Journal of
Food Quality

Journal of Food Quality ISSN 1745-4557

FORTIFICATION OF COMMERCIAL NIXTAMALIZED MAIZE (*ZEA MAYS* L.) WITH COMMON BEAN (*PHASEOLUS VULGARIS* L.) INCREASED THE NUTRITIONAL AND NUTRACEUTICAL CONTENT OF TORTILLAS WITHOUT MODIFYING SENSORY PROPERTIES

DANIELA TREVIÑO-MEJÍA, DIEGO A. LUNA-VITAL, MARCELA GAYTÁN-MARTÍNEZ, SANDRA MENDOZA and GUADALUPE LOARCA-PIÑA[1]

Programa de Posgrado en Alimentos del Centro de la República (PROPAC), Research and Graduate Studies in Food Science, School of Chemistry, Universidad Autónoma de Querétaro, Querétaro, Qro 76010, México

[1]Corresponding author.
TEL: +52-442-192-1304;
FAX: +52-442-192-1307;
EMAIL: loarca@uaq.mx

Received for Publication October 25, 2015
Accepted for Publication September 2, 2016

10.1111/jfq.12251

ABSTRACT

Common beans have been used to fortify maize tortillas increasing nutritional properties but affecting sensorial properties. The aim of this study was to evaluate the physicochemical and nutraceutical composition; and acceptability of tortillas formulated with maize and common bean. The physicochemical characterization showed no significant differences between common bean-fortified (CBMF) and maize (CMF) tortillas regarding texture, rolability and puffing. Nutritionally, CBMF had higher protein (10.89%) and dietary fiber (12.76%) levels than CMF tortilla (9.47 and 5.78%, respectively). Compared with CMF tortilla, CBMF had higher content of bound phenolics (2.13 and 1.84 mg eq. gallic acid/g, respectively). CBMF tortillas contained higher flavonoids concentration (62.59 mg eq. rutin/g) than CMF tortilla (33.73 mg eq. rutin/g). According to the sensory evaluation, there were no differences of general acceptance. The results suggest that the addition of bean to maize flour increased the nutraceutical value in tortillas without modifying their sensory attributes.

PRACTICAL APPLICATIONS

Tortillas are widely consumed in Latin American countries. Tortilla flour industry is well positioned in the market; however, looking for a healthier lifestyle, the consumption of tortillas is starting to decrease. The fortification of maize tortillas with common bean in the formulation proposed in this work represents an alternative of nutritional improvement for this product maintaining its sensory and technological properties.

INTRODUCTION

Tortillas are staple food developed by Mesoamerican civilizations. They are made from fresh ground nixtamal (masa) or nixtamalized corn flour (NCF). To obtain nixtamal, maize (*Zea mays L.*) is cooked in water with lime, steeped for 12–16 hours and then washed and ground to obtain the masa of which tortillas are made (Flores-Farías *et al.* 2000). This process induces nutritional changes in tortillas; for instance, it reduces the protein solubility of zein, major protein of maize with a low nutritional value, and also increases glutelin solubility (Paredes-López *et al.* 2008). Nixtamalization entails to an increase in maize amino acids bioavailability. Maize is deficient in lysine and tryptophan essential amino acids, resulting in a low nutritional quality protein (Mahan *et al.* 2014). This deficiency has led the attention of research to improve the nutritional value of tortillas by supplementing them with pulses flours among others, however, this supplementation decrease significantly consumers acceptance. For this reason, new formulations of pulse-fortified tortillas keeping their organoleptic properties are required. Common bean (*Phaseolus vulgaris* L.) is the most consumed pulse in Mexico and Latin America, along with maize; they constitute the base of Mexican diet (Hervert-Hernández *et al.* 2011). Common bean has lysine and tryptophan; therefore, corn and beans could form a blend to generate highly nutritious and healthy foods. The proteins of corn and beans complement each other, providing significant amounts of each respective limited amino acids. The maize tortilla and common beans have been considered as nutraceutical foods due to its content of bioactive compounds, including phenolic compounds, resistant starch, dietary fiber and bioactive peptides. Previous studies have used common bean as a fortifying ingredient in maize tortillas increasing their nutritional content, but also affecting undesirably their physical and sensory properties (Tovar *et al.*, 2003; Sáyago-Ayerdi *et al.*, 2005; Mora-Avilés et al., 2007; Grajales-García *et al*, 2012). Therefore, the objective of this study was to characterize the physicochemical and nutraceutical content as well as the acceptability, and physical properties of tortillas made with maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.) flours.

MATERIALS AND METHODS

Materials

Common bean cv. Bayo Madero was donated by the INIFAP, Mexico. Nixtamalized maize flour was obtained from Maseca® (GRUMA, San Pedro Garza, Monterrey, N.L., Mexico). Amyloglucosidase, protease, α-amylase, methyl red, standards raffinose, stachyose, verbascose, vainillin, (+)-catechin, Folin–Cioucalteau reagent, Trolox and ABTS radical were obtained from Sigma-Aldrich Co (St. Louis, MO). All other reagents were obtained from J.T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ) unless otherwise indicated.

