooking time. When the grain is old and dry, more lime is added and cooking time increases. Dry matter losses are around 15%, but this depends on the soundness and cleanness of the material when first cooked, since variability from 8.9% to 21.3% was reported (18). Tortilla diameter and thickness are family traits and yellow maize is preferred in some regions and white in others. The process at rural levels has not changed, with the exception of the grinding process of the cooked maize, the most work-demanding step in the process.

Industrial Technology

Factors such as migration of people from rural to urban areas increased the demand for ready-made tortillas and for precooked flour to be made into tortillas. This, in turn, led to the development of special equipment to process raw maize into lime-treated maize, dough, tortillas, and to the establishment of the industrial production of tortilla flour in Mexico and other countries. The mechanized production in Mexico acquired importance soon after the Second World War, according to Deschamps (3). One avenue was a small family owned-home industry, where the process was carried out as described earlier, but using larger and mechanical equipment to supply a relatively larger market. This development was made possible through the use of a rotary mill and a tortilla maker designed by Romero in 1908. This equipment was later replaced by a more efficient model designed by Peralta, Celerio, and Verastegui (5). Basically, the dough is passed through a rotating metal roll which cuts the flat dough into a tortilla. This falls on a moving belt or continuous cooking griddle, falling into a receptable at the end of the belt. These small operations may use whole maize, in which case it is cooked in large receptacles as previously indicated, or they may make the dough from commercially available tortilla flour.

The second option is the large industrial conversion of maize into an instant, precooked tortilla flour. The industrial process has been described by various

workers (3, 19, 20), and patents have been obtained (21); however, it is based for all practical purposes on the method used traditionally in rural areas. More recently, the process has been expanded into a mechanized process to produce tortillas.

Table 1 provides a brief description of the steps in the industrial process utilized in Mexico and other countries for manufacturing lime-treated maize flour (20). After maize is bought, it is selected and cleaned. Selection for grain quality is made after inspecting and sampling by the person in charge of receiving the maize. At this time, batches of maize with a high percentage of defective grains are rejected, and the price paid for batches which are accepted is set according to defects found in the raw material. Maize is also selected according to its moisture content, since grain high in moisture will be subject to storage problems. During the cleaning stage impurities, such as dirt, cobs, leaves, etc., are removed. The selected and cleaned maize is sent to the silos and warehouses for storage as previously mentioned.

From the warehouses or silos, maize is then conveyed to the units for lime treatment. This process involves the cooking of the maize with lime water to convert it into nixtamal using either a batch or a continuous process. After cooking and steeping, the lime-treated maize is washed with pressurized water or by spraying and it is ground into a dough (masa) which is then transferred to a dryer and made into a flour. The rough flour, consisting of particles of all sizes, is forced through a sifter where the coarse particles are separated from the fine ones. The coarse particles return to the mill for regrinding and the fines, which constitute the final product, are sent to the packing units and packed in lined paper bags.

According to Del Valle (20), one complete unit must have equipment for the following operations: lime treatment, milling, drying, and sifting, with a daily production capacity of 30 to 80 tons of flour. To increase production capacity, a commercial enterprise must install several parallel units. The use of the units is more a tradition than a technical necessity since it would be perfectly feasible to design plants with a capacity lower than 30 and higher than 80 tons per day.

Table 1. General Description of the Industrial Process of Producing Maize Flour for Tortilla

- 1. Receiving, cleaning, and storage of maize.
- 2. Preparation of nixtamal through the controlled cooking of maize in lime water.
- 3. Milling of nixtamal.
- 4. Drying of the milled nixtamal.
- 5. Sifting of the dry-milled nixtamal. Course particles are returned to the mill for regrinding, and the fine flour is the final product.
- 6. Packaging of the final product.

The concept of a unit is based on economic aspects of the process, since plants that are very large or very small apparently would not be practical.

The industrial yield of alkali-cooked maize flour fluctuates between 86% and 95% depending on the type of maize, its quality in terms of whole kernels, and the lime treatment conditions. Industrial yields have been reported to be higher than those at the rural and semiindustrial level, possibly due to the quality of the grain processed (18, 22, 23).

Tortilla flour is a fine, dry, white or yellowish powder, with the characteristic odor of maize dough. This flour, when mixed with water gives an adequate dough for the preparation of tortillas, tamales, atoles (thick gruels), and other food structures.

Maize flours made in Mexico must conform to the official quality regulations of the Department of Standards and Regulations of the Secretariat of Industry and Commerce of the Mexican Government. The physical and chemical specifications are given in Table 2. It should be pointed out that all maize tortilla-flour producers in Mexico comply with the specifications of this regulation (20).

When the flour has a moisture content of 10-12% it is stable against microbiological contamination. If moisture content is over 12% it is easily attacked by molds and yeast. The problem of bacterial attack is almost nonexistent since the minimum moisture required for growth of these organisms is so high that if the flour reached it, it would already be transformed into masa. Another problem related to the stability of the flour is rancidity, which is not normally present unless the flour is packed at high temperatures. The minimum time required for the flour to spoil is from 4 to 6 months during the winter, and 3 months during

Table 2. Quality Regulations Set by the Mexican Government for Lime-Treated Maize Flour

Chemical specifications				
Maximum moisture	10% (at packing time)			
Ash	2%			
Mimimum protein	7.5%			
Maximum crude fiber	3 %			
Ether extract	5%			
Lignin	Negative reaction			

Physical specifications

- 1. Tortillas must pass the folding test (i.e., on folding, they should not show cracks).
- 2. The finess of the flour must be such that a minimum of 85% will pass through mesh 60 (250 μ m).

the summer in Mexico. Nevertheless, the flour is usually sold to the consumer within 15 days after being sold to retailers and wholesalers. The shelf-life of the flour is 1 month (20).

Tortillas made from lime-treated maize flour can be made at home or at tortilla factories. The flour is mixed with water and blended until the adequate consistency for making tortillas is attained. Tortillas are made from this dough by special machines which can produce from 30 to 120 tortillas per minute. It must be pointed out that while the flour has been a great boon to households, and to small and large tortilla factories, its use in rural areas is not widespread.

In Guatemala, around 250 MT of maize are processed for tortilla flour production. These amounts are significantly lower than the amount processed in Mexico, since the population is smaller and the number of small tortilla factories is also very low. Around 90% of the production is sold in urban areas, and 75% is used for tortilla making (24). Other countries where lime-treated maize flour is produced are Costa Rica (25) and the United States (26, 27). In Costa Rica (25) tortilla consumption per person is around 25.6 kg. Approximately 62% is commercial tortilla, 30.6% is homemade from commercial flour, and 7.4% homemade from grain. The technology is well advanced in the United States, as reviewed by Serna-Saldivar, Gómez, and Rooney (28).

Technological Modifications

The traditional method of cooking maize with lime to make tortillas at the rural level is time consuming (around 14 to 15 h) and requires hard labor. From 70% to 80% of the time is taken by the cooking and soaking operations, which in a sense is a convenience for the rural housewife. It can be decreased substantially, to 2 or 3 h and even less. At the industrial or commercial level, the grinding and dehydration steps are major cost factors. The lime-cooked maize contains around 56% moisture, which must be decreased to 10-12% in the tortilla flour. In any case, the availability of an instant tortilla flour has many advantages such as convenience for the housewife, less labor, lower use of energy, a safe and stable product, and the possibility to use it as a carrier of nutrients.

Any method which may decrease both time and cost, and still yield an acceptable tortilla, would be advantageous. Efforts in this respect have been carried out by a number of workers. Bressani, Castillo, and Guzmán (29) evaluated a process based on pressure cooking at 5 and 15 lb pressure/in.² under dry and moist conditions for 15, 30, and 60 min, without the use of lime. None of the treatments had any effect on chemical composition and true protein digestibility, but all reduced the solubility of the nitrogen. Pressure cooking at 15 psig under dried conditions reduced the nutritional quality of the product, particularly when carried out for 60 min. The pressure-cooking method without lime did not reduce

crude fiber content, which is one of the particular effects of lime, and the calcium content was obviously significantly lower in comparison with dry dough (masa) prepared by the traditional process. Khan and co-workers (23) conducted a comparative study of three methods: the traditional, a commercial, and a laboratory pressure cooker procedure. For each process maize was undercooked, optimally cooked, and overcooked, for the purpose of measuring some of the physical and chemical changes which may take place. Although the traditional method gave the greatest loss of dry matter from the grain, it gave the best tortillas in terms of texture, color, and acceptability. The pressure-cooking procedure yielded a sticky dough and undesirable tortillas. According to the authors, the commercial method was the least desirable.

This study allowed the authors to propose a method to evaluate the completeness of cooking. Bedolla et al. (30) tested various methods of cooking maize and sorghum, as well as mixtures of the two grains. They found that the methods of cooking affected the total dry matter lost during processing into tortillas. The methods tested included the traditional, steam cooking as tested by Khan et al. (23), and a cooking method using a reflux-condensing system. Manipulation of cooking conditions can result in lower processing times. For example, Morad et al. (31) concluded that a 40% reduction in cooking time was achieved by presoaking the grain before alkali cooking. In these studies it was found that dry matter losses, water uptake, Ca content, and enzyme-susceptible starch increased, whereas amylograph maximum viscosity decreased for both presoaked or raw maize upon cooking. The decrease in viscosity and the increase in the other parameters were faster for the presoaked maize.

The previous modifications to transform maize into tortilla utilized a wet medium. Dry-heat processes have also been studied. Johnson, Rooney, and Khan (32) tested the micronizing process to produce sorghum and maize flours.

Micronizing is a dry-heat process which uses gas-fired infrared generators to heat the grain. Rapid internal heating takes place, cooking the grain from the inside out. The previously mentioned authors studied this process for production of tortilla flour, claiming that it would be quick and economical as compared to the traditional method. The procedure applied to whole maize consisted in heating the grain to the point of eversion in 20-30 sec with gas-fired infrared burners. After heating the grain, it was transferred to rollers, emerging as flakes. This changed the bulk density of maize from 56.0 lb/bushel to 23.0 lb/bushel. The results showed that micronized-maize tortillas had a texture and rollability comparable to tortillas made from a commercial maize tortilla flour and from white maize. Alkali added to the micronized maize increased yellowness, pH, and flavor, with simultaneous darkening. The micronized-maize flour showed greater water uptake, loss of bireifringence, and enzyme susceptibility, as compared to commercial maize flour. With respect to starch gelatinization the micronized flour showed a lower temperature of initial viscosity increase and also of

peak viscosity. Although it made acceptable tortillas, the technology has not been adopted.

A different process was tested at the pilot-plant level by Molina, Letona, and Bressani (33). In this process, maize flour-was mixed with water at a 3:1 ratio with 0.3% lime added on the basis of maize weight. The dough, after mixing, was passed through a double-drum drier heated with steam at either 15, 20, or 25 psig (93°, 99°, and 104°C surface temperature) at 2, 3, or 4 rpm. The process produced an instant tortilla flour with physicochemical and organoleptic characteristics identical to those of the reference sample prepared by the traditional method, but differing from the commercial product.

