

## Comparative study on the nutritional and antioxidant properties of two Mexican corn (*Zea mays*) based meals versus processed cereals

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**SUMMARY.** The present study was conducted to analyze the chemical composition, total phenolics content and antioxidant capacity of two whole corn (*Zea mays*) based meals traditional from Mexico: "traditional pinole" and "seven grain pinole"; and compare it with information available from ready to eat cereal products based on refined corn and whole grain cereals. Proximate analyses (moisture, ash, fat, protein and fiber) were carried out according to the procedures of AOAC, sugars content was determined by HPLC method; calcium and iron were quantified using atomic absorption spectroscopy. Total phenolic compounds were determined by Folin-Ciocalteu spectrophotometric method; the antiradical capacity was determined by DPPH colorimetric method and total antioxidant capacity was determined by FRAP method. Traditional and seven grain pinole presented higher energy content and nutrient density (protein and fat) than processed cereals. Calcium content was higher in processed cereals than pinole; seven grain pinole presented the highest concentration of iron. Polyphenolic concentration was higher in both kinds of pinole compared to processed cereals; traditional pinole presented the highest antioxidant activity measured by DPPH and FRAP methods. The results provide evidence about the important nutrient and antioxidant content of traditional and seven grain pinole compared to processed cereals based on corn and other grains. It is recommended their incorporation in to regular diet as a healthy food, with a good protein level, low sugar content and good antioxidant capacity.

**Key words:** Whole grain, processed cereals, proximate composition, total phenolic, antiradical capacity.

**RESUMEN. Estudio comparativo sobre las propiedades nutricionales y antioxidantes de dos alimentos mexicanos basados en maíz (*Zea mays*) en comparación con cereales procesados.** En el presente estudio se analizó la composición química, el contenido de compuestos fenólicos totales y la capacidad antioxidante de dos alimentos elaborados a base de granos enteros de maíz (*Zea mays*) típicos de México: "pinole tradicional" y "pinole de los siete granos"; y se comparó con la información nutricional ya disponible de dos cereales procesados. El análisis proximal se realizó de acuerdo a los procedimientos de la AOAC, el contenido de azúcares se determinó por HPLC; el calcio y hierro se cuantificaron utilizando espectroscopía de absorción atómica. Los compuestos fenólicos totales se determinaron espectrofotométricamente por el método de Folin-Ciocalteu; la capacidad antirradical se determinó por el método colorimétrico del DPPH, y la capacidad antioxidante total se determinó por el método FRAP. El pinole tradicional y el pinole de los siete granos presentaron una mayor densidad de nutrientes (proteína y grasa) y mayor contenido calórico en comparación con los cereales procesados. El contenido de calcio fue mayor en los cereales procesados; el pinole de los siete granos presentó la mayor concentración de hierro. La concentración de compuestos fenólicos fue mayor en ambos tipos de pinole comparado con los productos procesados; el pinole tradicional presentó la mas alta actividad antioxidante medida por los métodos de DPPH y FRAP. Los resultados muestran evidencia sobre el importante contenido de nutrientes y compuestos antioxidantes del pinole tradicional y pinole de los siete granos; se recomienda su consumo regular, por ser alimentos con un buen nivel de proteína, bajo contenido de azúcar y con una buena capacidad antioxidante.

**Palabras clave:** Granos enteros, cereales procesados, composición proximal, compuestos fenólicos totales, capacidad antirradical.

### INTRODUCTION

Whole grains, or foods made from them, contain all the essential parts (bran, germ, and endosperm) and naturally-occurring nutrients of the entire grain seed. If the grain has been processed (e.g., cracked, crushed, rolled, extruded, and/or co-

oked), the food product should deliver the same balance of nutrients that are found in the original grain seed (1). Whole grains represent a rich source of nutrients, and have been the basis of human diet for thousands of years (2); their regular ingestion has been negatively associated with morbidity and mortality due to different chronic-degenerative diseases, such as chronic inflammation, heart failure,

hypertension, obesity, type-2diabetes, and some types of cancer, among others (2, 5). The protective effect of whole grains consumption is likely mediated through the different phytochemicals present in them, such as dietary fiber, oligosaccharides, phenolic compounds, phytoestrogens, antioxidants, vitamins and minerals, which may act together in a synergistic way (2, 5). Recommendations for a healthy diet suggest consuming three or more ounce-equivalents of whole grain products per day; at least half of the portions of cereals should come from whole grains (6, 7). However, most of the grain products consumed are refined, which means that the bran and germ have been removed; and hence an important part of the phytochemicals has been lost, and only the starchy endosperm remain in white flour used to prepare them (2). Some of the lost nutrients are added in a concentrate form, especially some minerals and vitamins, but some other, such as stanols, fiber and natural antioxidants can not be replaced.