Common Bean Flour (CBF)

Raw common beans were cleaned, rinsed, and then cooked using a method previously described (Aparicio-Fernández *et al.* 2005). Briefly, 500 g of non-soaked beans were placed in a beaker with 2500 mL of distilled water and boiled (94C) covered until they were suitable for consumption (~2.5 h), according to the finger compression test. An aliquot of 150 g of cooked beans (including broth) was freeze-dried, ground and stored for further analysis. The remaining cooked seeds and broth were transferred to a flat tray, spread until reaching 1 cm of thickness and allowed to dry for 12 h at 60C in a Felisa oven (Jalisco, Mexico). This dehydrated (dry heated) sample was ground and sieved through a 40-mesh Montinox (0.25 mm) screen; and stored protected from light in polyethylene bags at 4C until analysis.

Physicochemical Analysis of Maize and Common Bean Flour

Particle Size of the Maize/Common Bean Flour. Initially, several formulations of the blend of flours were tested in order to select the optimal ratio of the common bean and maize flours. The physicochemical properties (rollability, puffing and texture) were used for the optimization (data not shown). The optimal composition was 80% nixtamalized corn flour (NCF) and 20% common bean (CBF), the blend of these flours was called maize/common bean flour (CBFM). This formulation was used throughout the study. The size particle distribution of the CBMF and CBF were evaluated according to Bedoya and Rooney (1984). A 100 g of flour was placed in a vibratory column of US mesh (14, 20, 30, 40, 60, 80 and 100) (Ro-Tap® RX-29, W.S. Tyler, Mentor, OH) for 15 min. After agitation, the fractions retained in each of the different screens were separated and weighted.

Water Absorption Index (WAI). The method described by Anderson (1982) was used for this analysis with slight modifications. Briefly, 2.5 g of dehydrated flour was suspended in 30 mL of distilled water in a 50 mL centrifuge tube, stirred with vortex (1 min) and centrifuged at 3,000 × g for 10 min at 25C. The supernatant liquid was discarded and tubes were draining for 10 min on paper towel. Sample was weighted for WAI calculation. WAI is expressed as percentage of obtained gel per gram of dry solid (g/g).

Viscosity Profile. Viscosity profile was according to AACC standard program for RVA analysis (Approved Method No. 61-02; AACC 2000) with modification made by Ménera-López et al. (2013). A rheometer (Physica Anton Paar model MCR-101, Australia) was used to determine the viscoamilographic curve; 3 g of each sample were adjusted to obtain 14% moisture. Distilled water was then added to keep the total weight of water and sample constant at 18 g. The rotating paddles were held at 50C for 2 min to stabilize the temperature and ensure uniform dispersion, then heated to 92C at a rate of 5.6C/min and held constant at that temperature for 5 min. The samples were then cooled to 50C at 5.6C/min. A plot of time (h) versus viscosity (cP) was obtained.

Caloric Content. The caloric content were determined using a calorimeter DSC550 (Instrument Specialists Incorporated, Twin Lakes, WI) previously calibrated with Indium. A 3 mg of flour was weighted directly in aluminum capsules and distilled water was injected using a micro-syringe. After sealing the capsules containing the samples, they were equilibrated for 15 min at room temperature. The samples were heated from 30C to 120C in a heat rate of 10C/min. An empty capsule was used as reference. Results are expressed as Cal/g of product (1 Joule/g is equal to 0.24 Cal/g).

Physicochemical Analysis of Tortillas

Tortillas Elaboration. The masa was prepared mixing flour with water at 30C in a mixer (Blazer, Mexico) until obtain an adequate consistency. The masa was die-cut in a Die-cut Tortilla System (Casa Herrera, Mexico). Masa discs with 1.8 mm thick, 12.5 cm of diameter and 28 ± 2 g of weight were obtained. The masa discs were heated in a metallic plate 1.6 mm thick to $250C \pm 10C$ with cooking times of 17 s to form the thin layer of the tortilla, it was turned over and heated 50 s to form the thick layer, and turned again and heated 17 s to allow tortillas to inflate.

Puffing. Tortillas' puffing is a subjective test described by Figueroa *et al.* (2001), and consists in a visual test of the puffing of tortillas throughout their cooking. In order to report the puffing, a scale of 1 to 3 was used, where 1 corresponded to those tortillas with a complete puffing, 2 corresponded to those tortillas with an intermediate puffing, and 3 to those tortillas with no puffing.

Rollability. Since rollability is an important quality parameter in tortillas, the methodology described by Bedoya and Rooney (1984) was used, which determines the tortilla rollability according to their rupture. The test was performed after 30 min of tortillas elaboration. Tortillas were rolled in a glass stick of 2 cm diameter, and the degree of breakage was observed. A scale from 1 to 5 was used to evaluate tortillas rollability according to following parameters: 1 = 0%, 2 = 25%, 3 = 50%, 4 = 75% and 5 = 100%.

