Formulation, Physicochemical, Nutritional and Sensorial Evaluation of Corn Tortillas Supplemented with Chía Seed (*Salvia hispanica* L.)

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Abstract

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Composite flours containing 5%, 10%, 15%, and 20% of chia seed flour and corn were used for tortilla formulations. The effects of chia powders supplementation on the physicochemical and sensorial characteristics as well as starch digestibility of the tortillas were evaluated. Nutritionally, all chia tortillas had significantly higher levels ($P < 0.001$) of protein, lipids, and total dietary fibre than the control. The reduced enzymatic starch hydrolysis rate and predicted glycemic index recorded for the chia seed-added tortilla indicated slow digestion features. Sensory evaluation did not show significant ($P > 0.05$) differences in the attributes among tortillas. Owing to the increase in the total dietary fibre, lower digestion, and predicted glycemic index values, chia seed-added tortilla can be considered as a nutraceutical food. Therefore, the newly developed tortilla supplemented with chia seed flour could represent a valuable staple in improving the nutritional value of the original food product.

Keywords: nutraceutical; glycemic index; Fibre-rich

Changes in human diets over the past years, particularly in terms of the dietary fat intake and its effect on human health, have become a major interest in the nutrition research (Risérus *et al.* 2009). Epidemiological and scientific evidence has shown a strong relationship betweem the total fat intake and its composition and a number of diseases, including coronary heart disease, cancer, diabetes, and depression (Kris-Etherton *et al.* 2002). There is growing consensus that dietary habits adopted by Western societies over the past 100 year have contributed to the increased risk of not only coronary heart disease, but also hypertension, diabetes, and cancer (Okuyama *et al.* 1997; Henning & Watkins 1998). During the last century, the emergence of processed foods, grain-fattened livestock, and hydrogenated vegetable fats has reduced the intake of ω-3 fatty acids while increasing the intake of ω-6 fatty acids and saturated fats (Simopoulos 1999), because many of these oils are used in the production of many of

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the processed foods. They are also found extensively in a wide range of foods consumed every day.

However, the nutritional community's attention has recently been drawn to a novel whole grain, chia seed, whose oil content ranges from 25% to 35% with high concentrations of polyunsaturated fatty acids as well as a complement of vitamins and minerals (Chicco *et al.* 2009; Coates & Ayerza 2009). Whole grains are important sources of many nutrients such as dietary fibre, resistant starch, trace minerals, vitamins, folic acid, selenium, zinc, phenolic compounds, and other compounds including phytoestrogens and antioxidants, which may contribute to the apparent cardioprotective effects of whole grains (Djoussé *et al.* 2007). Not only that, it is touted as having the highest fiber content out of any food, being beneficial for type 2 diabetics (Vuksan *et al.* 2007), reduction of cholesterolaemia, modification of the glycemic and insulinaemic responses, changes in intestinal function and antioxidant activity (Borderías *et al.* 2005; Esposito *et al.* 2005). Previous research has shown that sugars are progressively liberated from tortilla mixed with whole seed during digestion, leading to a standard increase in postprandial blood glucose and insulin response (glycemic index; Islas-Hernández *et al.* 2007; Rendón-Villalobos *et al.* 2009). Low-glycemic index foods have been shown to positively affect lifestyle through various mechanisms, including the improvement of glucose tolerance (Liljeberg *et al.* 1999) and helping maintaining a better cognitive performance throughout the day (Papanikolaou *et al.* 2006), and can also be used in the treatment of obesity, type 2 diabetes mellitus, and in weight management (Anton *et al.* 2008; Rendón-Villalobos *et al.* 2009). Chia seeds from *Salvia hispanica* L., *Salvia colurnbria* Benth, *Salvia polystachya*, and other *Salvia* members of the family Labiatae have long been used as a food ingredient, oil source, medicine, and are especially well known by American Indians and rural Mexicans (Cahill 2003; Reyes-Caudillo *et al.* 2008). Economic historians have suggested that *S. hispanica* as staple food was as important as maize and in some areas even more important (Cahill 2003).