Extrusion cooking has also been evaluated as an additional technology to produce tortilla flour. Bazúa, Guerra, and Sterner (34), using a Wenger X-5 Extruder, processed ground maize mixed with various lime concentrations (0.1-1.0%). Tortillas made from the extruded dough were compared with those made by the traditional process for organoleptic properties, as well as for lysine, tryptophan, and protein content. No appreciable differences were noted at comparable use levels of calcium hydroxide. Either the traditional process or the extruded modification induced losses of tryptophan related to the amount of lime added. At the 0.2% addition, 8% of the tryptophan was lost, while at 1% lime, over 25% of the total tryptophan was lost. Some lysine losses were also observed. The organoleptic results suggested it is possible to make culturally acceptable tortillas using extrusion as an alternative process to the lime-heat treatment by wet process.

PHYSICOCHEMICAL CHARACTERISTICS OF MAIZE FOR TORTILLA

Preparation and the Role of Lime in the Process

Grain quality is a concept acquiring more importance in plant-breeding programs aimed at increasing acceptance of genetically improved seeds by farmers, consumers, and food processors. The grain quality characteristics include yield, technological properties, and when possible, nutritional characteristics. Technological properties include stability during storage, efficiency of conversion into products as affected by processing conditions, and, of course, acceptability to the consumer. The technological quality of maize for tortilla preparation is of little importance to the small farmer in least developed countries (LDC), who seldom uses seed other than those he keeps from harvest to harvest. Furthermore, the rural housewife knows how to adjust cooking conditions based on the type of maize she will process for consumption. But maize is now being converted into a tortilla flour using industrial processes, where the grain being used may be of several varieties and may come from various producers and different

environments. These grains may have a different structure or may have been poorly handled after harvest, factors which influence the yield and physicochemical and organoleptic as well as culinary properties of the final product. This would appear to be of more importance in countries such as the U.S. where maize tortilla is becoming a very popular food (10, 26-28).

That physical characteristics of maize are important became clear some time ago, when it was shown by Bressani, Paz y Paz, and Scrimshaw (18) that the yield of dry matter as dried-maize dough or flour was affected by the maize cultivar. These authors showed—from rural home studies—that dry matter losses from white maize averaged 17.2%, with a variability of 9.5% to 21.3%—as compared to yellow maize, where dry matter losses averaged 14.1%, with a range from 8.9% to 16.7%. These results indicated that the maize type influences the yield of dry matter, although the study was not conducted for the purpose of establishing grain quality maize characteristics for tortilla preparation. A more specific study was presented by Cortez and Wild-Altamirano (35). These authors conducted a series of measurements on 18 cultivars of maize (including two cultivars of popping maize) produced in Mexico. A standard cooking procedure was used, with 1.5% lime with respect to kernel weight, at 80°C, and a steeping time of 12 h. Cooking time was determined by the ease of seed coat removal. Evaluations conducted on the cooked maize included measurement of the volume of 1 kg of maize, the yield of dough from 1 kg of grain, and the moisture content of the dough. The latter was further evaluated by measuring the dough's strength and water absorption. The dehydrated dough was ground to 60 mesh and evaluated for moisture, color, specific volume, and other physical characteristics using a mixograph. The tortilla made from the dough of each maize sample was further evaluated for extensability, volume, plasticity, softness, and roughness of the surface. From this extensive study, the authors concluded that maize varieties or cultivars of higher weight per volume, higher endosperm hardness, and higher moisture, produced the best tortillas. The authors also concluded that the Swanson mixograph was very useful for evaluating differences in maize types for tortilla preparation. Maize cultivars with a high protein content tended to produce a betterquality tortilla. Cooking time among the 18 samples of maize tested ranged from 30 to 75 min, and dry matter losses ranged from 10% to 34%. Maize cultivars with hard endosperm and weight/hL were best for tortilla making. Rooney and Serna-Saldivar (10) have also indicated that maize with hard or corneous endosperm texture requires a longer cooking time, but its cooking follows a more predictable fashion than the cooking of soft maize according to Ellis, Freidman, and Mehlberg (36). Bedolla and Rooney (26) stated that the texture of the dough is affected by the endosperm texture and type, drying, storage, and soundness of the maize kernel. Martínez-Herrara and Lachance (37) established a functional relationship between kernel hardness and the time needed for the cooking process. The term alkaline cooking index, derived from sensation of softness,

cooked appearance, and seed coat disintegration, was found to have an equivalent in kernel hardness. The same investigators (32) reported that within a maize variety, higher calcium hydroxide concentration slightly decreased cooking time. Furthermore, the linear relationship between variety initial hardness and time at the terminal point of cooking, makes it possible to predict cooking time of maize varieties when the initial hardness is known. Khan et al. (23) and Bedolla and Rooney (26) used a specially designed shear cell developed by Desrosier (cited in Ref. 26) to measure a parameter termed "nixtamal shear force" (NSF), which measured kernel hardness. The measurement was related to both cooking time and processing method. These authors were able to show that the NSF measurement could detect small differences in maize with similar endosperm texture and could predict optimum cooking time for tortilla making.

Dry matter losses resulting from lime cooking constitute a good index of maize quality for tortilla preparation. Jackson et al. (38) reported that greater losses were obtained from stress-cracked and broken kernels than from sound kernels. Therefore, they concluded that any protocol for assessing maize quality for alkaline cooking should include measures of broken kernels, potential for breakage and ease of pericarp removal. Specific studies on the effects of drying and storage on maize quality for tortilla making are not readily available in the literature. Bressani et al. (39) reported on quality protein maize (QPM) storage and tortilla quality. The Nutricta QPM selection was stored under a number of field or rural conditions. Containers made of cloth not treated with insecticides allowed insect infestation, thus inducing higher dry matter losses during cooking; however, the protein quality was not affected.

As was indicated in the introduction of this document, possibly the most interesting feature of the process to convert maize into tortilla is the use of an alkaline medium, particularly calcium hydroxide. The most obvious effect of lime addition is its effect in facilitating seed coat removal during cooking and steeping. According to Trejo-González et al. (40), lime addition maintains the alkaline pH needed to hydrolyze the hemicelluloses of the pericarp. Its uptake by the kernel follows water uptake (31, 40), but its rate of increase is lower than that of water. Morad et al. (31) also showed that presoaking of the kernels before cooking led to higher calcium content in the grain. Calcium content of masa was affected by lime levels, and also by cooking-steeping temperatures (41). Various authors (18, 22, 26, 31, 35, 37, 40, 41) have shown in one way or another that lime uptake during alkaline cooking was affected by maize physical and chemical characteristics.

Martinez-Herrera and Lachance (37) found that higher Ca(OH)₂ concentrations slightly decreased the cooking time, but differences were not statistically significant. These authors also reported an interaction between maize variety and Ca(OH)₂ concentration; however, the coefficient of variance was high (29.1%) and attributed to inherent variability in the kernels of the different maize varieties.

Bedolla and Rooney (26) found that increases in cooking time, cooking temperature, lime concentration, and steeping time produced lower viscoamylograph peak viscosities at both 95° and 50°C, which was interpreted to mean a higher degree of starch gelatinization. Trejo-González et al. (40) showed that calcium was bound in some way to the starch of the maize kernel. Other effects noted included changes in color, greater solid losses with increasing amounts of lime, aroma, flavor, and a delay in the development of acidity, thus extending shelf-life (22, 32). If added in exceedingly large amounts, lime affects organoleptic properties of the tortilla, particularly if the maize had been stored for a long time.

Chemical and Nutritional Changes Resulting from Processing

Chemical Composition

As already indicated, the conversion of maize into tortilla involves a process wherein water, heat, and calcium hydroxide are used. All three factors influence the chemical composition of processed maize. Nutrient losses are caused by leaching in the case of water, by the time and temperature of cooking, by the alkalinity provided by lime, and by the steeping time after cooking. The changes which take place are caused by both physical and chemical losses. The chemical losses may be due to destruction of some nutrients and to chemical transformation of others.

Dry Matter Losses

From studies on maize cooking in rural homes using traditional methods, Bressani et al. (18) reported physical losses from maize to dough of 17.1% for white corn and of 15.4% for yellow. Cooking procedures influence the loss of solids. Bedolla et al. (26, 30) reported losses of 13.9% and 10.0% for white and yellow maize using the traditional process and 7.0% and 5.7% for steam cooking. In other studies where variations in processing techniques were evaluated, Khan et al. (23) found losses of 7-9% for commercial processing, of 9-11% for pressure cooking, and from 11% to 13% for the traditional method. These workers reported that dry matter loss increased as cooking time increased. Likewise, the integrity of the maize kernel influences losses. Jackson et al. (38) found that dry matter losses using the traditional cooking procedure were higher (10.8-12.1%) for broken kernels as compared to normal kernels (6.3-8.9%). Besides the integrity of the kernel and the heating process used, other factors such as length of steeping influence dry matter losses. Pflungfender et al. (22) reported that long steeping caused larger losses than short steeping time. Quality protein maize (QPM) of hard endosperm behaves similarly to common maize in terms of dry matter losses. Recently, Bressani et al. (42) reported losses of 17.1% for the Nutricta

QPM selection as compared to 17.6% from a white tropical maize. Sproule et al. (43) found a 9.6% dry matter loss from QPM as compared to a 10.4% loss for food-grade maize. The dry matter losses depend, then, on a number of variables such as the type of maize (hard versus soft endosperm), kernel integrity (whole or broken kernels), cooking procedure (traditional, steam cooking, pressure cooking, commercial), the levels of lime used, cooking time, and steeping length, as well as other operations such as the rubbing done to eliminate the seed coat during washing of the kernels. The process eliminates physical fractions of the kernel, mainly the seed coat, the tip cap, and possibly the aleurone layer and small amounts of germ. Paredes-López and Saharopulus (44), using scanning electron microscopy, showed that the outside surface of the lime-treated maize had important structural deterioration. They indicated that the aleurone layer was retained as well as some pericarp layers and that the germ remained attached to the endosperm. Thus, some of the chemical changes which have been observed can be explained by the chemical compounds present in these three or four physical fractions of the kernel. The dry matter content of the losses has been analyzed by Pflugfelder et al. (22), who reported 64% nonstarch polysaccharides (fiber), 20% starch, and 1.4% protein.

Nutrient Losses

Studies on the losses of nutrients during the transformation of maize into tortilla are not abundant, even though significant changes take place (17, 18, 45). The amount of ether-extractable substances is 33% in yellow maize and 43% in white maize (18). This is difficult to explain, although it could partially be accounted for by the loss of the pericarp, the aleurone layer, the tip cap, and part of the germ, physical fractions of the kernel containing ether-extractable substances. Crude fiber losses were reported to be around 46% for white maize and 31% for yellow maize (18). Lime treatment at 96°C for about 55 min hydrolyzes the pericarp, which is removed during washing, pulling with it the tip cap. This could account for much of the fiber loss. Nitrogen losses amount to about 10% and 5% for white and yellow maize, respectively. Again, this may be partially due to the physical loss of the pericarp and tip cap. Various studies have indicated that the tortilla may have a slightly higher protein content than the original maize, on an equal moisture basis. This may be caused by a concentration effect, since soluble sugars from the kernel are lost. Ash content increases due to the absorption of lime, which induces a significant increase in calcium content (17, 18, 45). Significant losses take place in thiamine (52-72%), riboflavin (28-54%), and niacin (28-36%). The carotene content in yellow maize decreased by 15-28% (18, 45).

