Nixtamalized maize supplementation with a sardine protein concentrate to improve the biological value of tortillas

Mardía López-Alarcón,¹ Irene Montalvo-Velarde,² Lilia Bernal-Gracida³ and María de Lourdes Barbosa-Cortés¹

¹Instituto Mexicano del Seguro Social, Centro Médico Nacional Siglo XXI, Pediatrics Hospital; ²Universidad La Salle, Faculty of Chemistry;

³Universidad Nacional Autónoma de México, Faculty of Chemical Sciences, Department of Biochemistry, Ciudad de México, Mexico

Abstract

Introduction: Most part of the rural population in Mexico obtains almost half its energy from corn tortilla, and its sources of protein are mainly of vegetal origin. **Objective:** To obtain a concentrate of sardine protein (SP) to supplement corn flour, and to identify which concentration provides corn tortillas with a better biological value, without modifying its physical and sensorial characteristics. **Method:** Obtainment of the SP concentrate, preparation of tortillas with corn flour and different SP concentrations, assessment of tortillas physical and sensorial characteristics by untrained panelists, assessment of biological quality in a murine model (growth and protein efficiency ratio [PER]). Parametric statistics was used. **Results:** A protein concentrate of 70.48 g/100 g was obtained. Smoothness, blistering, foldability and quality of the tortillas prepared with mixtures containing 0.63-3.75% of SP were comparable to those of tortillas prepared with non-supplemented flour. The growth of rats fed supplemented tortillas was superior; the difference was significant with $\geq 3.75\%$ concentrations ($p < 0.05$). The PER of tortillas with 3.75% of SP was 2.41, which was comparable to that of the reference protein (casein). **Conclusion:** SP-supplemented corn flour at a 96.25:3.75% ratio improves the biological value of tortillas without modifying their physical and sensorial characteristics.

KEY WORDS: Nixtamalized maize. Sardine. Protein concentrate. Corn tortillas.

Introduction

Even when overweight and obesity are top priority health problems in Mexico, National Nutrition Surveys in Mexico show that children undernutrition remains an important problem, especially in rural areas.¹⁻⁴ For example, an analysis of nation-wide stunting rates shows an important descending trend but, in rural areas, prevalence remains high, with a rate higher than 20 %.⁴ In fact, rural areas from the south of the country maintain a prevalence of low height that is 13.9 percentage points higher than national average (27.5 versus 13.6 %).⁴ As for diet, the most recent National Nutrition Survey reports that, although in the country there is an excess in energy consumption deriving from dairy and non-dairy sweetened foods,

less than 50 % of the rural population consumes the recommended foods that provide quality proteins such as eggs, non-processed meat and dairy products, and only around 60 % consume the recommended energy intake and with high fiber content. These dietary deficits are even more serious in school-age children than in adolescents.⁵

The main nutritional influence on growth in developing countries is protein and energy consumption.⁶ In addition, protein requirements increase to the extent energy requirements are not met.^{7,8} On the other hand, in developing countries, main protein sources are often vegetables and grains, which provide low biological value proteins.⁸ In Mexico, particularly in rural areas, most part of the population acquires at least half of its energy from corn (maize) tortilla, and protein sources are mainly of vegetable origin, which

Correspondence:

Mardía López-Alarcón
E-mail: marsau2@prodigy.net.mx

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makes it to be of low quality.⁹ It is important taking into account that corn protein has low lysine and tryptophan concentrations, in addition to an imbalance in the leucine and isoleucine proportion, which are factors that interfere with linear growth.^{10,11} Analyzing this as a whole, it is probable that in developing countries' populations, dietary protein use is inefficient owing to an energy deficit, and even if protein intake is adequate, the consumed protein is of low biological value and to a large extent growth-limiting for originating almost entirely from corn.