Therefore, the purpose of this study was to develop nutritionally enhanced tortillas by incorporating an ingredient with well-documented nutritional functionality (chia seed) in a corn tortilla, in an attempt to create a low-glycemic index (GI) and fibre-rich product while preserving the sensory acceptability and physicochemical properties.

MATERIAL AND METHODS

General. Corn tortillas were produced in the Quality Control Laboratory, CEPROBI-IPN. Nixtamalised corn flour (NCF) (MASECA™, León, Mexico) and whole chia seed (Agrobeck International de México™, SPR de RL de CV, Guadalajara, Jalisco, México) were obtained from a selected supermarket. Whole seeds were ground in a Tekmar™ analytical mill (Model A-10; Tekmar Co., Cincinnati, USA).

Tortilla preparation. Corn tortillas were produced using the method described by Rendón-Villalobos *et al.* (2009). NCF (100 g) (either NCF or composites) with weight adjusted to 14% of moisture content added at different levels (5%, 10%, 15%, and 20% of chia seed powder). The dry ingredients plus shortening were mixed for 2 min before water addition. Once water was added, the batter was further mixed to form masa, and cut into pieces of 35 grams. These pieces were rounded and pressure-molded and extruded into thin circles to make tortillas 1 mm thick. Thereafter, the tortillas were transferred onto a hot plate preheated at 250°C and baked for 30 s on the first side, flipped, and baked for 40 s on the second side, and flipped and baked for another 10 s on the first side. The baked tortillas were cooled on a rack for 1 min and then frozen at −50°C, 5 Pa, for 48 h in a freeze dryer (VirTis Consol 25SL; VirTis, Gardiner, USA).

Proximate analyses. The freeze-dried tortillas were analysed for their moisture content by AOAC method 925.10 (AOAC 2000). Due to the different moisture contents of the samples, all calculations were made on a dry matter basis. Ash and fat were analysed according to the approved methods 08-01 and 30-25, respectively (AACC 2000). Nitrogen content was determined by the Kjeldahl method 46-13 and was multiplied by a factor of 5.85 to estimate protein content (AACC 2000). Total dietary fibre (TDF) was evaluated using the AOAC method 985.29 (AOAC 2000). All analyses were performed in triplicate. The samples were gelatinised with a heat-stable α-amylase (pH 6, 100°C, 30 min) and digested enzymatically first with protease (pH 7.5, 60°C, 30 min) and then with amyloglucosidase (pH 6, 60°C, 30 min) to remove protein and starch. TDF was precipitated with ethanol, and after washing and drying, the residue was weighed. The results were corrected for protein and ash contents.

In vitro digestibility tests. Total starch content was determined by the method of Goñi *et al.* (1997). In brief, a 50 mg sample was dispersed in 2M KOH to hydrolyse all the starch (30 min) and subsequently incubated (60°C, 45 min, pH 4.75) with amyloglucosidase; glucose content was then determined using the glucose oxidase/peroxidase assay (SERA-PAK™ Plus; Bayer de México, S.A. de C.V., Ciudad de México, Mexico). Total starch content was calculated as glucose (mg) × 0.9; potato starch was used as a reference. Potentially available starch content was assessed following the multienzymatic protocol of Holm *et al.* (1986) using heat-stable α-amylase and amyloglucosidase. In brief, the sample (300 mg, dry basis) was suspended in 20 ml of distilled water and incubated with heat-stable *α*-amylase in a boiling water bath for 20 minutes. This mixture was then diluted to 100 ml with distilled water. To 0.5 ml of this suspension, amyloglucosidase and 0.1M sodium acetate buffer (pH 4.75, 1.0 ml) were added. The mixture was incubated at 60°C for 30 min, diluted to 10 ml with distilled water and analysed for glucose using the glucose oxidase/peroxidase assay. The *in vitro* rate of hydrolysis was measured using hog pancreatic α-amylase according to Holm *et al.* (1985). Each assay was run with 500 mg of available starch. Predicted Glycemic Index (pGI) was calculated from the α-amylolysis curves, using the empiric formula GI = 39.21 + 0.803 H_{90} (r = 0.91, P < 0.05) established by Goñi *et al.* (1997).