Fat and Fatty Acids

As indicated in the previous section, ether-extractable substances of 33% and 43% were reported by Bressani et al. (18) from yellow and white maize, respec-

tively, as processed in Guatemalan rural homes. Pflugfelder et al. (22) reported losses of 11.8% to 18.1%, and suggested this may be partially due to the vigorous handling of cooked maize at the industrial plant. Pflugfelder et al. (41) found that of the total masa lipid, 25-50% was free and partially emulsified. Bedolla et al. (30) found ether extract values of 5.0%, 3.1%, and 3.6% in raw maize, cooked maize, and tortilla; respectively, or about a 28% change. This loss has not been fully explained; however, it may result either from the loss of the seed coat, the tip cap, the aleurone layer, and possibly, part of the germ; or from the loss of ether-soluble substances, not necessarily fat. Even though ether-extractable substances are lost in the process of converting maize into tortillas, the fatty acid makeup of the fat does not change either for common maize or for QPM, as shown in a report by Bressani et al. (42). Relatively larger differences were found between maize samples, either raw or processed, than between the raw maize and its tortilla, suggesting that the alkaline cooking method does not alter the fatty acid makeup of the fat.

Fiber Fraction

The crude fiber content of maize—as determined by the AOAC methodology decreases as the kernel is converted into tortillas as various investigators [Bressani et al. (18), Bedolla et al. (30), Saldana and Brown (45)] have found. All these authors have explained how and why such a loss takes place. With the availability of newer methodology to determine fiber, Reinhold and García (46) using the Van Soest method reported a significant increase in neutral detergent fiber of 6.60% in tortilla, and acid detergent fiber of 3.75% on a dry weight basis. These values were significantly different than those found in the dough, which averaged 5.97% and 2.98%, respectively. No difference was reported for hemicellulose, with dough containing 3.18% and the tortillas 2.89%. Using the same method, Bressani, Ortiz, and Breuner (47) found 10.8% NDF in maize and 9.0% in tortilla, as well as 2.79% of ADF in raw maize and 3.00% in tortilla. Hemicellulose averaged 8.0% in maize and 6.0% in tortilla, while lignin was 0.13% in maize and 0.15% in tortilla. Similar values were reported by García-López and Wyatt (48) and by Reinhold et al. (49). Using the method of Asp et al. (50), Acevedo and Bressani (51) detected a decrease in insoluble fiber from raw maize (13.0%) to the dough (6.0%) and an increase to tortilla (7.0%). Soluble fiber increased from 0.88% in raw maize to 1.31% in the dough, with a further increase to 1.74% in the tortilla. Similar findings were reported by Bressani et al. (42) in 10 samples of common maize and 1 sample of QPM converted into tortilla, as well as by Sproule et al. (43). The decrease from raw maize to dough is due to the loss in seed coat which takes place as indicated previously. However, the increase from dough to tortillas may be due to browning reaction products as has been reported for wheat baked products (52), and as suggested by Reinhold et al. (49).

Ash

The ash content changes have not received much attention. Most of the findings have shown an increase in total ash content from maize to tortilla (17, 18, 45), which is to be expected since lime is used for cooking. Along with the increase in total ash, there is a significant increase in calcium content. According to Pflugfelder et al. (41), calcium content in the dough is influenced by lime levels, cooking/steeping temperatures, and maize characteristics. The changes in other minerals are variable and may possibly depend on the purity of the lime used. In one study by Bressani et al. (42, 47), magnesium content increased by 8% to 35% from maize to tortilla; there was no change in sodium and a small decrease in potassium. Iron content values also increased; however, this was possibly due to contamination. Phosphorus content also increased from maize to tortilla as reported by a number of workers (17, 18, 45). One aspect which is of nutritional interest is that Ca-to-P ratio, which is about 1 to 20 in maize, is approximately 1 to 1 in the tortilla (18). Data on a number of minerals in maize and tortillas have been provided by Bressani, Ortiz, and Breuner (47). Their results confirm values for some of the mineral elements reviewed here.

Carbohydrates

Maize and its tortilla contain significant amounts of soluble carbohydrates. During the alkaline process, losses of starch of around 5% have been reported, and these substances are recovered in the solids lost [Pflugfelder et al. (22)]. In this respect, sugars in maize were 2.4%, which decreased to 0.34% in tortilla [Bressani, Ortiz, and Breuner (47)]. Robles et al. (53) found that alkali-cooking and soaking of maize caused large increases in viscosity, and cooking time had a significant effect in pasting properties, although there was no extensive gelatinization of the starch. Differential scanning calorimetric studies yielded similar gelatinization endotherms for untreated maize and nixtamal flours. Morad et al. (31) found that the process increased enzyme-susceptible starch, which increased as cooking time was increased.

Protein and Amino Acids

With respect to protein, the alkaline process induces some important changes. Most workers (17, 18, 45) report a small increase in N content which is due to a concentration effect. Bressani and Scrimshaw (54) found that the solubility of all protein fractions is decreased from raw maize to tortilla, with an increase in the insoluble fraction. The method used (54) was extraction of the nitrogen from raw maize and its tortilla by water, sodium chloride, 70% alcohol, and sodium hydroxide. The solubility of the water, salt, and alcohol protein fractions decreased significantly, with the alcohol-soluble proteins affected the most. Only a small decrease of around 13% in the solubility of the alkali-soluble fraction

was detected. Because of this, the insoluble nitrogen fraction increased from 9.4% in maize to 61.7% in tortillas.

Similar changes were observed by Ortega, Villegas, and Vasal (55) using the Landry-Moureaux protein fractionation technique (56), for both common and QPM maize. The solubility of true zeins decreased 58% in the tortillas prepared from normal maize, and 52% in QPM tortillas. These are similar to the decreases of 53% to 67% reported for zein from common maize by Bressani et al. (54). Ortega et al. (55) indicated that hydrophobic interactions may have been involved in the change in protein solubility observed. Sproule et al. (43) reported a decrease in the albumin plus globulin-nitrogen (expressed as percentage of total nitrogen) from maize to tortilla, and similar results were reported by Vivas, Waniska, and Rooney (57).

With respect to amino acids, in vitro enzymatic studies indicated total nitrogen and α -amino nitrogen were released faster from maize than from tortillas (54). An interesting observation was that when the α -amino nitrogen released was expressed as percentage of the total nitrogen release, tortilla values were higher at the end of 12 h hydrolysis with pepsin, than those observed with raw maize. The percentage of α -amino N was similar from maize and tortillas after 60 h hydrolysis with trypsin and pancreatin. With respect to essential amino acids, after 60 h hydrolysis with pepsin, trypsin, and pancreatin, the percentage of enzymatic released amino acids as compared to the acid hydrolyzed amino acids, suggested a faster release from tortillas than from maize. This information was recorded up to 36 h except for leucine, phenylalanine, tryptophan, and valine, which were released at about the same rate. At 60 h hydrolysis the amino acid concentrations between the maize and tortilla hydrolyzate reached comparable levels, with the exception of methionine [Bressani and Scrimshaw (54)]. These authors reported losses of arginine (18.7%), histidine (11.7%), lysine (5.3%), leucine (21.0%), cystine (12.5%), and small amounts of glutamic acid, proline and serine.

Sanderson et al. (58) reported small losses of arginine and cystine from alkaline treatment of common and high-lysine maize. These same authors found 0.059 and 0.049 g/100 g of protein of lysino-alanine from common and from high-lysine maize, respectively, but none was found in raw maize. In commercial masa, they found 0.020 g of lysino-alanine/100 g of protein, while in tortillas the level found was 0.081 g/100 g of protein.

Ortega, Villegas, and Vasal (55) found a small loss in tryptophan in both normal (11%) and QPM (15%) tortillas. On the other hand, they reported minimal losses in lysine from normal and QPM maize, similar to those previously reported (54, 58). Higher losses for both amino acids have been reported recently by Bressani et al. (42) from normal and QPM (Nutricta) maize converted into tortilla by rural processing.

Ortega, Villegas, and Vasal (55) also indicated that on the basis of the very small loss of lysine in the alkaline product, minimal amounts of lysino-alanine were probably present in common and QPM tortillas.

Vitamins

As indicated earlier, losses in thiamine, riboflavin, niacin, and carotene take place upon processing maize into tortilla by lime cooking (17, 18, 45). Of all vitamins, however, the one which has attracted the most attention of researchers has been niacin, because of its relationship to pellagra. Although the biological implications of the lime-cooking process for niacin availability and pellagra are discussed in the next section, the changes in concentration of niacin due to the process are presented in this section. Bressani, Gómez-Brenes, and Scrimshaw (59) reported that the seed coat of maize contains 4.2 mg niacin/100 g of seed coat, while in the germ and endosperm this is around 2 mg/100 g. However, since the endosperm is the major fraction of the kernel, about 79.5% of the kernel niacin is provided by the endosperm, and 10% each by the germ and seed coat. After lime-cooking, the endosperm contributed around 68% of the total niacin, and the germ around 5.5%. Of the total, 26% was found in the cooking water. They also found that the percentage of niacin extracted in water from the raw grain was 68.5% of the total; and from lime-cooked maize, 76%. Furthermore, they reported that enzymatic hydrolysis with pepsin yielded 69% of the niacin of all the samples, and after trypsin and pancreatin hydrolysis, yields of 78% and 100% of niacin, respectively, were found. This information was interpreted to mean that niacin is slightly more available from lime-treated maize as compared to raw maize, but the pellagragenic effect of maize could not be attributed to the presence of bound or unavailable niacin in maize.

Nutrient Availability

Although the lime-cooking process to convert maize into tortillas induces some important losses in nutrients, the process also induces important changes in nutrient availability.

Calcium

Due to the use of calcium hydroxide in converting maize into tortillas, calcium content in the product increases significantly, up to 400% (17, 18, 40, 41, 45). The results of bioavailability studies conducted by Braham and Bressani (60) in experimental animals showed the calcium in lime-treated maize to be somewhat less (85.4%) available than calcium from skim milk (97.0%). Nevertheless, calcium bioavailability increased when lime-treated maize was supplemented with its limiting amino acids, lysine and tryptophan. Recently, Poneros and Erdman, Jr. (61) confirmed the high bioavailability of Ca from tortillas with or without the addition of ascorbic acid. As pointed out in a previous section, the use of calcium hydroxide improves the Ca/P ratio in the tortilla, which possibly favors the utilization of the calcium ion by the animal. This is an important finding for populations who do not consume diets high in this essential mineral.

Furthermore, the finding that better quality in maize protein favors calcium bioutilization is of nutritional significance, and it represents an additional reason for the commercial production of QPM for people who depend on maize for their nutrition.

Amino Acids

The release of essential amino acids from maize and alkali-cooked maize is significantly influenced by the lime treatment, as shown by Bressani and Scrimshaw (54) using in vitro enzymatic digestions with pepsin, trypsin, and pancreatin. These studies revealed that at the end of the pepsin digestion, the amount of α -amino nitrogen as percentage of total digested nitrogen was twice as high from tortilla (43.1%), as compared with maize (21.4%). At the end of the pepsin digestion, higher levels of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and tryptophan were found from the tortilla hydrolysate than from maize, suggesting a faster release from the proteins. These authors proposed that the difference in rate of release could be due to the significant decrease in the solubility of the prolamine protein fraction in tortillas, as compared to maize. However, Serna-Saldivar et al. (62), working with ileum-cannulated swine found that at this level in the intestinal tract, the digestibility of most of the essential amino acids was somewhat higher from water-cooked maize as compared to lime-cooked maize. Ortega, Villegas, and Vasal (55), Sanderson et al. (58), and Paredes and Saharapulus (44) have suggested that during processing of maize, hydrophobic interactions, protein denaturation, and crosslinking of proteins, were probably responsible for changes in the solubility of these components, which could affect amino acid release during enzymatic digestion.