Previous, very old studies carried out in children,^{12,13} adults¹⁴ and experimental animals¹⁵ showed that nixtamalized maize nutritional value can be enhanced by simultaneously adding the 3 limiting amino acids: lysine, tryptophan and isoleucine, which owing to its high cost is hardly feasible at the population level. In animal models, improvement of maize tortilla quality has also been demonstrated when supplemented with flour of legumes such as beans, soy, chickpeas, fava beans and lentils, but its use in humans has been insufficient to improve the population nutritional status.^{16,17} One study carried out in Mexico in human subjects, where tortillas were prepared with a blend of corn and soy flour, reported improvement in tortillas biological value, but no effect was demonstrated in children nutritional status.¹⁸

Supplementation with high biological value protein extract, such as that obtained from animal sources, in order to improve corn tortilla quality, has already been attempted.¹⁹⁻²¹ Supplementation with fish flour has already been successfully carried out for animal feed, but its use in humans has not been accepted, mainly because grinded fish has been used (rather than protein extract), and the odor is therefore very strong and the tortilla characteristics are not acceptable for human consumption.^{20,21} For example, in 1957, whole yellow corn protein efficiency ratio (PER) was reported to improve from 0.61 to 1.34, and growth in rats was increased from 21.5 to 71.1 g, when a mixture of grinded fish was added in order to obtain a diet with comparable protein amounts.²⁰ In humans, one study carried out with complementary diets for infants based on rice or corn, supplemented with different sources of protein such as beans, sardine, or both, demonstrated that amino acid scores, digestibility and efficiency rate were better in the diet with sardine mixtures.²¹ It is important taking into account that, in both studies,^{20,21} supplementation was with mixtures of fish flour or whole fish and, in the study in humans, the effect on growth was not analyzed.

This way, evidence suggests that an adequate approach to the enhancement of corn nutritional quality is supplementation with high biological value protein, and fish protein appears to be a good option. In Mexico, sardines are cheap, abundant and contain high-quality protein;²² therefore, it would be a good alternative. Although, ideally, incorporation of sardines to the diet would be more adequate, cultural and economic factors have prevented this food from being added to the Mexican diet, and it is therefore appropriate assessing the addition of a protein concentrate to corn flour in order to, eventually, improve children's growth.

However, it is essential assessing if addition of the protein concentrate to the corn flour does not modify tortilla physical and sensory characteristics, so that it is accepted for use in humans.

The purpose of this study was focused on obtaining a sardine protein (SP) concentrate to be added to corn flour at different proportions and identifying the blend that provides tortillas with acceptable characteristics for human consumption. In addition, to assess the biological value of tortillas prepared with the supplemented mixtures in an animal model by analyzing the tortillas PER and rats growth.

Method

Several experiments were carried out to obtain a sardine protein (SP) extract. Tortillas were prepared with blends of corn flour and different SP concentrations, and their physical and sensory characteristics were evaluated by a group of non-trained panelists. Subsequently, the tortillas biological quality was analyzed in an animal model by assessing growth curves in rats and PER.

Frozen sardines (*Sardina pilchardus*) were used, which were obtained from a local market and stored at -80 °C until being used. Once thawed, heads and intestines were removed from the sardines, and flesh and bones were grinded with a domestic grinder (KitchenAid, Hobart). The obtained blend was sieved through a mesh with 6-mm holes and then through another with 4-mm diameter holes. The obtained material was combined with water at a 1:4 ratio (w/v) and liquefied to obtain a homogeneous mixture. All this process was carried out at the Faculty of Chemical Sciences Biochemistry Laboratory of the National Autonomous University of Mexico.

The aqueous compound obtained was adjusted to an alkaline pH (12.0) using NaOH 2N. The stabilized mixture was centrifuged at 8000 g for 20 minutes at

4 °C in order to separate the soluble proteins. Neutral lipids remained on the upper part, and solid material, mainly connective tissue and bone, went to the bottom. This way, the half part of the mixture containing the solubilized proteins could be obtained. pH was adjusted to 5.5 to favor protein precipitation using HCL 2N and protein was finally recovered after centrifugation at 8000 g for 20 minutes at 4 °C and drying with atomizer (air input temperature at 40 °C and air output at 65 °C, at a rate of 30 mL minute⁻¹). Protein content was determined with the Bradford method (1976), and concentration was calculated using bovine serum albumin as standard.