Sensory evaluation. Fresh tortillas were evaluated in a sensory evaluation laboratory under white light for colour, flavour, taste, aroma intensity, and general acceptability on a 3 point hedonic scale in which score 1 represented the attributes most disliked and score 3 the attributes most liked. A panel of seventeen judges (untrained but familiar with corn tortillas) consisting of randomly selected students and staff members was used. Coded samples were presented on white plates. The judges were asked to rinse their mouths with tap water between the samples.

Statistical analysis. All determinations were carried out at least in triplicate and the values were averaged and given as the means with the standard errors of the means (± SEM). One-way analysis of variance (ANOVA) was used to analyse the data. Tukey's multiple range test at a significance level of $P \leq 0.05$ was used to identify significant differences in the means of the parameters measured. Statgraphic Plus™ version 5.1 and Microsoft Excel™ 2007 were used for all statistical analyses.

RESULTS AND DISCUSION

Proximate analyses

The moisture value in the chia seed-added tortilla decreased by 43.17–41.83% at all levels of substitution; however, there were no significant differences (P > 0.05) in relation to moisture content in tortillas made with different percentages of chia seed powder (Table 1). This pattern might be related to the hydrophobic character imparted by the oil present in chia seed. Rendón-Villalobos *et al.* (2009) showed that the reduction in the moisture content in tortilla resulted from increased levels of substitution of flax seed flour with corn. According to Agama-Acevedo *et al.* (2004, 2005) the moisture content in tortillas ranges between 35%

Table 1. Average chemical composition (%) of tortilla containing different ground chia seed levels

Sample	Moisture	Fat[1]	Protein[1,2]	Ash[1]	TDF
Control	47.12 ± 0.12^{a}	4.08 ± 0.11^{a}	9.01 ± 0.22^{a}	1.29 ± 0.11^{a}	16.45 ± 0.23^{a}
A	43.17 ± 0.28^{b}	6.25 ± 0.05^{b}	9.41 ± 0.30^{a}	1.34 ± 0.02^{a}	17.66 ± 0.33^{b}
B	43.33 ± 0.20^{b}	7.90 ± 0.01^{c}	9.98 ± 0.01^{a}	1.52 ± 0.02^{a}	21.28 ± 0.45^{c}
C	42.50 ± 0.29^{b,c}	9.45 ± 0.15^{d}	11.15 ± 0.59^{b}	1.64 ± 0.01^{a}	23.24 ± 0.32^{d}
D	41.83 ± 0.13^{c}	10.95 ± 0.15^{e}	12.48 ± 0.02^{c}	1.76 ± 0.05^{a}	25.71 ± 0.67^{e}

A = Tortilla + 5 % chia seed; B = Tortilla + 10 % chia seed; C = Tortilla + 15% chia seed; D = Tortilla + 20% chia seed

Results are means of three replicates ± SEM (n = 9); means with the same superscript letters within a column are not significantly different at P > 0.05 level by Tukey's multiple range test

[1]composition expressed on a dry weight basis

[2]N × 5.85

and 50%, depending on the conditions during the nixtamalisation. The maize variety used may also influence the final product moisture, since blue corn tortillas exhibit a lower moisture content (34.7%) than white tortillas (38.3%) prepared under identical nixtamalisation conditions (Hernández-Uribe *et al*. 2007). The addition of chia flour to NCF was expected to increase the protein content of the final product, since chia seed generally contains more proteins than cereals (Vuksan *et al*. 2007), and these might be important to increase the essential amino acid level that is deficient in corn. In fact, even at the lowest concentration chia tortillas were on average 9.41% richer in protein than the control. Significant increases were observed at all levels of substitution; however, 15% and 20% substitutions resulted in more evident changes. Chia seed flour has 20% higher protein level (Ayerza & Coates 1999) than that in the NCF (9.33%) (Agama-Acevedo *et al*. 2004), which influenced this parameter. Figueroa *et al*. (2001) observed a 3% protein content increase when the tortilla was fortified with 4% of defatted soy. There were no significant ($P > 0.05$) differences in the total ash content in either type of tortillas supplemented with different levels of chia powders (Table 1). The ash content found in the chia seed-added tortilla was higher than that reported for conventional tortillas (Agama-Acevedo *et al*. 2004). Islas-Hernández *et al*. (2007) reported similar values for tortilla fortified with amaranth, 1.4% in tortilla from 100% maize and 1.5% for corn/amaranth tortilla. The fat contents of the chia seed/tortilla were higher than that of the control (Table 1), showing an increase to 200%, that could be attributed to the high concentrations of oil content of the chia seed (Chicco *et al*. 2009; Coates & Ayerza 2009). This is important from the nutritional point of view, chia seed containing a short-chain omega-3 fatty acid, α-linolenic acid (ALA), which has been reported to be useful in the prevention and treatment of coronary artery disease, hypertension, and type 2 diabetes (Simopoulos 1999).