Niacin

The alkaline treatment of maize has been reported to destroy its pellagragenic factor, and evidence from a number of researchers (17, 59, 63-72) has suggested that pellagra is due to an imbalance of the essential amino acids, increasing the niacin requirement of the animal. This point has been extensively debated between those who claim that niacin in maize is bound and not available to the animal, and those who favor the improved amino acid balance induced by the alkaline-cooking process. Lime treatment results in a release of the bound niacin. However, Pearson et al. (66) have shown that boiling maize in water has the same effect; that is, it increases niacin availability. Bressani, Gómez-Brenes, and Scrimshaw (59) found that *in vitro* enzymatic digestion liberated all the niacin, both from raw maize and from tortillas. They concluded that differences in amino acid balance rather than in bound niacin were responsible for the difference between raw and lime-processed maize in biological activity and pellagragenic action. Lime treatment of maize improves amino acid balance, as demonstrated by Cravioto et al. (73) and by Bressani and Scrimshaw (54). Other workers have

shown that experimental animals grow better when fed lime-treated rather than raw maize (53, 63-71, 73). Using cats as experimental animals—which cannot convert tryptophan into niacin—Braham, Villareal, and Bressani (74) showed that niacin from raw and lime-treated maize was utilized to an equal extent, suggesting its availability is not affected by processing.

Dietetic Fiber

In a previous section, it was shown that upon processing maize into tortillas by lime-cooking, total dietary fiber (TDF) decreased in the dough stage and then increased in the tortilla to levels only slightly below those found in raw maize [Bressani et al. (42), Acevedo and Bressani (51)]. In these studies the levels of TDF in tortilla averaged 10% on a dry weight basis. If a person consumed around 400 g of tortilla dry weight, the TDF intake would be 40 g, a value significantly higher than that recommended as an intake. Even small children would consume relatively large amounts of DF. This can affect the availability of iron as suggested by Reinhold et al. (46, 49) and by García-López and Wyatt (48). Likewise, zinc nutrition could be affected as suggested by Solomons et al. (75). The other mineral which could be affected is calcium; however, Braham and Bressani (60) and Poneros and Erdman Jr. (61) found it to be relatively well available from tortilla, with availability increasing as the protein quality was improved through the addition of the limiting amino acids. An excess of Ca, rather than DF, could be responsible for Zn availability, as has been indicated in a number of studies (76).

Protein Quality Maize and Nutrient Bioavailability

It was shown by Braham and Bressani (60) that Ca retention was greater from tortilla supplemented with lysine, its limiting amino acid, and with a mixture of amino acids required to meet the needs of growing rats. Therefore, protein quality is an important factor in bioavailability of nutrients from maize and its lime-treated product. As already stated, niacin availability also improves when protein quality is improved, and in studies with QPM, Bressani et al. (70) showed better utilization of niacin. The same observation has been made in the utilization of carotene, which is higher if yellow maize is lysine supplemented (77).

Nutritional Quality Changes

Changes in nutritional value—particularly that of protein, in going from raw maize to tortillas—have been studied mainly with experimental animals. Even though chemical losses in some nutrients take place upon lime-cooking of maize, protein quality evaluation shows that there is a small but consistent improvement in protein quality of the tortillas, as compared to the raw maize. Table 3 summarizes the results of various studies where the raw maize and the tortilla made

Table 3. Protein Quality of Maize and Its Tortilla

Type of maize	Protein quality					
	Maize	Tortilla	Casein	Ref.		
Common	1.13 ± 0.26^a	1.27 ± 0.27^a	_	71		
Common	1.49 ± 0.23	1.55 ± 0.23	$2.88 \pm 0.20^{\circ}$	70		
QPM (opaque-2)	2.79 ± 0.24	2.66 ± 0.14	2.88 ± 0.20	70		
Common	1.38	1.13	2.50	124		
Common tropical	0.99 ± 0.25	1.41 ± 0.11	2.63 ± 0.17	42		
Common highland Xetzoc	0.96 ± 0.19	1.41 ± 0.20	2.63 ± 0.17	42		
Common highland Azotea	1.02 ± 0.19	1.41 ± 0.17	2.63 ± 0.17	42		
Common highland Sta. Apolonia	0.71 ± 0.20	0.98 ± 0.17	2.63 ± 0.17	42		
QPM Nutricta	1.91 ± 0.23	2.12 ± 0.12	2.63 ± 0.17	42		
Common	59.5 ^b	59.1 ^b	69.4 ^b	124		
	51.2°	49.4 ^c	64.5 ^b	124		

PER.

from it have been evaluated. As seen, the protein efficiency ratio (PER) of tortillas is in general somewhat higher than that of the raw maize, although some studies have reported otherwise. The difference may be due to processing conditions, particularly the concentration of lime added, which is lower in rural home cooking than at the industrial level. Obviously, the chemically determined amino acid pattern of the tortilla is no better than the pattern in raw maize. Therefore, the only explanation is that the process increases the availability of key amino acids, providing a better absorbed pattern. An indication of this is found in the results of feeding studies with young rats reported by Bressani, Elías, and Braham (71). Both raw maize and its lime-cooked dough were supplemented with increasing levels of lysine alone (from 0% to 0.47% of the diet), with maximum PER for maize at a level of addition of 0.31% and for a maximum PER for its limecooked dough at a level of 0.16. At all levels of supplemental lysine the dough gave higher PER values than the raw maize. Tryptophan supplementation alone was also tested, and in this case 0.025% addition gave highest PER for maize, with no response for the dough. The addition of the two amino acids at a level of 0.41% lysine with tryptophan varying from 0.05% to 0.15% improved the quality of both materials, but higher for the dough. These results were interpreted to mean that the quality of lime-treated maize is superior to that of raw maize. This explanation is supported by the in vitro studies reported by Bressani and Scrimshaw (54) showing a faster release of EAA from tortillas in comparison with maize, even though Ortega et al. (55) reported in vitro protein digestibility in maize, dough, and tortillas to be 88%, 91%, and 79%, respectively.

^bBiological value.

NPU.

For QPM the respective values were 82%, 80%, and 68%. Recently, Serna-Saldivar et al. (62) reported on dry matter, gross energy, and nitrogen digestibilities of maize cooked with and without lime. No différence was found between processing treatments for dry matter and gross energy digestibility values. Cooking maize with lime, however, reduced nitrogen digestibility from 76.5% to 72.8%. These values were measured near the end of the small intestine in pigs. Values for dry matter, gross energy, and nitrogen digestibility increased when measured over the pig's total digestion tract. From nitrogen balance studies, the same authors reported nitrogen retention as a percentage of intake: 45.8% for maize cooked without lime, and 41.2% for the lime-cooked maize. Nitrogen retention as percentage of absorbed nitrogen was 48.2% for the lime-cooked maize and 52.9% for the maize cooked with water alone. Digestible and metabolizable energy were similar between maize processed with and without lime. The authors concluded that the lime-cooking process decreased the nutritive value of maize. In a study with rats Serna-Saldivar et al. (78) reported an increase in percentages of dry matter and gross energy digestibilities from maize to nixtamal (dough) and to tortillas; however, protein digestibility decreased—in vitro studies correlated in vivo values. Braham, Bressani, and Guzmán (72) showed better weight gain and feed efficiency in Duroc-Jersey pigs fed lime-treated maize, as compared to raw maize. In studies with dogs, lysine and tryptophan addition to limecooked maize improved nitrogen balance to values obtained with skim milk (79, 80). It was further shown that if isoleucine, threonine, methionine, and valine (81, 82) were also added, nitrogen retention increased above the values measured with lysine and tryptophan. Lime-treated maize has also been evaluated in children (83, 84, 85). Nitrogen balance results have shown a high response to lysine and tryptophan addition, which, in turn, is dependent in protein level of intake. At low levels lysine alone improved quality, but as nitrogen intake increased, the addition of tryptophan with lysine became important. All studies, therefore, suggest that in lime-treated maize, lysine is slightly more deficient than tryptophan, and the contrary seems to be the case for raw maize. Nevertheless, for a significant improvement in nutritional quality of the protein lime-treated maize, both of these amino acids are required.

OTHER EFFECTS OF THE LIME-COOKING PROCESS ON MAIZE

Lysino-alanine Formation

In 1969 De Groot and Slump (86) demonstrated that alkali treatment of proteins gave rise to peptides such as lysino-alanine, lanthionine, and ornithine, which are not biologically available and have detrimental effects on protein quality. Consequently, the effect of the alkaline-cooking process to convert maize into tortilla has received some attention from various researchers. Stenberg, Kim, and Scwende (87) reported commercial samples of masa flour, tortillas, and taco

shells, to contain 480, 200, and 170 μ g/g of lysino-alanine. Sanderson et al. (58) also found that lanthionine and ornithine formed during alkaline cooking of maize. These authors (58) found no lysino-alanine (LAL) in common or in highlysine raw maize; however, 0.059 and 0.049 g% of the protein was found in the respective alkali-treated maize products. A commercial masa contained 0.020%, and tortillas contained 0.081% on a protein basis. These authors also reported lanthionine and ornithine values in the masa prepared from the two types of maize. Chu, Pellet, and Nawar (88) reported values of 133.2 μg LAL/g protein when maize was processed with 4.1 mol/kg of lime, for 30 min, at 170 °F. The use of sodium hydroxide under equal conditions yielded higher levels of LAL. Since higher levels of LAL were obtained with NaOH and KOH, they suggested that Ca ions may, in some way, interfere with the mechanism of LAL formation. It is difficult to evaluate the significance of LAL formation during tortilla making among people who eat relatively large amounts of this food on a daily basis. However, since tortillas have been eaten for a long time, it would seem that the small amounts do not interfere with nutritive value or cause any pathological effects.