To make the tortillas, nixtamalized maize flour was used, and were prepared with non-supplemented flour and with flour supplemented with different SP concentrations. This way, dough blends with the following corn flour: SP were obtained: 92.5:7.5, 95.0:5.0, 96.25:3.75, 97.5:2.5, 98.75:1.25 and 99.37:0.63. The tortillas were made using a conventional press and were cooked by placing them on a hotplate for 2 minutes, approximately one minute for each side (Fig. 1).

To assess the tortillas physical and sensory characteristics, 20 untrained panelists who were not allergic to fish were invited, and subjective rating scales were used. Freshly made tortillas were evaluated using those prepared with non-supplemented corn flour as reference.

Flavor, taste, color, odor, softness (by applying pressure with the fingertips on the tortilla and observing the resistance to such pressure) and smoothness (absence of roughness on the tortilla surface) were rated, as well as puffing (formation of blisters at the last phase of cooking) and folding tests (by rolling up the tortilla and observing if the taco external part showed cracks) (Table 1). At the end, overall quality was assessed by asking if the tortilla was not acceptable, moderately acceptable or acceptable.

For the biological tests, only moderately and highly acceptable diets were considered.

For the assessment of the tortillas chemical characteristics, first the moisture content was determined according to the Association of Analytical Communities (AOAC). The moisture content was determined by drying the samples at 105 °C in a conventional oven for 3 hours and calculating the percentage of weight difference between the moisturized sample and the dry sample.²³ Then, the samples were dried at 60 °C and grinded in a grain grinder and the flour was placed in hermetically sealed and labeled glass jars and kept in a fresh place for chemical analysis.

Figure 1. Press and hotplate used to make the tortillas.

Table 1. Scale for sardine protein-supplemented corn flour tortilla sensory evaluation

Softness ()
0-1 = Moderately soft, resistant to pressure
2-3 = Soft, pressure with little difficulty
4-5 = Very soft, easily pressured
Smoothness ()
2 = No roughness
1 = Roughness
Folding ()
2 = Flexible, rolls up, no cracks on taco exterior
1 = Moderately flexible, shows cracks but can be rolled up
0 = Brittle, it cracks so much that a taco cannot be formed
Puffing ()
2 = Blisters on the entire surface
1 = Blisters, but not on 99% of surface
0 = No blisters
Flavor ()
3 = Characteristic, same as corn tortilla
2 = Preferential, different but likable
1 = Acceptable, less likable than preferential
0 = Not acceptable, unpleasant
Color ()
3 = Characteristic, same as corn tortilla
2 = Preferential, different but likable
1 = Acceptable, less likable than preferential
0 = Not acceptable, unpleasant
Odor ()
3 = Characteristic, same as corn tortilla
2 = Preferential, different but likable
1 = Acceptable, less likable than preferential
0 = Not acceptable, unpleasant
Overall quality ()
16-20 = Acceptable, excellent quality
11-15.99 = Moderately acceptable, regular quality
5-10.99 = Not acceptable, bad quality

Protein content was analyzed with the Kjeldahl method, ash content, with incineration in a muffle furnace, raw lipid content, by extraction with solvents (Soxhlet), raw fiber, by acid and alkaline hydrolysis, and total carbohydrate content, by percentage difference with regard to the above determinations. The energy content was calculated based on the reference values (4 kcal/g of protein, 9 kcal/g of fat and 4 kcal/g of carbohydrates.

Table 2. Reference diet proposed by AOAC* for biological tests

Ingredient	g/100 g of diet
Protein	10
Vegetable oil	8
Mineral salts	5
Mixture of vitamins	1
Water	5
Cellulose	1
Carbohydrates: sucrose or dextrin	70

*Energy: 392 kcal/100 g. Reference 23.

Biological tests

PER was determined in the non-supplemented corn flour and in the blends selected in the sensory evaluation tests, using as comparator a casein diet, which is regarded as the protein quality reference.