TDF content was significantly ($P < 0.001$) affected by chia powders substitution levels and showed an increase (56%). This implies that this product might be of interest for the food industry, considering its potential application as a functional ingredient in confectionery, bakery, or in the preparation of low-fat, high-fibre dietetic products. Chia seed is a good dietary fibre (DF) source (Reyes-Caudillo *et al*. 2008) and has become an important component in the daily diet. Due to this increase in TDF, chia seed-added tortilla might be considered a nutraceutical food. The high fiber content of chia seed may improve satiety, decrease energy intake, and promote weight loss (Vuksan *et al*. 2007). Dietary guidelines recommend a minimum daily intake of DF of 25 g (equivalent to 12.5 g DF per 1000 calories consumed), which is considerably higher than the estimated intakes in Western countries (Marlett *et al*. 2002). Therefore, there is a need to increase the fibre intake, which has prompted the consumption of dietary supplements or fibre-enriched food products. DF components like pectins, gums, cellulose, and others have been used as functional ingredients by the food industry, with an extensive market of food by-products as DF sources.

***In vitro* digestibility tests**

The total starch (TS) content decreased when chia seed flour was incorporated in the tortillas (Table 2). This effect is due to the low starch content present in this seed, which results in dilution of the starch in the composite tortilla. The TS values at 20% substitution were significantly lower among all levels of substitution ($P < 0.001$) as compared to the tortilla control. Previous studies from our laboratory showed similar TS pattern in tortillas with other oilseed added (Rendón-Villalobos *et al*. 2009), and similar TS values were determined in white corn tortilla (74.9%), but

Table 2. Total starch (TS) and available starch (AS) content of tortilla containing different ground chia seed levels

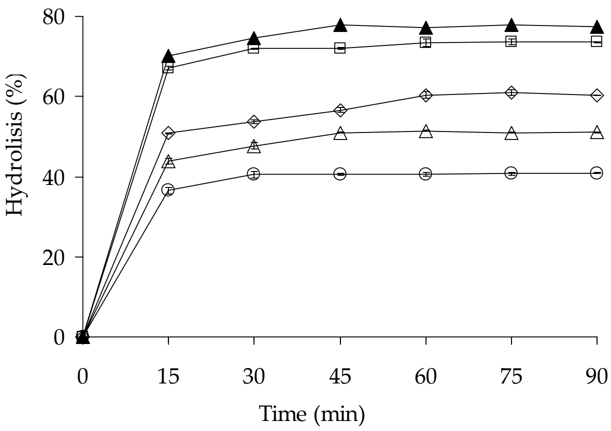
Sample	Total starch	Available starch
Control	74.94 ± 0.53^{a}	67.77 ± 0. 45^{a}
A	67.15 ± 0.57^{b}	65.76 ± 0.95^{b}
B	66.40 ± 0.49^{b}	63.51 ± 0.91^{c}
C	64.22 ± 0.37^{c}	61.66 ± 0.98^{d}
D	62.51 ± 0.45^{d}	58.99 ± 0.98^{e}

A = tortilla + 5% chia seed; B = tortilla + 10% chia seed; C = tortilla + 15% chia seed; D = tortilla + 20% chia seed