Texture: Tensile Strength and Cutting Force. *Tensile Strength.* This test simulates the resistance to rupture of tortillas with the hands. Three tortillas were selected randomly and a piece "I" shaped (8.7 cm long, 3.7 cm wide in the sides and 1.5 cm wide in the middle) was cut from the middle of the tortillas (Mauricio *et al.* 2004). The piece was fastened with two tension clamps (TA-96). The tension test was performed using a TA-XT2 (Texture Technologies Corp, United Kingdom) texture analyzer to a speed of 2 mm/s and a distance of 10 mm. Maximum tension strength until rupture of the sample was determined.

Cutting Force. This test simulates the cut of tortillas with human teeth. It was determined by sextuplicate with a texture analyzer (TA-XT2, Texture Technologies Corp, United Kingdom), using a 3 mm blade (TA-90), the blade speed was 2 mm/s and a deep of 15 mm.

The tensile strength and cutting force were expressed as the peak force (N) required breaking or cutting the strip.

Proximal Analysis. AOAC procedures were used to determine moisture (method 925.10), lipid (method 920.39), ash (method 923.03) and nitrogen (method 920.87) contents of the ground bean samples (AOAC 2002). Moisture was assessed based on weight loss after oven drying at 105C until constant weight was reached. Nitrogen content was determined using the micro-Kjeldahl method and equipment (RapidStill, Labconco, Kansas City, MO) with sodium sulfate as catalyst. Protein content was calculated as nitrogen × 5.85. Lipid content was obtained from Soxhlet extraction for 6 h with petroleum ether. Ash content was calculated from the weight of the sample after incineration in a muffle furnace at 550C for 5 h.

Nutraceutical Composition

Total Dietary Fiber. Dietary fiber fractions, containing soluble dietary fractions (SDF), and insoluble dietary fractions (IDF) were determined following the method proposed by Shiga *et al.* (2003). Briefly, 1 g of each sample was placed in different flasks, 50 mL phosphate buffer (0.08 mol/L, pH6) added, and pH adjusted to 6. Samples were placed in a water bath at 95C, 0.1 mL α-amylase added to each, and incubated for 30 min with manual stirring every 5 min. The flasks were cooled rapidly and the samples adjusted to pH 7.5. After the addition of 0.1 mL of protease (5 mg/mL phosphate buffer), samples were placed in a water bath at 60C for 30 min. Samples were cooled and the samples' pH was adjusted to 4. After pH adjustment, samples were placed in the water bath at 60C for 30 min, and 0.3 mL of amyloglucosidase was added. Samples were incubated for 30 min under constant agitation.

Then, 100 mL ethanol at room temperature were added at a 1:4 sample/ethanol ratio and the mixture was incubated at room temperature for 24 h. Samples were filtered at a constant weight and residues were washed three times with 10 mL of distilled water. The residues were then placed in an oven at 90C for 2 h and weighed. The TDF was determined gravimetrically and it was considered as PE. To quantify IDF, the ethanol was not added. The SDF was calculated by subtracting the IDF proportion from TDF.

Quantification of Resistant Starch. Resistant starch was quantified following the gravimetric method of Saura-Calixto et al. (1993). Briefly, polysaccharides extract (0.1 g) was homogenized with 6 mL of 2M KOH and placed in a shaker (Maxi Mix II, Thermolyne type 37600 mixer, San Francisco, CA) for 30 min at 25C under constant agitation.

Acetate buffer (3 mL, 0.4 M, pH 4.75) and 5 mL of 2N HCl were added and the pH adjusted to 4.75, if needed. Subsequently, 60 μL of amyloglucosidase were added and the tube placed in a shaking bath at 60C for 30 min. Sample was centrifuged (15 min at 3,000 × g) after incubation. The pellet was resuspended in 10 mL distilled water and centrifuged twice, freeze dried and weighed. The fraction obtained corresponds to resistant starch.

Oligosaccharides Quantification. Oligosaccharides were extracted from tortillas. The method was based on the procedure described by Diaz-Batalla *et al.* (2006). Tortillas samples (0.5 g) were homogenized in 10 mL distilled water and placed in a water bath shaker at 80C for 60 min. The extracts were recovered, frozen and lyophilized. To quantify the oligosaccharides, 7 mg of sample were dissolved in deionized water (1 mL), filtered and subjected to HPLC analysis. The sample (20 μL) was injected into an Agilent HPLC system model HP-1100 (Agilent Technologies, Inc., Santa Clara, CA) with a refractive index detector (IDR, 61362A) and fitted with a Zorbax NH2 precolumn (4.6 × 12.6 mm, 5 μm) and Zorbax column (250 × 4.6 mm). Water/acetonitrile (50:50) was used as mobile phase at 1 mL/min. Standard curves were determined using raffinose, stachyose and verbascose standards.