Consumption of whole grains could be enhanced through the regular ingestion of traditional foods produced from complete grains. One of the staple grains in Mexico is corn (*Zea mays* L.) which is consumed in many ways, including sweet and salted preparations (8). One of the most antique preparations, from Mexico, based on corn is called "pinole"; it is a prehispanic powdered meal, traditionally prepared with roasted corn and some times sweetened, and additioned with cacao, cinnamon or anise. The way to consume it is mixed with liquid: water, juice or milk, to prepare porridge or a thick drink. Pinole represents a basic food for infants for some indigenous groups in Mexico (8, 10); different kinds of pinole, made up with corn and mixtures of grains, have been developed; for example, a kind of pinole with corn and chickpea (*Cicer arietinum* L.) is prepared in Guanajuato, Mexico; with faba bean (*Vicia faba* L.), rice (*Oriza sativa* L.) and pumpkin seeds (*Cucurbita pepo* L.) is prepared in Zacatecas, Mexico. In Mexican highlands a kind of pinole is prepared with corn or amaranth (*Amaranthus* spp.) (10). "Pinole de los siete granos" (seven grain pinole) is sold in Durango, Mexico; it is prepared with five cereal grains and two legumes, regular consumers of this product refer it as a health promoter. This product is typically made from whole grains, so they provide all the nutritional and phytochemical benefits to consumers. There is little information on proximate composition of traditional and other enriched pinole products

(10,11); however, there is no information about the chemical composition of "seven grain pinole" and on the antioxidant properties of pinole, and that is why the objective of this research was to analyze the proximate composition, iron and calcium content of traditional and seven grain pinole and compare the information with data available from commonly consumed breakfast cereals prepared prepared with corn (corn flakes) and a mixture of corn and other grain (multi-grain cereal). Additionally, phenolic content and antioxidant capacity of traditional and seven grain pinole and processed cereals were analyzed and compared.

## MATERIALS AND METHODS

*Corn based meal samples.* Two different types of corn based meals were analyzed: 1) "traditional pinole" (TP), prepared with roasted cacahuatzintle corn (*Zea mays*) and cinnamon, it was purchased from local market in Lagos de Moreno, Jalisco, Mexico; 2) "seven grain pinole" (SGP), prepared with a mixture of corn (*Zea mays*), faba beans (*Vicia faba* L.), lentils (*Lens culinaris*), rice (*Oriza sativa* L.), amaranth (*Amaranthus* spp.), granola and wheat (*Triticum* spp.), was purchased directly to the manufacturer in Ocampo, Durango, Mexico. For comparisson, the nutritional information available of two different breakfast cereals was utilized; corn flakes, a refined corn cereal (RCC), and multi-grain cereal (MGC) were chosen because their ingredients are similar to those in TP and SGP, because they are typically consumed with milk in the same way of pinole, and because they are among the most consumed breakfast cereals in Mexico. All the products were analyzed for phenolic content and antioxidant capacity. Two different batches of each product (TP, SGP, and processed cereals) were sampled from the first and second semesters of 2009.

*Chemicals.* All chemicals used were of high-purity reagent grade. Throughout all analytical work, ultrapure water (Labconco, USA, 18.2 M $\Omega$ /cm) was used. Atomic absorption spectrometry standards (Ca and Fe), hydrogen peroxide, boric acid, sodium hydroxide, nitric acid, HPLC solvents (methanol, ethyl acetate, acetonitrile), butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, Folin-Ciocalteu reagent, acetate buffer, 2,4,6-tripyridyl-s-triazine (TPTZ), FeCl<sub>3</sub>•6H<sub>2</sub>O, and acetate buffer were from Sigma Chemical Co. HPLC standards

(sucrose, fructose, glucose and maltose) were from WVR International.

## ANALYSES

*Proximate composition.* Nutritional composition of pinole samples was determined according to standard AOAC methods (12). Crude protein was determined by the microKjeldahl method using 6.25 as the conversion factor (Method 960.52). Crude fiber was determined by Method 962.09E; while fat and ash contents were determined by Soxhlet extraction and dry ashing methods, respectively (Methods 920.39C, and 923.03). Total carbohydrates were calculated by subtraction of the five main constituents (moisture, ashes, fat, protein and fiber) to 100%. Gross energy was determined by calculation using Atwater's conversion factors (7).