Mycotoxins and Alkaline-Cooking of Maize

The presence of mycotoxins on a variety of cereal grains and other foods and feedstuffs is today well recognized, and maize is no exception. In Mesoamerica where maize is such an important food, the grain is harvested twice a year in the tropical areas. One harvest takes place in August when it is still raining, and ideal conditions of moisture (RH) and temperature for the growth of fungi exist. Martínez and co-workers (89) reported the presence of six different fungi in maize samples obtained from different markets throughout the country in Guatemala. The frequency for Aspergillus versicolor was 57.1%; for A. wentii, 32.1%; for A. ruber, 26.8%; for A. echinulatus, 25.0%; A. flavus, 25.0%; and Chaedosporium sp., 26.8%. On the basis of the total samples, 44% had Penicillium sp., 31% Fusarium moniliformis, 10% A. echinulatus, and 7% A. versicular. This finding suggests that this problem existed when the Mayan and Aztec civilizations initiated maize production and consumption of alkali-processed tortillas. De Campos et al. (90, 91) confirmed these studies and reported on aflatoxin levels found in maize in Guatemala. Because of the significance of the presence of mycotoxins in cereal grains, the effect of calcium hydroxide cooking of maize has received attention. Martínez et al. (92) fed infected maize, raw and alkali-processed, to chickens and rats. The maize was infected by Fusarium, with Penicillium sp., with Aspergillus niger, and with Aspergillus flavus. They found a high mortality of birds fed on the raw contaminated maize. However, no mortality was reported in the group of chickens fed the same maize that had been processed with calcium hydroxide. In young rats, the raw contaminated grain

reduced weight gain and caused some mortality. The same grain processed with lime induced no mortality, and weight gain and feed efficiency were equal to those found in the controls. Adult rats were also affected by the contaminated maize, but not by maize processed with lime. The study did not report levels of mycotoxins before and after processing. Martínez (93) reported on studies of tortilla samples collected in Mexico City. He found that 15% to 20% of the samples contained aflatoxins in the spring samples of 1978 and of samples collected in the rainy season of 1977-1978. Furthermore, he reported concentrations of aflatoxin B₁ to vary from 50 to 200 ppb. He also indicated that lime-cooking of maize reduced aflatoxin concentrations from 50% to 75%. Martínez (93) and De Campos, Crespo Santos, and Olszyna-Marzys (91) reported that lime concentrations up to 10% were no more effective in reducing aflatoxin levels than a 2% concentration. Ulloa-Sosa and Schroeder (94), reported that the tortillamaking process was not effective in removing aflatoxins from aflatoxin-contaminated maize. Nevertheless, others have obtained different results. For example, Solorzano Mendizábal (95) found that maize inoculated with A. flavus and with A. parasiticus produced high levels of aflatoxins, which were reduced by lime cooking up to 100% in some cases, but most often, up to 80% of the initial values. Lime concentration varied from 0.6% to 8%, and analyses were done on maize, masa, tortillas, and the cooking waters. In another study, de Arriola et al. (96, 97) using QPM Nutricta, found that the lime levels normally used to prepare nixtamal in Guatemala do not reduce aflatoxin levels in contaminated grain sufficiently to make it safe for human consumption.

Levels of 2% of lime and above gave high aflatoxin reduction, but the tortillas were not acceptable. Aflatoxin B₁ was reported to be reduced the most. Torreblanca, Bourges, and Morales (98) found relatively high aflatoxin levels in both maize and tortillas in a study conducted in Mexico City. Aflatoxin B₁ was found in 72% of the maize tortilla samples tested; furthermore, 24% of the samples gave positive reaction to zearalenone. Carvajal et al. (99) also found mycotoxins in maize and tortillas in Mexican samples, and indicated that aflatoxins, zearalenone, and deoxynivalenol were not destroyed by the lime treatment, or by temperatures of 110°C.

More recently, Price and Jorgensen (100) found that the alkaline-cooking process reduced aflatoxin levels from 127 μ g/kg in raw maize to 68.6 μ g/kg in the tortilla. The authors concluded the process was ineffective, since the lower value obtained is still much above the value that has been established as acceptable, which is around 20 μ g/kg. These authors found that acidification—as it occurs in the intestinal tract—increased aflatoxin levels. Abbas et al. (101) reported on the effect of 2%-lime-cooking of maize on the decomposition of zearalenone and deoxynivalenol. They found significant reductions of levels of zearalenone and DON. Furthermore, 15-acetyl-DON was completely destroyed. The percentage of reduction ranged from 58% to 100% for zearalenone, 72-82% for DON, and 100% for 15-acetyl-DON.

Results obtained by various authors are somewhat conflicting, since some of them report partial reduction in some mycotoxins, while others report total reduction. In many studies, the mycotoxins levels in maize were relatively high, requiring more vigorous processing conditions, in terms of lime concentration.

Microbiological Aspects of Tortilla and Tortilla Flour

Studies on the microflora in lime-cooked maize tortillas are very limited. A study conducted by Capparelli and Mata in 1975 (102) showed that the main contaminants of tortillas, as made in the highlands of Guatemala, were Coliforms, Bacillus cereus, two species of staphylococcus, and many types of yeasts. Nevertheless, when tortillas were first cooked, bacterial counts were around 103 or fewer organisms per gram, which is a safe level of consumption. As they are cooked about 5 min on the hot plate—they are placed hot in a basket, often covered with a cloth, which captures the vapor from the tortilla, creating an environment appropriate for microbial growth. After some 10 h under these conditions, the surface of stacked tortillas becomes slimy and they are not acceptable for consumption. Although in rural areas there are many opportunities for contamination from maize to tortillas, the factors which possibly contribute the most are the water used during conversion of cooked maize to masa, and the mill used to grind the cooked maize. In another study, a greater increase in bacterial counts was reported for tortillas fortified with soybean flour and vitamins, as compared to unfortified tortillas (103). In this case the mill used to grind the cooked maize to make the dough (masa) was chlorinated. This helped lower the bacterial count in the soy-supplemented maize. The tortillas also had a lower bacterial count when the mill was chlorinated. The rate of increase in bacterial number also decreased upon chlorination. Higher bacterial counts were reported by Valverde et al. (104) in the dough and tortillas made from QPM Nutricta, as compared to common maize, showing the effect of nutritional quality on bacterial growth.

This problem of relatively high moisture content has limited market possibilities for tortillas and is responsible for the very short shelf-life. Nevertheless, there is a demand for tortillas in urban areas where they are marketed under refrigerated conditions. A number of attempts have been made to improve the shelf-life of tortillas. Rubio (105-110) received six patents which included various additives: epichlorohydrin and poylcarboxylic acid and their anhydrides (1972); hydrophilic inorganic gels (1973), ascorbic acid and its salts as well as the methyl, ethyl, butyl, and propyl esters of para-hydroxy benzoic acid (1974) and acetic and propionic acid (1975).

Peláez and Karel (111) developed a shelf-stable intermediate-moisture tortilla with a water activity of 0.86, which was free from microbial growth, including S. aureus, and from yeast and molds, and enterotoxin production. This was achieved through the use of glycerol, corn solids DE-42, and salt, as well as the

mycostatic agent K-sorbate. Protection with appropriate packaging was claimed for at least 30 days and the appearance, texture, and other characteristics were similar to regular tortillas with a water activity of 0.97. Hickey, Stephens, and Flowers (112) reported relatively good protection of flour tortillas with low levels of sorbates or propionates added to the dough, and with a spray of sorbate on the surface after cooking on the hot plate. Both sides had to be sprayed for good protection.

More recently, Islam, Lirio, and Del Valle (113) claimed that by using calcium propionate the shelf-life of tortillas at room temperature ranged from 2 to 5 days, and with dimethyl fumarate the shelf-life was 2-11 days, under the same storage conditions and in polyethylene bags. Similar results have been reported by Tellez-Girón and co-workers (114).

Although advances have been made in extending the shelf-life, this still constitutes a problem which limits the sale of tortillas commercially.

SUPPLEMENTATION OF NUTRITIONAL QUALITY

Amino Acid Supplementation

As already indicated in preceding sections of this review, raw maize proteins have been shown to be of a low nutritive value due to deficiencies in the essential amino acids lysine and tryptophan (71). There are many studies conducted with experimental animals which demonstrate that the addition of both these amino acids improves the quality of the protein. Some workers have even found that besides lysine and tryptophan, isoleucine is also deficient, possibly because of an excess of leucine in maize proteins (115). Similar kinds of data have been obtained when lime-treated maize is supplemented with lysine and tryptophan in studies with experimental animals (71). These results have been confirmed in nitrogen balance studies conducted with children (83-85). Selected results are shown in Table 4. In these studies, children were fed in different nitrogen balance experiments 3.0, 2.0, and 1.5 g protein/kg body wt/day, exclusively from limetreated maize. The basal diet of maize was supplemented with tryptophan; lysine; tryptophan and lysine; tryptophan, lysine, and isoleucine; and lysine, tryptophan, isoleucine, and methionine. The results indicate that positive nitrogen balance was achieved when the children were fed 3.0 g protein (P)/kg/day. Lysine addition gave a positive balance when 2 g P/kg/day were given, but tryptophan alone at this level of protein intake did not improve the quality of limetreated maize. The two amino acids added together with and without isoleucine, induced positive balance when protein intake was dropped to 1.5 g P/kg/day. These data confirm results obtained in growing rats and demonstrate that lysine and tryptophan limit the quality of lime-treated maize. Furthermore, the findings show these two amino acids are extremely important at low levels of protein intake, a situation which takes place particularly with growing children. Although

Table 4. Nitrogen Balance in Children Fed Lime-Treated Maize at Various Levels of Intake, With and Without Amino Acid Supplementation

Dietary treatment	Protein intake level (g)						
	2.9 Nitrogen (mg/kg/day)		2.1 Nitrogen (mg/kg/day)		1.5 Nitrogen (mg/kg/day)		
							Intake
	Basal diet (B)	469	14	326	-5	238	-10
B + Try	465	33	327	-17	_		
B + Lys	482	38	335	24	239	-4	
B + Try + Lys	461	83	328	36	239	30	
B + Try + Lys + Ileu	475	108	335	40	240	46	
Milk	458	70	364	73			

Note. Amino acid levels used: DL-Try: 0.34%; L-Lys HCl: 0.56%; DL-Ileu: 0.45%. Taken from Refs. 83-85.

of a lesser practical importance, the results indicate a more inefficient use of the protein at high levels of intake, since the addition of the two amino acids at the lower levels of protein intake gave a nitrogen retention significantly higher than at the higher level of protein intake. These considerations have been overlooked often when the importance of protein quality was overshadowed by indicating energy intake to be more important.

Supplementation with Protein Sources

The results shown from animal and human studies with limiting amino acid addition served as the basis for evaluation of different types of protein supplements to improve the quality of lime-treated maize. Protein supplementation of lime-treated maize flour has been published by many researchers (17, 79, 80, 116-124) using different food sources. Most of the supplements tested have several characteristics in common. First, they all have a relatively high protein content and are good sources of lysine, with the exception of cottonseed protein and sesame oil meal; second the latter is a good source of methione; and third, with the exception of casein and/or milk and fish protein concentrate, the rest are of vegetable origin. The improvement in quality of protein in tortilla flour is in most cases a synergistic response of some improvement in quality resulting from lysine and tryptophan, and some response due to a higher level of protein, both provided by the supplement. Since soybean protein in different forms is the supplement to tortilla flour most often tested by different investigators, and because it is about the only one also tested in children (121) with comparable results as those in studies with rats, its importance and effects are reviewed in this section.

Results shown in Figure 2 indicate that maximum PER is achieved upon addition of 4-6 g% soybean protein, whether from whole soy, soyflour (50%), soy protein concentrate, or soy protein isolate. For reasons of availability, cost, and practical applications in developing countries, the results with whole soybean are discussed. The 4- to 6-g% level of supplementary protein can be provided by either 15% whole soybean or 8% soybean flour (121), which have resulted in comparable protein quality improvement. The advantage of using 15% whole soybeans is that the supplementation process can be done at the home level with soybeans produced by the family; it is very economical and, besides obtaining a food product of higher protein quantity and quality, the soybeans provide some additional energy from the oil they contain.