Free Phenolic Compounds Extraction. The extract was obtained according to the methodology described by Cardador-Martinez et al. (2002). Tortilla sample (1 g) was placed in a flask and 10 mL of methanol were added and shake for 24 h. The suspension was centrifuged to 5,000 rpm for 10 min. The supernatant was recovered and kept for further analysis and the residue were extracted for bound phenolic compounds.

Bound Phenolic Compounds Extraction. Bound phenolics were extracted according to Adom and Liu (2002) with slight modifications. The residue from free phenolic compounds extraction was suspended in 10 mL of 2 M NaOH at room temperature. Nitrogen was flush into the sample to displace the air in the sample tube. The sample was hydrolysated at 95C and 25C in a water bath shaker for 30 and 60 min, respectively. HCl was added to the sample tube in order to neutralize the sample and the resulting solution was extracted five times with 10 mL of ethyl acetate and the obtained volume was evaporated. The residues were suspended in 2 mL of methanol and stored at −20C until evaluation.

Total Flavonoids Quantification. Flavonoids were quantified according to the procedure described by Oomah *et al.* (2005). Briefly, 50 μL of the methanolic extract was mixed with 180 μL of methanol and 20 μL of a solution of 1% 2-aminoethyldiphenylborate in a 96-well microplate. The absorbance of the solution was monitored at 404 nm with a microplate reader (Thermo Scientific, Multiskan Ascent model 51118307). Extract absorption was compared with a rutin standard at concentrations ranging from 0 to 50 μg/mL. Flavonoid content was expressed as milligram rutin equivalent per gram of sample.

Condensed Tannins Quantifications. The condensed tannins, expressed as milligram (+)-catechin equivalents per gram of sample (mg/g), were analyzed according to the colorimetric method of Deshpande and Cheryan (1985) modified for use with a 96-well plate. Briefly, 200 μL of vanillin reagent (0.5% vanillin, 4% HCl in methanol) was added to 50 μL of methanolic extract and placed in a 96-well plate; each sample was tested in triplicate. Condensed tannins were quantified at 500 nm in a microplate reader (Thermo Scientific, U.S.A. Multiskan Ascent, model 51118307) using (+)-catechin (up to 0.2 mg/mL) as a reference standard.

Total Polyphenol Quantification. Total phenolic content was determined according to a procedure described by Singleton and Rossi (1965) with slight modifications (Farhat *et al.* 2013). A 500 µL of the methanolic extract was added to 460 µL distilled water. The solution was reacted with Folin–Ciocalteu reagent (1:10) for 4 min, and then 2 mL saturated sodium carbonate solution (about 75 g/L) was added into the reaction mixture. The absorbance readings were taken at 760 nm after incubation at room temperature for 2 h with a microplate reader (Thermo Scientific, Multiskan Ascent model 51118307). Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalent (mg GAE)/g of tortilla.

DPPH Radical Scavenging Activity. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a free radical used for assessing antioxidant activity. Reduction of DPPH by an antioxidant or by a radical species results in a loss of absorbance at 515 nm. Thus, the degree of discoloration of the solution indicates the scavenging efficiency of the added substance. Determination of antioxidant activity by the DPPH method was adapted for use with microplates. A solution of DPPH (150 µM) was prepared in 80% methanol. Samples (methanolic extract from section "Free Phenolic Compounds Extraction") or standard (20 µL) was added to a well in a 96-well flat-bottom visible light plate containing 200 µL of DPPH solution, absorbance at 520 nm was measured in a spectrophotometer (Spectramax Plus 384). The antioxidant activity was expressed as µmol of Trolox equivalent antioxidant capacity (TEAC) per gram sample by means of a dose-response curve for Trolox.

ABTS Radical Scavenging Activity. TEAC estimation was performed using the 2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay described by Loarca-Pina et al. (2010). A 20 μL of methanolic extracts from "Free Phenolic Compounds Extraction" was mixed with 230 µL of $ABTS^{+}$ (7 mM) radical solution. The absorbance was read at 570 nm at room temperature with a microplate reader (Thermo Scientific, Multiskan Ascent model 51118307). The measurement was performed in triplicate. The TEAC value was calculated employing a Trolox calibration curve and expressed as μmol of Trolox equivalents per gram of sample (μmol Trolox/g).

Sensory Evaluation

Tortilla squared pieces pre-heated at 45C were presented in one plate to taste. The testing panel was composed of 120 consumers (aged between 18 and 35 years) who were habitual tortilla consumers. Plain water was used as a palate cleanser between samples. The flavor, odor, color, texture and general acceptance were assessed by consumers. The consumers were asked to indicate his/her degree of liking/disliking using a 9-category hedonic scale (1 – dislike extremely to 9 – like extremely).