Recently weaned (21 to 23 days from birth) Sprague-Dowley rats were used, grouped in 6-animal batches for each assessed diet. The animals were placed in individual cages and fed the problem and control diets for 21 days. During this time they were freely supplied water and food, with weight and ingested food being measured every 5 days in order for PER to be calculated using the following formula:

$$PER = \text{weight gain (g)} / \text{protein consumption (g)}$$

For the preparation of the isoproteic and isoenergetic diets, the formula proposed by AOAC was taken as a basis (Table 2).

Statistical analysis

The Minitab statistical program, version 17, was used for statistical analysis, with a one-way ANOVA being used for PER to be compared between the different diets and a two-factor one to assess growth in the different groups over time. For the comparison with the control-diet (non-supplemented maize flour), Dunnet post hoc test was used, and for the comparison between all diets, the Bonferroni test for simultaneous comparisons.

Results

In the experimental phase to obtain the protein concentrate, a compound containing 70.48 g of protein in 100 g of the product could be obtained (Table 3). Subsequently, tortillas were prepared with the proposed

Table 3. Proximate chemical analysis of the raw materials used for the preparation of supplemented tortillas

Determination	Protein extract	Corn flour
Moisture	9.42	52.77
Protein	70.48	4.12
Ash	2.35	0.83
Raw fat	7.16	2.08
Raw fiber	0.0	1.74
Carbohydrates	10.59	38.46
Kilocalories	388.72	189.0

concentrations, which were observed to puff up and fold similarly as the tortillas prepared with non-supplemented corn flour from the 96.25:3.75 blend on (Fig. 2). The sensory tests analysis showed that softness, puffing and folding of the tortillas prepared with the lowest SP concentrations (3.75 to 0.63 %) were comparable to those of the tortillas made with non-supplemented corn flour. As a consequence, overall quality of these tortillas was rated at as least regular. Although the sardine odor was perceptible at the 3.75 % SP concentration, the panelists considered it was not unpleasant (Table 5).

Proximate chemical composition was determined in the tortillas prepared with non-supplemented corn flour and in those made with corn flour with different SP concentrations added; protein addition was observed to be apparent from the lowest dose used on (0.63 %) (Table 4).

As regards the biological tests, the rats receiving casein or tortillas supplemented with 3.75 to 7.5 % of SP grew more than those fed non-supplemented tortillas. The groups of animals that received the lowest protein concentrations also grew better than those fed on non-supplemented tortilla, although the difference did not reach statistical difference (Fig. 3).

Since the tortillas prepared with blends with high SP concentrations were inadequate in the sensory evaluation, PER was only obtained for the blends that were rated as regular and excellent. Table 5 shows that PER with the 96.25:3.75 blend was similar to that of casein, which was consistent with the results obtained in the rat growth analysis (Table 6).

Discussion

This study demonstrates that the quality of corn tortilla can be improved by supplementing it with

Figure 2. Puffing comparison between tortillas made with the different corn flour and sardine protein blends (corn flour: SP) and tortillas made with non-supplemented flour.

Table 4. Proximate chemical analysis of tortillas prepared with different blends of corn flour and sardine protein*

Parameter	92.5:7.5	95.0:5.0	96.25:3.75	97.5:2.5	98.75:1.25	99.37:0.63	100
g/100 g, wet basis							
Moisture	46.38	42.10	45.44	44.29	46.02	48.09	46.89
Protein	10.41	9.09	7.79	6.85	5.29	5.16	4.46
Ash	1.25	1.32	1.19	1.20	1.07	1.06	1.04
Raw fat	1.38	1.26	1.27	1.17	1.17	0.94	1.14
Raw fiber	1.15	1.79	2.05	1.75	2.02	2.11	1.96
Carbohydrates	39.43	44.44	42.26	44.74	44.43	42.64	44.51
Kcal	211.8	225.5	211.6	216.9	209.4	199.7	206.1
g/100 g, dry basis							
Protein	19.42	15.70	14.28	12.29	9.80	9.95	8.40
Ash	2.34	2.28	2.19	2.16	1.99	2.05	1.96
Raw fat	2.58	2.18	2.32	2.11	2.17	1.82	2.14
Raw fiber	2.14	3.10	3.76	3.15	3.74	4.06	3.70
Carbohydrates	73.52	76.74	77.45	80.29	82.30	82.12	83.80
Kcal	211.8	225.5	211.6	216.9	209.4	199.7	206.1