Whether processed at home or at the industrial level, it has been demonstrated that the nutritional quality improves, with the process being capable of destroying all trypsin inhibitor activity as well as that of urease in the soybean (117, 118, 122). It has been shown that the tortilla made with 15% soybean is acceptable to rural consumers, and that it has many of the functional properties of the tortilla

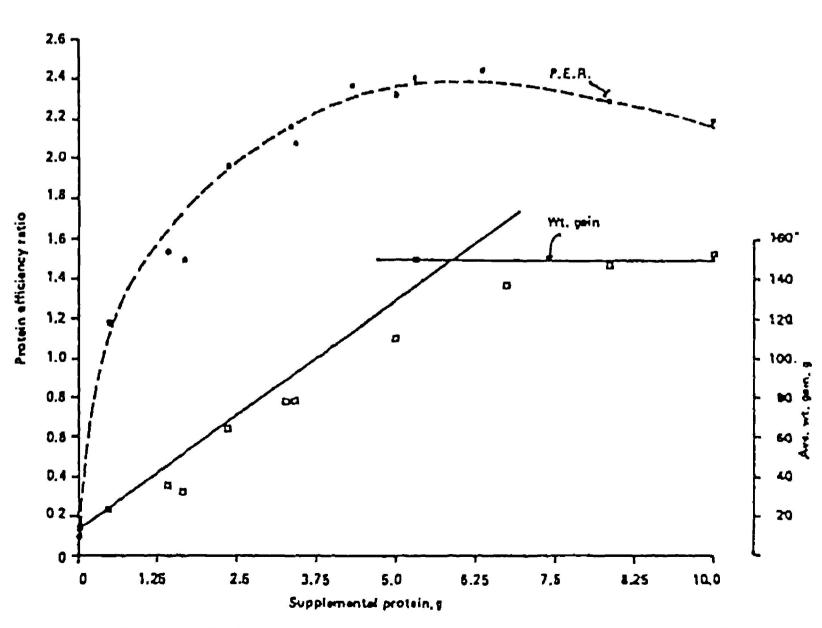


Figure 2. Protein quality improvement of maize by soy protein supplementation (Incap 77-224).

without soybeans, except that it is more flexible and softer. Many attempts have been made to introduce such a technology both at the industrial and at the home level. However, it has not been a sustainable approach, due to various reasons such as the unavailability of whole soybeans in villages, the cost of soybeans, and possible changes in organoleptic characteristics. These, however, have not been indicated. With the relative increase in industrially produced lime-treated maize flour, fortification with protein sources and other nutrients is efficiently accomplished by a dry-mixing operation, as is done with other cereal flours. The problem is not so much with the technology, but with the lack of legislation which, if implemented, could make maize tortillas a better-quality food, as it is done with wheat flour in many countries throughout the world. The studies that have been described led to the development of a dry supplement to tortilla flour which contained: 97.5 g% of soybean flour (50% protein); 1.5% L-lysine HCl; 26.8 mg% thiamine, 16.2 mg% riboflavin, 19.3 mg% niacin, 0.60% ferric orthophosphate, 0.031% vitamin A 250, and 0.133% corn starch. The quantity recommended for addition to tortilla flour was 8% by weight (125). The effectiveness of this supplement was partially tested by Urrutia et al. (126), and preliminary data suggested some improvement in the nutritional status of young children.

Supplementation With Green Vegetables

One form of masa consumption in some countries consuming lime-cooked maize is the tamalito. This is made from masa, wrapped in the husks of the maize ear, and place over steam. Tamalitos are often used instead of tortillas and have the advantage of remaining soft for a longer period of time as compared to the regular tortilla. There are a number of preparations, some of which include the young leaves of native vegetables, such as chipilin and amaranthus. Chemical and nutritional studies have demonstrated that around 5% of these leaves improves the protein quantity of the quality of the dough (14). The reason is that these leaves have relatively high levels of protein rich in lysine and tryptophan.

Supplementation With Other Grains

Sorghum is another grain which has been processed by lime-cooking in Mexico and Central America, particularly in areas where maize does not produce well. Sorghum tortillas, however, are not of the organoleptic or nutritional quality of maize tortillas. Many efforts have been made to use blends of both cereal grains with successful results (57, 62, 78, 123, 124). Alternative approaches include the use of blends of common maize with germinated maize (127), since germination has been reported to increase lysine. Mixtures of tortilla flour and rice, and of tortilla flour and wheat flour, have also been studied. The rice/maize

products have a higher nutritive value than the wheat/maize tortillas (128). More recently, blends of amaranth grain with lime-cooked maize flour have been shown to have an improved protein quality due to the much higher lysine and tryptophan content of amaranth as compared to maize. The product has been reported to be of an acceptable organoleptic quality (129). Other added products that provided foods with acceptable sensory attributes are potato, rice, and pinto beans (130).

Quality Protein Maize

Improvement in the protein quality of lime-treated maize with protein supplements rich in lysine and tryptophan is discussed in the previous section. But protein quality improvement of maize has been achieved through genetic means, first giving origin to the well-known opaque-2 maize and more recently, to what is known as QPM (quality protein maize). These materials contain higher levels of lysine and tryptophan, as well as a better amino acid balance, as compared to common maize (131). The difference between the original opaque-2 maize and the new generation of QPMs is that the latter have significantly improved agronomic characteristics and retain the high nutritive quality of opaque-2 maize. Yield performance, resistance to insects, hard grain, and many other desirable characteristics have been introduced into QPMs so that they can be utilized to make lime-treated maize tortillas with a high protein quality. This has been shown by various workers (39, 42, 43, 55, 132, 133).

The high protein quality of lime-treated opaque-2 maize as tested in children has been demonstrated (132). Recently, the high protein quality of QPM has also been shown in nitrogen balance and growth studies with children (133, 134). The lime-cooking process, as was shown, induces some protein quality improvement in common maize; however, the quality improvement in QPMs is not as marked (42). Recent studies by Ortega et al. (55) have shown that QPM treated with lime yields a tortilla retaining the high nutritional value of the raw maize, although some small losses of lysine and of tryptophan occur. However, the protein quality was still significantly higher than that of common maize. Losses of lysine by lime-cooking of QPM were around 13-15%, and losses of tryptophan around 18-20%. These, however, are not different from those reported for common maize tortillas (54). Results of Bressani et al. (42) indicate that QPM processed at the home level may have higher losses of these amino acids; however, the protein quality is still significantly higher than that of regular maize tortillas.

The nutritional significance of QPM as lime-treated dough or tortilla for children has really not been fully appreciated. Reports indicate that 3-year-old children consume around 226 g/day of tortillas; and lactating children (6-11 mo), from 4 to 23 g (7, 8). It is because of the low intakes that protein quality is so important. As stated in the early part of this section, addition of lysine and tryp-

tophan to lime-treated maize has a significant effect. This is much more important when it is realized that the other foods consumed do not provide the quality needed for human growth.

PHYSICOCHEMICAL CHARACTERISTICS OF TORTILLA FLOUR AND OTHER USES

Physicochemical Characteristics

Production of precooked instant tortilla flour is steadily increasing in Mexico, in some countries in Central America, and in the United States. This higher demand has stimulated research, not only in optimizing the original technology, but in developing alternative processes of conversion and in the identification of better grain types as described in an early section of this review. Because of these facts, it has become necessary to develop quality criteria for tortilla flour. Not only are these quality characteristics useful in the manufacture of tortilla flour by the various technologies proposed, but they should be useful in the diversification of its use in other food products. Attempts to develop some quality criteria for tortilla flour were made by Molina et al. (135). Two flours were evaluated, one produced at laboratory level, and one commercial flour produced in Guatemala. Quality characteristics included: soluble sugars, protein, calcium content, starch and damaged starch, available lysine, Zeleny sedimentation number, water absorption using a farinograph set at 300 BU, and maximum viscosity at 85 °C by amylography. The only differences between the two flours were in Zeleny sedimentation, water absorption, and maximum viscosity. A similar study was conducted by Bedolla and Rooney (27) using 12 tortilla-flour samples manufactured in the U.S. and 9 from Mexico. The quality criteria included chemical components such as moisture, protein, starch, and enzyme-susceptible starch. The differences between U.S. and Mexican products were small. Physical criteria included pH at 25 °C, bulk density in kg/hL, water absorption index, optimum water uptake, and particle size index. The only difference between samples found was in particle size index—higher for the Mexican tortilla flours. Other criteria included viscoamylography curves, with differences at random at the holding T of 95 °C (45-80 °C) and at 100 min at 50-61 °C.

A final set of criteria included rollability of the tortilla and color of the flour and tortilla. They concluded that highly acceptable tortillas were made from flours with a uniform particle size distribution (32% in U.S. sieve Nos. 60, 70, and 80), peak viscosity between 220 and 330 BU, pH of around 7.2, optimum water uptake index of 1.3 g water $70\,^{\circ}$ C/g dry flour, and a white to semiyellow color (L-82.0; b = 12.0). Similar results were reported by Gómez et al. (136) and Ranhotra (137). Padua and Whiteney (138) applied the INSTRON test to evaluate the suitability of modifications in the commercial manufacture of limetreated maize flours and to evaluate the flours prepared from different varieties

of maize for tortilla making by means of rheological studies of the maize dough. They demonstrated that maize dough derived by lime-cooking of the grain behaves as a Bingham plastic and under specific conditions of mixing time, of holding time, and of moisture content, can be useful for the applications indicated. These criteria will help in the selection of maize varieties, alternative or conventional processing methods, quality of maize under storage, and other factors influencing the final quality of the product. The shelf-life of tortilla flour can decrease if not properly stored. Paredes-López and Mora-Escobedo (139) showed that tortilla flour stored under conditions of high temperature and relative humidity developed a high fat acidity, that the insoluble residue protein increased, and that available lysine decreased, as well as in vitro digestibility and protein quality. Negative changes were also found in sensory attributes. To help in this potentially damaging shelf-life problem, studies have been carried out to extend the life of the flour and of the tortilla. Some additives which have been shown to be effective include monoglycerides (140), calcium propionate and K sorbate (141), and many others (105-114).

Other Uses

The quality criteria of tortilla flour will be useful in finding alternative applications for tortilla flour. As indicated earlier, these flours are used for tortilla making, taco shell, chips, tamales and tamalitos, arepas, and other food preparations, including drinks or thin porridges (142)—particularly in Mexico and Central America.

It is a well known fact that the consumption of wheat flour products is increasing in developing countries [Fellers and Bean (143), Salazar de Buckle (144)]. This trend is increasing as urbanization and income increases take place in LDCs. Efforts have been made to reduce the use of wheat flour through the development of composite flours (143, 144). In tortilla-consuming countries and possibly in others as well, lime-treated maize as tortilla, tamalitos, and arepas, could be very useful in reducing consumption of wheat flour-derived food products, through actions directed to improve the presentation to consumers of the lime-treated maize products and through education programs. With respect to the first point, a number of studies have been conducted to extend the shelf-life of the ready-made tortilla, with successful results (105-114), as was indicated in a previous section of this review.

Although nutritive value is seldom a convincing approach to increase food purchases, there are some advantages in this respect, which favor lime-treated maize over wheat flour. As reported by Bressani and Elias (128), the protein quality of mixtures of tortilla and of bread flours decreases as the amount of bread flour increases in the mix. In other kinds of studies, Vargas, Muñoz, and Gómez (145) showed tortillas to have a higher protein quality than local breads,

and similar findings were found by Bressani and Jarquín (146). In this last study, using a multiple-point protein quality assay method, with young growing rats, tortilla gave an NPR value of 2.22, about 50% the value for casein (4.43), while wheat bread had an NPR value of 1.08, around 24.4% of the value for casein. Similar findings were also obtained using nitrogen-depleted adult rats.