Statistical Analysis

The analyses were performed in at least three different experiments and results are expressed as the mean ± standard deviation and analyzed through ANOVA. Statistical significance was determined using Tukey HSD test ($\alpha = 0.05$). The statistical analysis for sensory evaluation was assessed using the Chi-square test (χ^2, $\alpha = 0.05$). All the statistical analysis was performed in the software JMP version 7.0.

RESULTS AND DISCUSSION

Physicochemical Analysis of Flours

Only the particle size of CBF was assessed since the commercial maize flour (CMF) was already characterized. The results are shown in Fig. 1. Around 96.8% of the particles in CBF passed through 60-US mesh screen, these results are within regulation levels according to Mexican regulation NMX-F046-S-1980, which indicates that the flours destined to tortillas elaboration must have at least 75% of particles ≤250 µm. The values obtained for WAI oscillated around 1.53 ± 0.04 and 1.50 ± 0.02 mL water/g of flour (Fig. 2). The CMF and CBMF showed no significant differences between CMF and CBFM. Both parameters, particle size and WAI play an important role on tortillas texture; smaller particles and a high WAI will provide tortillas with more flexibility and softness (Flores-Farías *et al.* 2000).

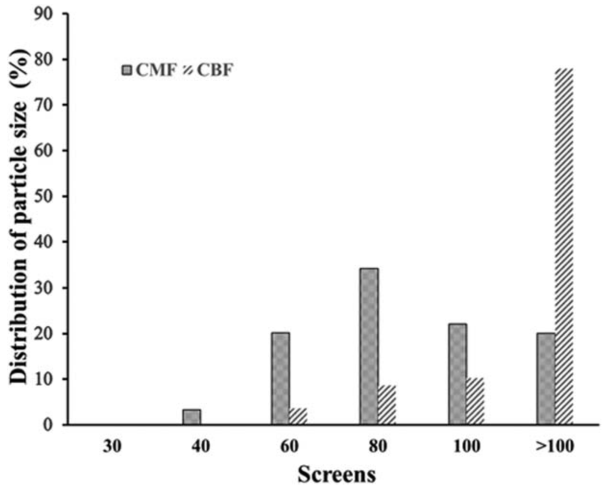

FIG. 1. PARTICLE SIZE CHARACTERIZATION OF COMMON BEAN FLOUR. CMF: COMMON BEAN/MAIZE FLOUR, CBF: COMMON BEAN FLOUR. THE UNITS OF X-AXIS ARE REPRESENTED IN US-MESH SIZE, WHICH MEANS THAT THOSE PARTICLES PASSING THROUGH THE 60 US-MESH WILL HAVE A SIZE SMALLER THAN 250 MICRONS.

Viscosity profile is an important parameter when determining the cooking variables of maize and its products. Pasting characteristics are used to predict their functional behavior during heating and cooling (Bello-Pérez and Paredes-López 2009). In Fig. 3 the viscoamylograph obtained for different flours including CBMF, CMF and CBF is shown. The pasting temperature, which is an indicative of the minimum temperature required for a correct cooking of the flours (Singh *et al.* 2009), was similar in the flours, therefore, the cooking conditions and equipment for maize tortillas will be appropriate for CBMF tortillas. Even though the pasting temperature was similar in the flours and the maximum viscosity was reached at similar times, the

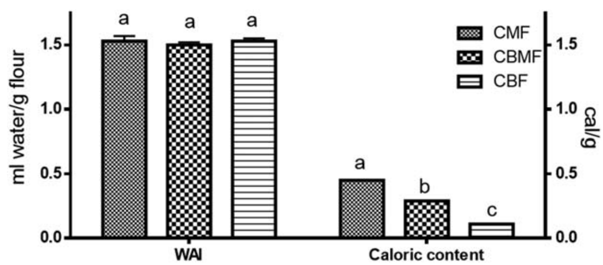

FIG. 2. WAI (WATER ABSORPTION INDEX, EXPRESSED AS ML OF WATER PER GRAM OF FLOUR) AND THERMAL PROPERTIES (EXPRESSED AS CALORIES PER GRAM) OF THE DIFFERENT FLOURS. CMF: COMMERCIAL MAIZE FLOUR, CBMF: MAIZE-COMMON BEAN FLOUR, CBF: COMMON BEAN FLOUR. THE RESULTS ARE EXPRESSED AS THE MEAN ± STANDARD DEVIATION OF THREE INDEPENDENT EXPERIMENTS. DIFFERENT LETTERS BY GROUP OF COLUMNS MEAN SIGNIFICANT DIFFERENCE ($P < 0.05$) AS DETERMINED BY TUKEY HSD TEST.