*The ratio corresponds to the corn flour: sardine protein proportion.

sardine protein without its physical and sensory characteristics being modified. Enhanced tortillas potentially would optimize children growth in populations

where maize tortilla constitutes the main source of energy and protein and where children's growth is deficient.

Table 5. Sensory evaluation of the tortillas prepared with different corn flour: sardine protein ratios

Parameter	92.5:7.5	95.0:5.0	96.25:3.75	97.5:2.5	98.75:1.25	99.37:0.63	100
Taste (0-3)	0.81	0.76	1.21	1.76	2.16	2.54	2.82
Color (0-3)	1.52	1.47	1.93	1.88	2.12	2.85	2.82
Odor (0-3)	0.71	1.00	0.93	1.71	1.82	2.54	2.76
Softness (0-5)	3.83	3.76	4.68	4.09	4.34	4.96	4.59
Smoothness (1-2)	1.19	1.06	1.79	1.88	1.94	2.00	2.00
Puffing (0-2)	1.00	1.00	2.00	2.00	2.00	2.00	2.00
Folding (0-2)	1.19	1.29	1.71	1.35	1.76	1.69	1.76
Total score	10.25	10.34	14.25	14.67	16.14	18.58	18.75

Figure 3. Growth curves of rats fed tortillas prepared with corn flour not supplemented or supplemented with different concentrations of sardine protein during the 21 days of experimentation. Eight groups of 6 rats fed on non-supplemented corn flour (CF), casein or sardine protein different concentrations were formed (two-way ANOVA).

The results are comparable with those in other studies that have demonstrated that the quality of corn tortillas improves when supplemented with legumes, both with whole-grain flour and with protein extract. However, the PER reported in legume-supplemented diets is approximately 1.8;^{22,23} this is important because, although there isn't a PER value of best quality, it is suggested for it to be at least similar to the casein PER of 2.5, regarded as the reference value.²³

The PER obtained in our study for the tortillas with a protein concentrate of 3.75 % was 2.44, suggesting that the use of a sardine protein concentrate is a better alternative than the use of legumes.

We recognize that other biological tests, such digestibility of the diets, were not performed in our study; however, the results observed in the rats' growth suggest that protein bioavailability of the different diets was adequate, since a direct association is observed

Table 6. Biological tests in the blends with the best physical and sensory ratings in comparison with non-supplemented and casein-supplemented tortillas

CF: SP blend	Experimental PER	Adjusted PER*
96.25:3.75	2.17	2.44
97.5:2.5	1.47	1.66
98.75:1.25	1.51	1.70
99.37:0.63	1.35	1.52
100 (NT)	1.25	1.41
Casein	2.22	2.50

CF: SP = corn flour: sardine protein. PER = protein efficiency ratio.

*Adjusted to casein PER of 2.50.

between the amount of added protein and rats' growth. In other words, the higher the protein concentration in the tortillas, the more the growth of the rats in a dose-response fashion (Fig. 3).

We also recognize that this study is in a preliminary stage prior to proposing corn flour routine supplementation with sardine protein. It is indispensable for a study to be carried out in a model of human beings in order to demonstrate that sardine protein addition to corn tortillas really has an impact on children growth. Furthermore, it will be necessary for cost-effectiveness studies to be carried out in order to calculate the cost of supplemented tortillas as related to children growth improvement and the implications of this. For example, if better growth is reflected on lower rates of infection, hospitalization, medical treatment, etcetera.

Finally, we consider that this result is relevant from the point of view of public health because it proposes an alternative to improve the diet and, consequently, the nutritional status of populations with nutritional deficits, particularly in places with high rates of under-nourishment and fish consumption is common, in order to improve the acceptance of foods.

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