On the basis of an increased shelf-life and of a product with higher nutritive value, well-planned and aggressive education programs could increase consumption of tortillas to replace bread. Furthermore; higher-income societies, who do not consume tortillas, could benefit further if they were told that consumption of tortillas would provide them with higher intakes of dietary fiber as compared to consumption of wheat bread.

Tortilla flour at a level of 56% is used in the high-protein food known as Incaparina (147), and 70% in a similar product known as Maisoy (118, 148). This last product, as dough or dried as a flour, has been used to make cookies of a high nutritive value when added to wheat flour to provide 15% dry beans in the mixture. The same can be done with tortilla flour to produce enriched flour (149, 150). Other products include bread and pastas. These are only a few examples which suggest that tortilla flour can be useful in providing acceptable food products to populations in maize-consuming countries.

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REFERENCES

- 1. R. Mac Neish, in "The Prehistory of the Tehuacán Valley" (D. Byers, ed.), University of Texas Press, Austin, Texas, 1967.
- 2. S. H. Katz, M. L. Hediger, and L. A. Valleroy, Science, 184, 765 (1974).
- 3. A. I. Deschamps, in "Aprovechamiento Industrial del Maíz en la Manufactura de Productos Alternos a los de Panificación, Originados en el Trigo," Congreso de Tecnología de Alimentos, Viña del Mar, Chile, 1985.
- 4. A. Chávez, "El Maíz en Nutrición de México," Simposio sobre Desarrollo y Utilización de Maices de Alto Valor Nutritivo, Colegio de Postgraduados, Chapingo, México SAG 1973.
- 5. Anonymous, "La Tecnología de la Tortilla," Consejo Nacional de Ciencia y Tecnología de México, 1979.
- 6. R. Bressani, in "Nutritional Improvement of Maize," Proceedings of an International Conference, INCAP, Guatemala City (R. Bressani, J. E. Braham, and M. Béhar, eds.), INCAP Publication L-4, 1972, p. 325.
- 7. B. García and J. J. Urrutia, in "Interacción entre Producción Agrícola, Tecnología de Alimentos y Nutrición," INCAP, Guatemala City, 1978, p. 116.

8. J. J. Urrutia and B. García, in "Interacción entre Producción Agrícola, Tecnología de Alimentos y Nutrición," INCAP, Guatemala City, 1978, p. 133.

- 9. INCAP, in "Evaluación Nutricional de la Población de Centro América y Panamá," INCAP V-25; Guatemala; V-26 El Salvador; V-27 Nicaragua; V-28 Costa Rica; V-29 Honduras; V-30 Panamá, 1969.
- 10. L. W. Rooney and S. O. Serna-Saldívar, in "Corn, Chemistry and Technology" (S. A. Watson and P. E. Ramstad, eds.), Amer. Assoc. Cereal Chem., St. Paul, MN, 1987.
- 11. I. A. Akinrele and C. C. A. Edwards, Brit. J. Nutr., 26, 177 (1971).
- 12. H. H. Mottern, T. S. de Buckle, and C. Pardo, Cereal Sci. Today, 15, 108 (1970).
- 13. R. Cuevas, E. Figueira, and E. Racca, Cereal Foods World, 30, 707 (1985).
- 14. R. Bressani, L. G. Elías, and C. de Bosque, in "Amaranth Newsletter," Edited in ALAN, INCAP, Cuatemala City, March 1988.
- 15. R. O. Cravioto, O. Y. Cravioto, G. Massiev H., and J. Guzman G., Ciencia, 15, 27 (1955).
- 16. R. Illescas, Soc. Mexicana de Historia Natural, 4, 129 (1943).
- 17. R. O. Cravioto, R. K. Anderson, E. E. Lockhart, F. de P. Miranda, and R. S. Harris, Science, 102, 91 (1945).
- 18. R. Bressani, R. Paz y Paz, and N. S. Scrimshaw, Agric. Food Chem., 6, 770 (1958).
- 19. O. Paredes-López and M. E. Saharopulus-Paredes, Bakers Digest, 57, 16 (1983).
- 20. F. R. Del Valle, in "Nutritional Improvement of Maize" (R. Bressani, J. E. Braham, and M. Béhar, eds.), INCAP Pub. L-4, Guatemala City, 1972, p. 57.
- 21. R. M. González and M. J. Rubio, Preparation of Tortilla Flour, U.S. Patent 3,369,908, Feb. 20, 1968.
- 22. R. L. Pflugfelder, L. W. Rooney, and R. D. Waniska, Cereal Chem., 65, 127 (1988).
- 23. M. N. Khan, C. C. DesRoisiers, L. W. Rooney, R. G. Morgan, and V. E. Sweat, Cereal Chem., 59, 279 (1982).
- 24. L. G. Elías and R. Bressani, in "Mesa Redonda sobre Procesamiento de Maíz para Consumo Humano," Instituto de Investigaciones Tecnológicas, Bogotá, Colombia, 1983.
- 25. V. F. Aguilar, G. C. Ivankovich, and L. F. Arias, "Hábitos de Consumo de los Derivados del Trigo y de sus Eventuales Sustitutos Autóctonos," 1988, p. 97.
- 26. S. Bedolla and L. W. Rooney, Cereal Foods World, 27, 219 (1982).
- 27. S. Bedolla and L. W. Rooney, Cereal Foods World, 29, 732 (1984).
- 28. S. O. Serna-Saldivar, M. H. Gómez, and L. W. Rooney, "The Chemistry, Technology and Nutritional Value of Alkaline Cooked Corn Products." To be published.
- 29. R. Bressani, S. V. Castillo, and M. A. Guzmán, Agric. Food Chem., 10, 308 (1962).
- 30. S. Bedolla, M. G. de Palacios, L. W. Rooney, K. C. Diehl, and M. N. Khan, Cereal Chem., 60, 263 (1983).
- 31. M. M. Morad, F. Y. Iskander, L. W. Rooney, and C. F. Earp, Cereal Chem., 63, 255 (1986).
- 32. B. A. Johnson, L. W. Rooney, and M. N. Khan, J. Food Sci., 45, 671 (1980).
- 33. M. R. Molina, M. Letona, and R. Bressani, J. Food Sci., 42, 1432 (1977).
- 34. C. D. Bazúa, R. Guerra, and H. Sterner, J. Food Sci., 44, 940 (1979).
- 35. A. Cortéz and C. Wild-Altamirano, in "Nutritional Improvement of Maize" (R. Bressani, J. E. Braham, and M. Béhar, eds.), INCAP Publication L-4, Guatemala City, 1972, p. 90.
- 36. E. B. Ellis, P. D. Friedemann, and L. O. Mehlberg, "Grain Quality for Food Processing," Proc. Annual Corn Sorghum Ind. Res. Conf., 38, 153 (1983).
- 37. M. L. Martinez-Herrera and P. A. Lachance, J. Food Sci., 44, 377 (1979).
- 38. D. S. Jackson, L. W. Rooney, O. R. Kunze, and R. D. Waniska, Cereal Chem., 65, 133 (1988).
- 39. R. Bressani, J. F. Medrano, L. G. Elías, R. Gómez-Brenes, J. M. González, D. Navarrete, and R. E. Klein, Turrialba, 32, 51 (1982).
- 40. A. Trejo-González, A. Feria-Morales, and C. Wild-Altamirano, Adv. Chem. Ser., 198, 245 (1982).

- 41. R. L. Pflugfelder, L. W. Rooney, and R. D. Waniska, Cereal Chem., 65, 262 (1988).
- 42. R. Bressani, V. Benavides, E. Acevedo, and M. A. Ortiz, "Changes in Selected Nutrient Content and in Protein Quality of Normal and Quality Protein Maize During Tortilla Preparation." Submitted for publication.
- 43. A. M. Sproule, S. O. Saldivar, A. J. Bockholt, L. W. Rooney, and D. A. Knabe, Cereal Foods World, 33, 233 (1988).
- 44. O. Paredes-López and M. E. Saharopulus, J. Food Tech., 17, 687 (1982).
- 45. G. Saldana and H. E. Brown, J. Food Sci., 49, 1202 (1984).
- 46. J. G. Reinhold and J. S. García, Amer. J. Clin. Nutr., 32, 1326 (1979).
- 47. R. Bressani, M. Breuner, and M. A. Ortiz, "Contenido de Fibra Acido Detergente y Minerales Traza en Maíz y en Tortilla." To be published.
- 48. S. García-López and C. J. Wyatt, J. Agr. Food Chem., 30, 724 (1982).
- 49. J. G. Reinhold, J. S. García L., and P. Garzón, Amer. J. Clin. Nutr., 34, 1384 (1981).
- 50. N. G. Asp, C. G. Johansson, H. Hallmer, and M. Siljestrom; J. Agric. Food Chem., 31, 476 (1983).
- 51. E. Acevedo and R. Bressani, "Contenido de Fibra Dietética de Alimentos Centroamericanos." Submitted for publication.
- 52. G. Ranhotra and J. Gelroth, Cereal Chem., 65, 155 (1988).
- 53. R. R. Robles, E. D. Murray, and O. Paredes-López, J. Food Sci. Tech., 23, 91 (1988).
- 54. R. Bressani and N. S. Scrimshaw, J. Agr. Food. Chem., 6, 774 (1958).
- 55. E. I. Ortega, E. Villegas, and S. K. Vasal, Cereal Chem., 63, 446 (1986).
- 56. J. Landry and T. Moureaux, Bull. Soc. Chim. Biol., 52, 1021 (1970).
- 57. N. E. Vivas, R. D. Waniska, and L. W. Rooney, Cereal Chem., 64, 384 (1987).
- 58. J. Sanderson, J. S. Wall, G. L. Donaldson, and J. F. Cavins, Cereal Chem., 55, 204 (1978).
- 59. R. Bressani, R. Gómez-Brenes, and N. S. Scrimshaw, Food Technol., 15, 450 (1961).
- 60. J. E. Braham and R. Bressani, Nutr. Bromatol. Toxic., 5, 14 (1966).
- 61. A. G. Poneros and J. W. Erdman, Jr., J. Food Sci., 53, 208 (1988).
- 62. S. O. Serna-Saldivar, D. A. Knabe, L. W. Rooney, and T. D. Tanksley, Jr., Cereal Chem., 64, 247 (1987).
- 63. W. A. Krehl, L. M. Henderson, J. de la Huerga, and C. A. Elvehjem, J. Biol. Chem., 166, 531 (1946).
- 64. J. Laguna and K. J. Carpenter, J. Nutr., 45, 21 (1951).
- 65. E. Kodicek, R. Braude, S. K. Kon, and K. G. Mitchell, Brit. J. Nutr., 10, 51 (1956).
- W. N. Pearson, S. J. Stempfel, J. S. Valenzuela, M. H. Utley, and W. J. Darby, J. Nutr., 62, 445 (1957).
- 67. R. L. Squibb, J. E. Braham, G. Arroyave, and N. S. Scrimshaw, J. Nutr., 67, 351 (1959).
- 68. E. Kodicek, R. Braude, S. K. Kon, and K. G. Mitchell, Brit. J. Nutr., 13, 363 (1959).
- 69. E. Kodicek and P. W. Wilson, Brit. J. Nutr., 13, 418 (1959).
- 70. R. Bressani, L. G. Elías, and R. A. Gómez-Brenes, J. Nutr., 97, 173 (1969).
- 71. R. Bressani, L. G. Elías, and J. E. Braham, Arch. Latinoamer. Nutr., 18, 123 (1968).
- 72. J. E. Braham, R. Bressani, and M. A. Guzmán, Metabolism, 15, 548 (1966).
- 73. R. O. Cravioto, G. H. Massiev, O. Y. Cravioto, and F. de M. Figueroa, J. Nutr., 48, 453 (1952).
- 74. J. E. Braham, A. Villarreal, and R. Bressani, J. Nutr., 76, 183 (1962).
- 75. N. W. Solomons, R. A. Jacob, O. Pineda, and F. E. Viteri, J. Nutr., 109, 1519 (1979).
- 76. W. R. Hoekstra, Federation Proc., 23, 1068 (1964).
- 77. C. de Bosque, R. Bressani, and E. Castellanos, "Efecto de la Calidad de la Proteína del Maíz sobre la Biodisponibilidad de los Carotenoides," PCCMCA, San José, Costa Rica, 1988.
- 78. S. O. Serna-Saldivar, D. A. Knabel, L. W. Rooney, T. D. Tankstey, Jr., and A. M. Sproule, J. Cereal Sci., 7, 83 (1988).
- 79. R. Bressani and E. M. de Villarreal, J. Food Sci., 28, 611 (1963).
- 80. R. Bressani and E. Marenco, J. Agr. Food Chem., 11, 517 (1963).