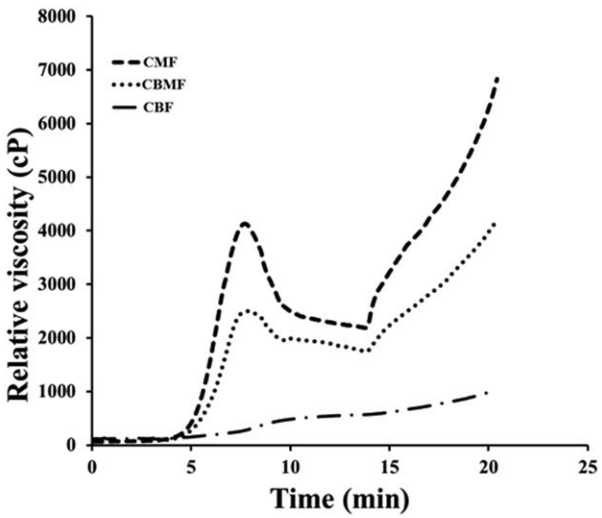

FIG. 3. VISCOAMYLOGRAPIC PROFILE OF COMMON BEAN AND MAIZE FLOURS. CMF: COMMERCIAL MAIZE FLOUR, CBMF: MAIZE/COMMON BEAN FLOUR, CBF: COMMON BEAN FLOUR.

incorporation of CBF in CMF represented a decreased of the viscosity in CBMF. This can be due to the amylopectin reduction in CBF and increasing of amylose, plenty found in common bean (Du *et al.* 2014). Indeed, it can be appreciated in CBF viscoamylograph that the maximum viscosity is low compared with the other flours, and slight rupture throughout heat-cooling cycles can be observed. According to Bello-Pérez and Paredes-López (2009), this indicates a restricted swelling of starch granules due to the high content of amylose.

On the other hand, a differential calorimetry scanning was performed using the flours; the calories provided for each flour are shown in Fig. 2. It can be observed that calories of CBMF were significantly lower than CMF; these results were expected since the caloric content of CBF had the lowest value. Nutritionally, this represents an advantage of tortillas made of CBMF.

Characterization of Tortillas

Tortillas yielded from 1.89 to 1.91 kg masa/kg tortilla, as shown in Table 1. As can be observed, there is no significant difference among treatments, which means that the addition of common bean flour does not affect the yield of tortillas. Weight loss is an important parameter when producing tortillas, if the weight loss is reduced; softer tortillas will be obtained (Figueroa *et al.* 2001). As well as in yield, CBMF tortillas were not significantly different to CMF tortillas (Table 1). These results are in accordance with those reported by Lecuona-Villanueva *et al.* (2012) for tortillas added with *Phaseolus lunatus* protein extract. Regarding puffing of tortillas, which is a desirable characteristic, CBMF tortillas had significantly higher values than CMF tortillas as can be observed in Table 1.

The rollability is a subjective measurement that provides information about the quality of tortillas to form rolls (Tacos) without fragmenting. Regarding rollability, values obtained for CBMF and CMF tortillas are shown in Table 1. As can be appreciated, CBMF tortillas had significantly higher values than CMF; the results suggested that as the content of CBF increased, the tortillas would become breakable. This could be due to the increase in the content of fiber when the CBF was added, according to Figueroa *et al.* (2001), an increase in the content of fiber will affect tortillas rollability. Moreover, results of texture analysis (cutting force and tensile strength) did not have significant differences as can be observed in Table 1. Although the addition of CBF to CMF produced more breakable tortillas compared only to CMF, the results of texture analysis indicated that at the moment of bite (simulated action of cut test) or rip with the hands (simulated action of tension test) the CBMF tortillas, no differences will be detected compared with CMF tortillas.

Proximal Analysis of Tortillas

In Table 2 the results of proximal analysis are shown. As expected, since common bean has higher values of protein than maize, the percentage of protein in CBMF tortillas increased significantly compared with CMF. These results represent a great advantage of the tortilla developed in this work since the addition of limited amino acids or various tryptophan- and lysine-rich protein sources to maize tortillas resulted in a significantly negative impact on sensory acceptability in previous works (Malovany *et al.* 2004).

Also, lipids content was decreased in the CBMF tortillas; this result was expected as the common bean has lower content of lipids.

TABLE 1. PHYSICAL CHARACTERIZATION OF TORTILLAS PRODUCED OF THE STUDIED FLOURS

	Weight loss (%)	Yield (kg masa/kg tortilla)	Puffing	Rolabillity	Tensile strength (N)	Cutting force (N)
CMF[1] Tortillas	24.67 ± 2.12^a	1.91 ± 0.09^a	1.6 ± 0.26^a	1.00 ± 0.00^b	1.03 ± 0.24^a	2.37 ± 0.22^a
CBMF[2] Tortillas	24.78 ± 1.07^a	1.87 ± 0.06^a	1.70 ± 0.19^a	1.38 ± 0.16^a	0.71 ± 0.06^a	2.01 ± 0.11^a

[1]CMF: Commercial maize flour.
[2]CBMF: Maize-common bean flour.
The results are expressed as the mean ± standard deviation of three independent experiments. Different letters within the same column mean significant difference ($P < 0.05$) as determined by Student *T* test.