*Sugars.* Sucrose and other simple sugars (maltose, glucose and fructose) were determined using an HPLC 2130 (Hitachi Instruments, Japan), with a refractive index detector (Bischoff 8120), with autosampler and Xcalibur software for data processing. The HPLC separations were carried out on a 250 × 4.61 mm i.d., 5- $\mu$ m Supelcosil LC-NH2 column (Supelco). The mobile phase consisted of acetonitrile:water 85:15 (v/v), a flow rate of 1500  $\mu$ L/min, and an injection volume of 20  $\mu$ L were employed (13). All assays were performed in triplicate.

*Minerals.* Previous to mineral analysis, pinole samples were digested in HNO<sub>3</sub>:30% H<sub>2</sub>O<sub>2</sub> (1:1, v/v) following the protocol described by Santoyo and others (14). Ca and Fe were determined by atomic absorption spectrometry (AAS) Perkin-Elmer model 2380 (Perkin-Elmer, Norwalk, CT, USA) with air-acetylene flame at a wavelength of 248.3 nm for Fe and 422.7 nm for Ca, and using calibration standards in eight concentration levels, from 0 to 6  $\mu$ g/L.

## ANTIOXIDANT PROPERTIES

*Sample preparation.* In order to develop the quantification of total phenolic compounds, DPPH and FRAP analysis, samples (TP, SGP, RCC and MGC) were subjected to extraction following the protocol described by Cardador-Martínez and others (15); briefly, samples were placed in flasks and mixed with methanol [1:50 (w/v) ratio]. The flasks were shaken for 24 h at 25 °C while wrapped in aluminum foil to protect from light. The samples were centrifuged (Sigma 2-16K, Germany) at 2000 rpm during 5 min. Analyses

were performed to the supernatants obtained.

*Phenolic content.* The amount of total phenolic was determined using the Folin-Ciocalteu modified method, according to Singleton and Rossi (16). A 0.1 mL aliquot of the extract was mixed with 0.1 mL of distilled water, 1.0 mL of 1N Folin-Ciocalteu reagent and 0.8 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub>. The mixture was allowed to stand in the dark for 30 min at room temperature. Absorbance was measured at 765 nm on a spectrophotometer (Sigma 2-16K116172, Germany), previously calibrated with a blank prepared in the same way substituting the extract by distilled water. The total phenolic content was expressed as gallic acid equivalents (GAE per gram) according to a calibration curve from 0.1 to 0.5 mg/mL of gallic acid.

*Antioxidant capacity.* For quantification of antioxidant capacity of whole grain (TP, SGP) and refined (RCC and MGC) samples two methods were utilized: a) the antiradical capacity was tested by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (15); and, b) the ferric reducing antioxidant power (FRAP) assay (17). For the DPPH method, 300  $\mu$ L of each extract were placed in test tubes, and 3 mL of methanolic DPPH solution (150  $\mu$ M) were added. Tubes were mixed and incubated in the dark at 25°C. After 30 min, the absorption was measured in 1 cm cuvettes at 520 nm, using a spectrophotometer (Jenway 6305, Germany). Antiradical capacity (ARC) was calculated as decoloration percentage:  $ARC = 100 \times [1 - (\text{absorbance sample} / \text{absorbance of control})]$ . For the FRAP assay, 2,700  $\mu$ L of the FRAP reagent, was mixed with 270  $\mu$ L of distilled water and 90  $\mu$ L of the test sample or the blank (methanol). Absorbance values were measured at 595 nm after 4 min of reaction at 37°C, on a spectrophotometer (Jenway 6305, Germany).

*Statistical Analyses.* The data were analyzed using SAS 9.1 statistical package. Analysis of variance was used to analyze the difference in the nutrient concentrations between pinole samples. Student's t test was used to compare means of proximate analysis and minerals; while Duncan's Multiple Range Test, at 5% level of probability, was applied to antioxidant properties results.