Results are means of three replicates ± SEM (n = 9); means with the same superscript letters within a column are not significantly different at $P > 0.05$ level by Tukey's multiple range test a lower amount was found in blue corn tortilla (68.5%) (Hernández-Uribe *et al.* 2007). Studies on laboratory-made tortilla reported TS levels in the range of 72% and 80% (Agama-Acevedo *et al.* 2004, 2005). The values of potentially available starch (AS) in the tortillas analysed ranged between 58.99% and 67.77% (Table 2) and showed the same tendency as TS, since they decreased when the chia seed flour level rose. These results show that the starch present in the tortillas is available for hydrolysis by the digestive enzymes used in this method, because it includes a boiling/Termamyl treatment that converts native resistant granules in digestible starch. The AS contents determined in chia seed-added tortilla were lower than those determined in tortilla prepared with nixtamalised corn flour (66–76%) (Agama-Acevedo *et al.* 2004) and in masa-made tortilla (67–76%) (Agama-Acevedo *et al.* 2005). The AS value is important due to its ability to supply glucose to the blood after the meal consumption. The consumption of chia seed-added tortilla might contribute to reduced carbohydrate intake and, consequently, it could be recommended for people with diabetes and/or overweight problems. In this sense, chia seed-containing tortilla might be considered a nutraceutical food. Fresh tortilla prepared with the blended corn/amaranth flours showed AS content of 73.3% which was lower than that determined in white corn tortilla (76.2%) (Islas-Hernández *et al.* 2007). A blend of tortilla with common bean exhibited a notably lower AS content (52.6%), suggesting that the incorporation of bean in tortillas reduces the blood sugar-raising potential of these traditional foods (Sáyago-Ayerdi *et al.* 2005).

Rate of enzymatic starch hydrolysis

The *in vitro* α-amylolysis reaction of control tortilla and the samples containing chia seed flour is represented in Figure 1. In addition, both tortilla control and chia seed-added tortilla curves showed no obvious peaks. At 15 min of reaction, the control tortilla exhibited 70% hydrolysis, but after 45 min, it was not different ($P > 0.05$) between control tortillas. The hydrolysis levels in all samples containing chia seed flour were significantly lower ($P < 0.0001$) from 30 min to 90 min, and exerted responses ($P < 0.02$) significantly different from one another. Regardless of the substitution level, the materials analysed presented hydrolysis



▲ with ground chia seed; □ 5%; ◇ 10%; Δ 15%; ○ 20%; error bars represent standard error of the means (n= 9)

Figure 1. *In vitro* starch hydrolysis of fresh NCF tortilla

indices that decreased when chia seed flour was incorporated in the tortillas as compared with the control tortilla. In this study, substituting NCF with 20% chia flour provided the lowest hydrolysis values of 36%, which resulted in the significantly slower digestion behaviour. Lower hydrolysis values (29–39%) were obtained for spaghetti with added chickpea flour, a result that seems to be due to the particularly slow digestion of the legume starch (Goñi & Valentín-Gamazo 2003). A pattern resembling that of control tortilla was found for amaranth-added tortillas, which exhibited hydrolysis values ranging between 75% and 80%, with no appreciable effect of the amaranth addition (Islas-Hernández *et al.* 2007). The rapid starch digestion behaviour of control tortillas was also observed by Tovar *et al.* (2003) and is an indicator of the effective gelatinising effect of nixtamalisation and tortilla making on corn starch. Maximum hydrolysis values between 72% and 80% were obtained in tortilla elaborated with nixtamalised corn flour (Agama-Acevedo *et al.*

Table 3. Predicted glycemic index (pGI) of tortilla containing different ground chia seed levels

Sample	pGI[1]
Control	101.41
A	98.29
B	87.65
C	80.20
D	72.06

[1]calculated from the equation proposed by Goñi *et al.* (1997)

Table 4. Sensory evaluation for control and chia tortilla

Sample	Colour	Flavour	Aroma	Taste	General acceptability
Control	2.96 ± 0.88^a	2.98 ± 0.65^a	2.88 ± 0.76^a	2.66 ± 0.77^a	2.84 ± 0.79^a
A	2.83 ± 0.10^a	2.94 ± 0.06^a	2.77 ± 0.11^a	2.53 ± 0.12^a	2.71 ± 0.11^a
B	2.29 ± 0.17^a	2.82 ± 0.10^a	2.70 ± 0.11^a	2.41 ± 0.15^a	2.56 ± 0.15^a
C	2.24 ± 0.16^a	2.88 ± 0.08^a	2.82 ± 0.10^a	2.24 ± 0.18^a	2.41 ± 0.15^a
D	2.12 ± 0.19^a	2.88 ± 0.12^a	2.70 ± 0.11^a	2.23 ± 0.17^a	2.47 ± 0.17^a