TABLE 2. PROXIMAL COMPOSITION OF FLOURS AND TORTILLAS EVALUATED

	Ash (%)	Protein (%)	Lipids (%)	Carbohydrates (%)
CBF[1]	5.16 ± 0.21[a]	20.70 ± 0.31[a]	2.85 ± 0.48[c]	71.29 ± 0.43[c]
CMF[2]	1.20 ± 0.12[c]	7.85 ± 0.79[d]	4.71 ± 0.15[ab]	89.38 ± 0.23[a]
CMF Tortilla	1.26 ± 0.11[c]	9.43 ± 0.44[c]	6.45 ± 0.18[a]	83.67 ± 0.66[b]
CBMF[3] Tortilla	1.81 ± 0.03[b]	10.89 ± 0.42[b]	4.13 ± 2.18[bc]	84.25 ± 1.34[b]

[1]CMF: Common bean flour.
[2]CMF: Commercial maize flour.
[3]CBMF: Maize-common bean flour.
The results are expressed as the mean ± standard deviation of three independent experiments. Different letters within the same column mean significant difference ($P < 0.05$) as determined by Tukey test.

Nutraceutical Analysis of Tortillas

A nutraceutical composition analysis was performed in order to know the content of bioactive compounds of elaborated tortillas focusing mainly in two groups: carbohydrates and polyphenols.

Carbohydrates. The content of insoluble and soluble fiber is presented in Table 3. As can be seen, the CBMF tortillas fiber content was significantly higher than CMF tortillas. This increase in the fiber content in tortillas is in agreement with the reported results from Grajales-Garcia et al. (2012), who also reported an increase of fiber when common bean was added to maize tortillas. It is well known the importance and the multiple benefits of including fiber as part of the diet (Slavin 2013); furthermore, in *in vitro* and *in vivo* studies, the chemopreventive effects of common bean non-digestible fraction carbohydrates and peptides extracts have been demonstrated in colon cancer models (Campos-Vega *et al.* 2012; Luna Vital *et al.* 2014).

Resistant starch has been recognized as a promoter of large bowel health preventing bowel inflammatory diseases and colorectal cancer (Topping *et al.* 2003). In Table 3 the content of resistant starch of produced tortillas is shown. The addition of CBF to CMF resulted in a significantly increase of resistant starch content. Bello-Perez et al. (2014) evaluated starch digestibility *in vitro* of fresh and stored tortillas made with a commercial flour. They found a directly relation in the content of RS and glycemic index. The high content of dietary fiber in the cell wall of common beans provide resistance to degradation during cooking process; also, the presence of certain antinutrient factors contribute to the low digestion of common bean starch, increasing its content (Bello-Pérez and Paredes-López, 2009).

Regarding oligosaccharides, in Table 3 can be observed the content of stachyose, since verbascose and raffinose were not detected. Stachyiose concentration in CBMF was significantly higher than CMF tortillas; this correlates with the analysis of stachyose in the flours, having CBF considerably higher content of the oligosaccharide than CMF (data not shown). The presence of stachyose in CBMF tortillas increase its nutraceutical potential, as stachyose can be fermented by gut microbiota into short chain fatty acids (SCFA), mainly acetate, propionate and butyrate, recognized as regulators of colonic pH, to be substrates for colonocytes and to prevent the formation of aberrant crypt foci in *in vivo* models (Kumar *et al.* 2013).

TABLE 3. FIBER AND OLIGOSACCHARIDE CONTENT OF THE FLOURS AND TORTILLAS EVALUATED

Sample	IF[4] (%)	SF[5] (%)	TDF[6] (%)	RS[7] (%)	Stachyose (mg/g tortilla)	Verbascose (mg/g sample)	Rafinosse (mg/g sample)
CBF[1]	21.11 ± 0.04[a]	1.40 ± 0.21[b]	22.51 ± 0.18[a]	5.52 ± 0.06[a]	29.85 ± 2.30[a]	ND	ND
CMF[2]	4.24 ± 0.07[c]	0.99 ± 0.08[b]	5.23 ± 0.15[c]	1.04 ± 0.09[c]	1.50 ± 0.15[c]	ND	ND
CMF Tortilla	4.66 ± 0.08[c]	1.13 ± 0.16[b]	5.78 ± 0.07[c]	1.13 ± 0.08[c]	ND	ND	ND
CBMF[3] Tortilla	10.47 ± 0.52[c]	2.30 ± 0.21[a]	12.76 ± 0.72[b]	2.79 ± 0.11[b]	7.10 ± 1.11b[c]	ND	ND

[1]Common bean flour.
[2]Commercial maize flour.
[3]Maize-common bean flour.
[4]Insoluble fiber.
[5]Soluble fiber.
[6]Total dietary fiber.
[7]Resistant starch.
The results are expressed as the mean ± standard deviation of three independent experiments. Different letters within the same column mean significant difference ($P < 0.05$) as determined by Tukey test.