## RESULTS

Significant differences were found between TP and SGP proximate composition, as shown in Table 1. TP

presented higher fat, fiber and total carbohydrate contents, while content of protein, ash and sugars were higher in SGP. Both kinds of pinole presented higher protein and fat content, compared to the refined products (corn and multi-grain cereals). Sugars content followed the order MGC > SGP > RCC > TP. Figure 1 shows the concentration of fructose, glucose, maltose and sucrose quantified by HPLC. Sucrose was the most abundant sugar present in both corn meals (1.44% in TP, 11.51% in SGP); while the content of fructose, glucose and maltose was lower than 1%. TP and SGP were more energetic compared to refined products (Table 1). Percentage of energy contribution of protein, fat and carbohydrates to total energy in all products was calculated; carbohydrates contributed the biggest portion of total energy, around 80% of energy came from carbohydrates in TP and SGP; protein contributed 7.92 and

10.64% of the energy in TP and SGP, respectively; and fat contributed 11.45 and 9.44% of energy, respectively. Protein contribution in energy of RCC and MGC (7.87 and 7.84%, respectively) was similar to TP (7.92%); RCC presented the lowest fat contribution to energy (4.13%). Calcium and iron contents quantified in TP and SGP are shown in Table 1, as well as data from processed cereals. SGP presented the highest concentration in both minerals analysed (37.91 and 21.56 mg/100g, for Ca and Fe, respectively); but lower content of calcium compared to both processed cereals which were added with vitamins and minerals, including calcium and iron. Content of total phenolics in samples analyzed ranged from 0.448 to 1.69 mg GAE/g sample (Table 2); whole grain products (TP and SGP) presented the highest content of total phenolic compounds, data were not statistically different between them; while RCC presen-

Table 1. Composition and energy contribution of "traditional pinole" and "seven grain pinole" compared to data reported for processed corn and multi grain cereals. (g/100 g)

	Whole corn products		Processed cereal products	
	Traditional pinole *	Seven grain pinole *	Refined corn cereal ‡	Multi-grain cereal ‡
Moisture	5.31 ± 4×10 <sup>-3a</sup>	4.22 ± 5×10 <sup>-3b</sup>	NR	NR
Protein	7.74 ± 0.01 <sup>b</sup>	10.37 ± 0.01 <sup>a</sup>	7.3	7.3
Fat	4.97 ± 4×10 <sup>-3a</sup>	4.09 ± 4×10 <sup>-3b</sup>	1.7	3.3
Fiber	2.13 ± 10 <sup>-3a</sup>	1.62 ± 2×10 <sup>-3b</sup>	4.0	5.0
Ash	1.06 ± 10 <sup>-3b</sup>	1.77 ± 10 <sup>-3a</sup>	NR	NR
Carbohydrates	78.79 ± 0.04 <sup>a</sup>	77.93 ± 0.03 <sup>b</sup>	81.6	78.3
Energy (kcal/100g)	390.9	390.0	370.9	372.1
Sugars	2.07 ± 0.15 <sup>b</sup>	12.27 ± 0.15 <sup>a</sup>	11.3	20.0
Ca (mg/100g)	31.89 ± 1.08 <sup>b</sup>	37.91 ± 6.22 <sup>a</sup>	660	660
Fe (mg/100g)	4.31 ± 1.82 <sup>b</sup>	21.56 ± 5.23 <sup>a</sup>	12.47	12.47

\* Values are expressed as mean ± standard deviation. Means within a row followed by different superscripts are significantly different using Student's t Test.

‡ Obtained from nutritional information label.

NR = not reported.

Table 2. Total phenolic content and antioxidant capacity (DPPH and FRAP) in "traditional pinole" and "seven grain pinole", compared to processed corn and multigrain cereals.\*

	Whole corn products		Processed cereal products	
	Traditional pinole	Seven grain pinole	Refined corn cereal	Multi-grain cereal
Total phenolic compounds ‡	1.58 ± 0.17 <sup>a</sup>	1.69 ± 0.07 <sup>a</sup>	0.448 ± 0.05 <sup>c</sup>	0.728 ± 0.04 <sup>b</sup>
Antiradical capacity †	27.87 ± 1.87 <sup>a</sup>	15.24 ± 1.63 <sup>c</sup>	2.37 ± 0.33 <sup>d</sup>	24.79 ± 2.01 <sup>d</sup>
Ferric reducing power **	21.25 ± 1.62 <sup>a</sup>	18.75 ± 2.33 <sup>b</sup>	12.66 ± 0.32 <sup>c</sup>	5.99 ± 1.05 <sup>d</sup>

\* Values are expressed as mean ± standard deviation. Means within a row followed by different superscripts are significantly different using Duncan's Multiple Range Test at p < 0.05.