- 81. R. Bressani, J. Nutr., 78, 365 (1962).
- 82. R. Bressani, J. Nutr., 79, 389 (1963).
- 83. N. S. Scrimshaw, R. Bressani, M. Béhar, and F. Viteri, J. Nutr., 66, 485 (1958).
- 84. R. Bressani, N. S. Scrimshaw, M. Béhar, and F. Viteri, J. Nutr., 66, 501 (1958).
- 85. R. Bressani, D. Wilson, M. Chung, M. Béhar, and N. S. Scrimshaw, J. Nutr., 80, 80 (1963).
- 86. A. P. De Groot and P. Slump, J: Nutr., 98, 45 (1969).
- 87. M. Sternberg, C. Y. Kim, and F. J. Schwende, Science, 190, 992 (1975).
- 88. N. J. Chu, P. L. Pellet, and W. W. Nawar, J. Agr. Food Chem., 24, 1084 (1976).
- 89. M. L. Martínez H., E. Schieber, R. Gómez-Brenes, and R. Bressani, Turrialba, 20, 311 (1970).
- 90. M. De Campos and A. E. Olszyna-Marzys, Bull. Environ. Contam. Toxicol., 22, 350 (1979).
- 91. M. De Campos. J. Crespo Santos, and A. E. Olszyna-Marzys, Bull. Environ. Contam. Toxicol., 24, 789 (1980).
- 92. M. L. Martínez H., L. G. Elías, J. F. Rodríguez, R. Jarquín, and R. Bressani, Arch. Latinoamer. Nutr., 20, 217 (1970).
- 93. R. R. Martínez, Veterinaria Mex., 10, 37 (1979).
- 94. M. Ulloa-Sosa and H. W. Schroeder, Cereal Chem., 46, 397 (1969).
- 95. M. del C. Solórzano Mendizábal, "Destrucción de Aflatoxinas durante el Proceso de Nixtamalización," Thesis to Facultad de Ciencias Químicas y Farmacia, Universidad de San Carlos de Guatemala, Mayo 1985.
- 96. M. C. de Arriola, E. de Porres, S. de Cabrera, M. de Zepeda, and C. Rolz, in "Aflatoxin and Tortilla Preparation in Guatemala" (M. S. Zuber, E. B. Lillehoj, and B. L. Renfro, eds.), Proceedings of the Workshop CIMMYT, Mexico, 1987, p. 298.
- 97. M. C. de Arriola, E. de Porres, S. de Cabrera, M. de Zepeda, and C. Rolz, J. Agr. Food Chem., 36, 530 (1988).
- 98. A. Torreblanca, H. Bourges R., and J. Morales, in "Aflatoxin in Maize and Tortilla in Mexico" (M. S. Zuber, E. B. Lillehoj, and B. L. Renfro, eds.), Proceedings of the Workshop CIMMYT, Mexico 1987, p. 310.
- 99. M. Carvajal, R. Rosiles, H. K. Abbas, and C. J. Mirocha, in "Aflatoxin in Maize and Tortilla in Mexico," Proceedings of the Workshop CIMMYT, Mexico, 1987, p. 318.
- 100. R. L. Price and K. V. Jorgensen, J. Food Sci., 50, 347 (1985).
- 101. H. K. Abbas, C. J. Mirocha, R. Rosiles, and M. Carvajal, Cereal Chem., 65, 15 (1988).
- 102. E. Capparelli and L. Mata, Applied Microbiology, 29, 802 (1975).
- 103. M. R. Molina, M. A. Baten, and R. Bressani, INCAP Informe Anual, 1977, p. 39.
- 104. V. Valverde, H. Delgado, J. M. Belizán, R. Martorell, V. Mejía Pivaral, R. Bressani, L. G. Elías, M. R. Molina, and R. Klein, "The Patulul Project: Production, Storage, Acceptance and Nutritional Impact of Opaque-2 Corns in Guatemala," INCAP Publication 1983, 179 pp.
- 105. M. J. Rubio, Tortilla and Process Using Carboxylicacid and their Anhydrides, U.S. Patent 3,694,224, 1972.
- 106. M. J. Rubio, Tortilla Process Using Epichlorohydrin, U.S. Patent 3,690,893, 1972.
- 107. M. J. Rubio, Tortilla and Process Using Hydrophilic Inorganic Gels, U.S. Patent 3,709,696, 1973.
- 108. M. J. Rubio, Tortilla and Process Using Sorbic Acid and its Salts, U.S. Patent 3,853,997, 1974.
- 109. M. J. Rubio, Tortilla and Process Using Methyl, Ethyl, Butyl and Propyl Esters of Parahydroxybenzoic Acid, U.S. Patent 3,853,998, 1974.
- 110. M. J. Rubio, Tortilla and Process Using Acetic and Propionic Acid, U.S. Patent 3,859,449, 1975.
- 111. J. Peláez and M. Karel, J. Food Proc. Preserv., 4, 65 (1980).
- 112. C. S. Hickey, D. O. Stephens, and R. S. Flowers, "Preservation of Flour Tortillas," Meeting of Institute of Food Technologists, Las Vegas, NV, June 1982.
- 113. M. N. Islam, M. E. Lirio, and F. R. Del Valle, J. Food Proc. Preserv., 8, 41 (1984).

114. A. Tellez-Girón, G. R. Acuff, C. Vanderzant, L. W. Rooney, and R. D. Waniska, J. Food Protection, 51, 945 (1988).

- 115. H. R. Rosenberg, E. L. Rohdenburg, and R. E. Eckert, J. Nutr., 72, 415 (1960).
- 116. R. O. Cravioto B. and M. Cervantes M., Ciencia (Mex), 24, 159 (1965).
- 117. F. R. Del Valle and J. Pérez-Villaseñor, J. Food Sci., 39, 244 (1974).
- 118. R. Bressani, B. Murillo, and L. G. Elías, J. Food Sci., 39, 577 (1974).
- 119. K. Franz, J. Food Sci., 40, 1275 (1975).
- 120. J. R. Green, J. T. Lawhon, C. M. Cater, and K. F. Mattil, J. Food Sci., 42, 790 (1977).
- 121. R. Bressani, L. G. Elías, and J. E. Braham, Improvement of the Protein Quality of Corn with the Soybean Protein, in "Nutritional Improvement of Food and Feed Proteins" (M. Friedman, ed.), Adv. Exptl. Med. Biol. 105, 1978, p. 29.
- 122. R. Bressani, J. E. Braham, L. G. Elías, and M. Rubio, J. Food Sci., 44, 1707 (1979).
- 123. C. E. Choto, M. N. Morad, and L. W. Rooney, Cereal Chem., 62, 51 (1985).
- 124. S. O. Serna-Saldívar, R. Canett, J. Vargas, M. González, S. Bedolla, and C. Medina, Ceral Chem., 65, 44 (1988).
- 125. R. Bressani, INCAP Informe Anual, 1970, p. 31.
- 126. J. J. Urrutia, B. García, R. Bressani, and L. J. Mata, in "Improving the Nutrient Quality of Cereals" (H. L. Ilcke, ed.), Second Workshop on Breeding and Fortification. USAID, Boulder, CO, 1976, p. 28.
- 127. Y. D. Wang and M. L. Fields, J. Food Sci., 43, 1630 (1978).
- 128. R. Bressani and L. G. Elías, INCAP Informe Anual, 1980, p. 10.
- 129. A. Sánchez-Marroquín, A. Feria-Morales, S. Maya, and V. Ramos-Moreno, J. Food Sci., 52, 1611 (1987).
- 130. A. M. Feria-Morales and R. M. Pangborn, J. Food Sci., 48, 1124 (1983).
- National Research Council, "Quality Protein Maize," National Academy Press, Washington, DC, 1988.
- 132. R. Bressani, J. Alvarado, and F. Viteri, Arch. Latinoamer. Nutr., 19, 129 (1969).
- 133. G. G. Graham, J. Lembcke, E. Lancho, and E. Morales, Pediatrics. To be published.
- 134. G. G. Graham, J. Lembcke, and E. Morales, Pediatrics. To be published.
- 135. M. R. Molina, M. Letona, G. de la Fuente, and R. Bressani, INCAP Informe Anual, 1974, p. 30.
- 136. M. H. Gómez, L. W. Rooney, R. D. Waniska, and R. L. Pflugfelder, Cereal Foods World, 32, 372 (1987).
- 137. G. S. Ranhotra, Cereal Foods World, 30, 703 (1985).
- 138. G. W. Padual and R. McL. Whitney, Cereal Chem., 59, 361 (1982).
- 139. O. Paredes-López and R. Mora-Escobedo, J. Food Tech., 18, 53 (1983).
- 140. T. J. Twillman and P. J. White, Cereal Chem., 65, 253 (1988).
- 141. T. Luke and C. Andrés, Food Proc., 42, 32 (1981).
- 142. N. E. Vivas, R. D. Waniska, and L. W. Rooney, Cereal Chem., 64, 390 (1987).
- 143. D. A. Fellers and M. M. Bean, Food Revs. Int., 4, 213 (1988).
- 144. T. Salazar de Buckle, Food Revs. Int., 4, 237 (1988).
- 145. E. Vargas, R. Muñoz, and J. Gómez, Arch. Latinoamer. Nutr., 36, 456 (1986).
- 146. R. Bressani and R. Jarquín, INCAP, unpublished data.
- 147. R. Bressani, in "Protein-Enriched Cereal Foods for World Needs" (M. Milner, ed.), American Association of Cereal Chemists, St. Paul, MN, 1969, p. 49.
- 148. R. Bressani and L. G. Elías, J. Food Sci., 31, 626 (1966).
- 149. R. Bressani and L. G. Elías, J. Agr. Food Chem., 17, 659 (1969).
- 150. R. Bressani, "Development and Consumption of Soybean Products," International Seminar on Vegetable Proteins, Instituto Mexicano de Aceites, Grasas y Proteinas, A. C. y Asociación Americana de Soya, Querétaro, México, 1986.