A = Tortilla + 5% chia seed; B = Tortilla + 1 % chia seed; C = Tortilla + 15% chia seed; D = Tortilla + 20% chia seed
Results are means of three replicates ± SEM (n = 17); means with the same superscript letters within a column are not significantly different at P > 0.05 level by Tukey's multiple range test

2004) and nixtamalised masa (Agama-Acevedo *et al*. 2005). Lower digestion rates were recorded in blue corn tortillas (58%) (Hernández-Uribe *et al*. 2007). Predicted glycemic indices (pGI) calculations were based on the 90 min degree of hydrolysis values of fresh tortillas (Goñi *et al*. 1997). The control sample showed a pGI of > 101 and those tortillas with added chia seed flour had much lower pGI values (72–98%; Table 3). The pGI indicates that chia seed-added tortilla may promote higher postprandial metabolic responses than standard corn tortillas. These results suggest that the intestinal glucose release after the consumption of chia seed-added tortilla might be lower than in the case when common white tortilla is ingested. In a recent study, fresh white corn tortilla had a pGI of 97.5 but the value for blue corn tortillas was significantly lower (85.8) (Hernández-Uribe *et al*. 2007). The tortillas prepared from different types of masa also exhibited greater pGIs than those recorded here (between 86.6 and 108.5) (Bello-Pérez *et al*. 2006). Taking into account that the composite tortilla also has a reduced AS content (Table 2), it may be concluded that this type of product represents a way to reduce the overall glycemic load (Foster-Powell *et al*. 2002), of a highly consumed traditional food item. Regarding GI, high GI diets are associated with high grade liver steatosis, particularly in insulin-resistant subjects (Valtueña *et al*. 2006), and a high intake of rapidly absorbed carbohydrate appears to play an important role in the risk of breast cancer in Mexican women (Lajous *et al*. 2005). Moreover, apart from the already cited benefits to metabolic variables, low GI diets have been often related to better cognitive and physical performances (Kirwan *et al*. 2001; Papanikolaou 2006).

Sensory evaluation

Tortilla prototypes were developed using a standard corn tortilla as the reference and modifying its formulation in the effort to include ingredients that are known to have a positive impact on the nutritional quality of the product. Tortillas were analysed for sensory acceptability, and the results are reported in Table 4. There were no significant differences (P > 0.05) in relation to the sensorial attributes between tortillas when the level of chia powder supplementation increased. The highest score corresponded to the most preferred tortilla for the corresponding attribute. The tortilla with 5% chia powder resulted as the most preferred prototype for all the parameters evaluated (colour, flavour, taste, aroma intensity, and overall acceptability), while the other prototypes were statistically equally acceptable (for all attributes) to a consumers panel. Generally, a "very good" flavour and aroma ("most liked") were been achieved for tortillas prepared with chia, indicating that the panelists preferred the inclusion of chia in maize tortillas compared to traditional tortillas; even though the incorporation of chia flour produced slightly dark products.

CONCLUSIONS

The effect of chia powder on some physicochemical and nutritional properties of chia seed flour-added tortilla was more significant with 15% and 20% substitutions. The *in vitro* digestibility of tortilla showed differences between the products, and the relative digestibility values appeared to be lower for chia powder added products. It is likely then, that these chia tortillas may lead to lower glucose liberation in the humans as well. However, *in vivo* measurements are needed to confirm the biological effects of the addition of chia to tortillas in human nutrition. According to the test panel, chia powder did not alter the sensory properties of tortillas. So far, we have developed and characterised a highly acceptable tortilla with a high nutritional value, regarding the most recent observation on the effects of low GI food items and high intake of dietary antioxidants. The uniqueness of our work is in having considered these two nutritional features at a time, thus creating a food product able to affect positively the health with two separate but strongly linked mechanisms, only very recently observed in the scientific literature.

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