‡ Quantified by Folin-Ciocalteu method, expressed as mg of gallic acid equivalents/g of sample.

† Antiradical capacity determined by DPPH method, expressed as percentage.

\*\* Ferric reducing power determined by FRAP method, expressed as μmol Fe<sup>2+</sup>/g.

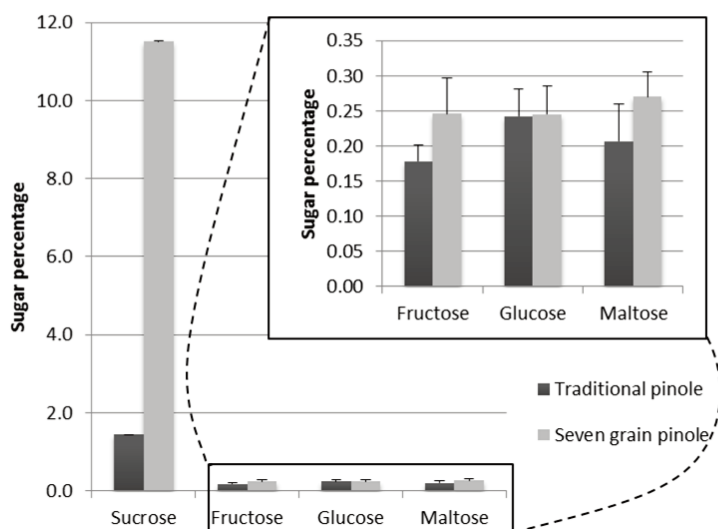


Figure 1. Sugars content: sucrose, fructose, glucose and maltose in "traditional pinole" and "seven grain pinole". Results are the mean  $\pm$  standard deviation based on three replications of two different batches.

ted the lower content. The antioxidant capacity of samples was determined through two different methods, one based on the capacity of sample components to quench free radicals (DPPH), and the other based on the capacity of samples to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ , namely their ferric reduction power (FRAP), the results are shown in table 2. Antiradical capacity of samples quantified by DPPH method ranged from 2.37 to 27.87%; and followed the decreasing order: TP > MGC > SGP > RCC; while the ferric reducing power of samples followed the order TP > SGP > RCC > MGC. As can be seen, TP presented the highest values of antioxidant capacity in both methods (27.87% of antiradical capacity by the DPPH method, and 21.25  $\mu\text{mol Fe}^{2+}/\text{g}$  by the FRAP method); while processed cereals presented the lowest values in DPPH (RCC 2.37%) and FRAP (MGC 5.99  $\mu\text{mol Fe}^{2+}/\text{g}$ ) tests.

## DISCUSSION

In general, data from proximate analyses of both pinole kinds (Table 1), were similar to pinole composition reported in Food Composition Table for Latin America (11), and were in agreement to corn products analyzed elsewhere (18). Both pinole products were prepared from whole grains, so they include germ and bran, which could explain the higher contents in fat over the refined products (2,7), and also they contain a

lower amount of added sugar; except for SGP in which the presence of granola, which contains honey, rised the sugar content to 12.27%; meanwhile processed cereals were added with refined sugar, partially inverted sugar syrup, and glucose syrup as stated in the label. Carbohydrate content represented the biggest portion of chemical components present in the two types of pinole analyzed and refined cereals (Table 1) and the main contributor to energy; results were similar to other reports (18) in which 80 to 82% of carbohydrates had been determined in corn products. Both processed cereals presented more than 80% of energy from carbohydrates, which reflects the fact of coming from refined grains, eventhough the description in label indicated the products contain 30.5 and 59% of whole grains, for RCC and MGC, respectively. Since these products are made

by hand and distributed unlabelled, there is no a portion size recommended for pinole; however, the way they are most commonly consumed is by mixing 3 tablespoons of powder (about 10g) with a glass of milk, juice or water to make a thick drink. In case of being eaten as porridge, a quarter cup of pinole (about 30 g) is mixed with liquid, a quantity similar to that of breakfast cereals portion. It is important to remark that TP and SGP, which are whole corn products, represent a healthy option for breakfast consumption since they provide higher protein concentration, lower sugar content (in the case of TP) compared to corn and multigrain processed cereals; and at least double of the healthy fat content present in the corn germ.

For TP and SGP, calcium quantification data (Table 1) were lower than the information reported in Food Composition Table for Latin America for traditional pinole (79 mg/100) (11), and higher than information reported by Figueroa-Cárdenas and others (19) for corn (7.7 mg/100 g). The concentration of calcium present in grains is variable, and the difference between both types of pinole, may be due to the grains included in their formulations. Iron content in SGP was almost three times the content of iron reported in Food Composition Table for Latin America for corn pinole (7.7 mg/100) (11). The higher content of iron in SGP could be attributed to the high content of it in legumes (lentils and faba beans) present in that product (7). The presence of

calcium and iron in diet is important because of their physiological effects, such as the bone and teeth building, muscles and nerve function, blood clotting and immune defense for calcium; and the myoglobin and immune function for iron; and that is one of the reasons processed foods are commonly fortified with these minerals (7), contrary to TP and SGP, which are produced in an artisanal way so they are not enriched. Processed corn and multi-grain cereals label stated that each cereal portion (30g) contained 22% of the recommended dietary ingestion (RDI) of calcium for Mexican population (900 mg/day) (20), which corresponded to 198 mg/30g or 660 mg Ca/100g; a higher content compared to both kinds of pinole analyzed. Iron content in SGP, was five times higher than the content in TP and 1.7 times the content reported in the processed cereals (22% of the RDI for Mexican population of 17 mg/day) (20).

Phenolic compounds are plant secondary metabolites with biological activity, commonly ingested by animals and humans through the diet. The presence of phenolic compounds in cereal products has been described especially in those prepared from whole grains, since they contain germ and bran, grain parts rich in phytochemicals (2, 21), and this could explain the presence of a higher phenolic content in the whole corn products analyzed in this research, compared to processed cereals. Both kinds of pinole, which are 100% whole grain products, presented more than triple and double phenolic compounds content compared to RCC and MGC, respectively (Table 2), which claim on the label to contain 30.5 and 59% of whole grains. Adom and Liu (22) reported a content of free phenolics of 0.36 mg GAE/ g of dehulled corn, a low content compared to TP, a product made from whole corn, but similar to RCC which is made from refined corn. MGC presented 1.6 times the phenolic content of RCC; and the values were similar to those reported by Yu and others (23) in four processed cereal samples based on wheat and oats (0.203-0.524 mg/g). The results suggest phenolic components are lost during cereal processing, because of the refining process which eliminates germ and bran. Antioxidant capacity of natural and processed cereals has been evaluated using different assays (22, 24). Results obtained by different authors present high variability, since antioxidant capacity values are influenced by analytical factors such as the kind of assay used, the oxidation substrate and the extraction method, among others; as well as, factors inherent to the sam-

ple; and that is why comparison among different results is difficult to carry out. Our results agree with the research of Adom and Liu (22), who investigated the antioxidant activity of raw grains, and found that corn, had the highest antioxidant activity followed by wheat, oats and rice. Products based on whole cereals are relatively high in antioxidant capacity, which is attributable to the presence of a number of nutrimental and non nutrimental antioxidant components (2, 24), including the polyphenolic compounds; in this research, concentration of phenolic compounds was not directly related to antioxidant capacity of samples, which proves that there are other components in grains that also have influence on their antioxidant capacity. Additionally, antioxidant capacity of foods can be increased during thermal treatment because of the formation of antioxidant non phenolic compounds such as Maillard products, which have been related to increased free radical scavenging properties (25). It is important to highlight that the antiradical capacity reported here may have been underestimated as, according to some authors (21, 22), the major portions of phytochemicals in the grains are present in the bound form and the results presented here come from soluble phytochemicals with antiradical capacity; further studies on the antiradical capacity of phytochemicals bounded to structural molecules are necessary to get a better picture of antiradical capacity of pinole and processed cereal products. It should be noted that polyphenolics are secondary metabolites which are synthesized in plants in response to environmental stress, and their concentration may vary among different batches of pinole.

## CONCLUSIONS

TP and SGP are whole grain cereal products with a greater amount of protein, healthy fat and total phenolic compounds than commonly consumed breakfast cereals, which are fabricated from refined cereals and added with high doses of sugar. The results provide information to suggest that consumption of TP and/or SGP could have a positive impact on nutrition or have beneficial health effects compared to the consumption of refined breakfast cereals, because of their whole grain content and their phytochemical contribution to diet. More research is needed in order to identify other antioxidant compounds, than phenolic compounds; and to evaluate their activity in vivo.

## ACKNOWLEDGMENTS

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