TABLE 4. PHENOLICS CONTENT OF FLOURS AND TORTILLAS EVALUATED

Sample	Total flavonoids (mg RE/mg)[4]	Bound phenolic compounds (mg GAE/mg)[5]	Free phenolic compounds (mg GAE/mg)	Total phenolics (mg GAE/mg)	Condensed tannins (mg CE/mg)
CBF[1]	24.54 ± 0.39[a]	4.38 ± 0.11[a]	4.88 ± 0.23[a]	9.26 ± 0.14[a]	5.99 ± 0.42[a]
CMF[2]	19.56 ± 0.70[bc]	1.84 ± 0.04[b]	5.26 ± 0.89[a]	7.10 ± 0.85[b]	4.67 ± 0.61[a]
CMF Tortilla	16.66 ± 0.17[c]	1.58 ± 0.09[c]	4.57 ± 0.14[a]	6.15 ± 0.10[b]	6.62 ± 0.51[a]
CBMF[3] Tortilla	19.97 ± 0.02[b]	2.03 ± 0.02[b]	4.64 ± 0.18[a]	6.67 ± 0.19[b]	5.16 ± 1.41[a]

[1]Common bean flour.
[2]Commercial maize flour.
[3]Maize-common bean flour.
[4]Milligrams equivalent of rutin per milligram of tortilla.
[5]Milligrams equivalent of gallic acid per milligram of tortilla.
[6]Milligrams equivalent of (+)-catechin per milligram of tortilla.
The results are expressed as the mean ± standard deviation of three independent experiments. Different letters within the same column mean significant difference ($P < 0.05$) as determined by Tukey test.

Polyphenols. The presence of total polyphenols in food is important since they have shown to exert physiological activities such as antioxidant, antimutagenic and antigenotoxic, among others (Cardona *et al.* 2013). In plants, certain polyphenols are able to interact with macromolecules of cell wall such as starch, cellulose, β-glucans and pentoses; however, they can be released through alkalis, acids or enzymatic treatments (Serpen *et al.* 2012). In order to assess the content of polyphenols of tortillas, both free and bound polyphenols were extracted. In Table 4 the content of bound, free and total polyphenols as well as total flavonoids and tannins is shown. Although common bean flour had higher content of total, free and bound polyphenols, no significant differences were found among CBMF and CMF tortillas. Regarding flavonoids content, the values in CBMF were significantly higher than CMF tortillas; this increase was in agreement with the results of the content of flavonoids in CBF which was higher than CMF, therefore, the increase of the content of CBF in CMF resulted in an increase of total flavonoid concentration. On the other hand, the concentration of condensed tannins was similar among flours showing no significant differences (Table 4).

Antioxidant Capacity. Two methods were performed to determine the antioxidant capacity of tortillas: DPPH and ABTS radical inhibition, which results are shown in Table 5. The results were slightly similar among methods, and it can be observed that the addition of CBF to maize tortillas resulted in an increase in the antioxidant capacity. The values of antioxidant capacity obtained were modestly higher than previous reported values for CBF (Ramírez-Jiménez *et al.* 2014). Antioxidant capacity is an important characteristic of food products, since it is well known for antioxidants to diminish or retard the formation of oxygen reactive species (ROS); the imbalance of the ROS concentration in human body has been related to cause oxidative stress, which in turn triggers non-communicable diseases (Gülçin, 2012). To counteract this, it is desirable in foods to increase the antioxidant capacity, goal achieved in this work.

Acceptability

In order to establish the acceptability of tortillas by consumers, a sensory evaluation was assessed. The results are presented in frequency bar plots (Fig. 4), which show the proportion of each one of 7 categories: 1 = Extremely

TABLE 5. ANTIOXIDANT CAPACITY OF ETHANOLIC EXTRACTS OF TORTILLAS AND FLOURS

	Bound phenolic compounds extract		Free phenolic compounds extract	
Sample	ABTS[5] (μmol/TEAC[4])	DPPH[6] (μmol/TEAC)	ABTS (μmol/TEAC)	DPPH (μmol/TEAC)
CBF[1]	4.37 ± 0.23[a]	4.14 ± 0.11[a]	2.82 ± 0.10[a]	1.46 ± 0.14[a]
CMF[2]	2.18 ± 0.001[c]	3.39 ± 0.27[b]	2.21 ± 0.08[b]	0.95 ± 0.06[b]
CMF Tortilla	3.17 ± 0.13[b]	2.29 ± 0.05[c]	2.03 ± 0.09[b]	0.93 ± 0.09[b]
CBMF[3] Tortilla	3.63 ± 0.32[ab]	3.58 ± 0.26[b]	2.16 ± 0.14[b]	1.06 ± 0.05[b]

[1]Common bean flour.
[2]Commercial maize flour.
[3]Maize-common bean flour.
[4]TEAC: Trolox equivalent antioxidant capacity expressed as μmol Trolox equivalents/g of tortilla.
[5]ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid).
[6]DPPH: 2,2-diphenyl-1-picrylhydrazyl.

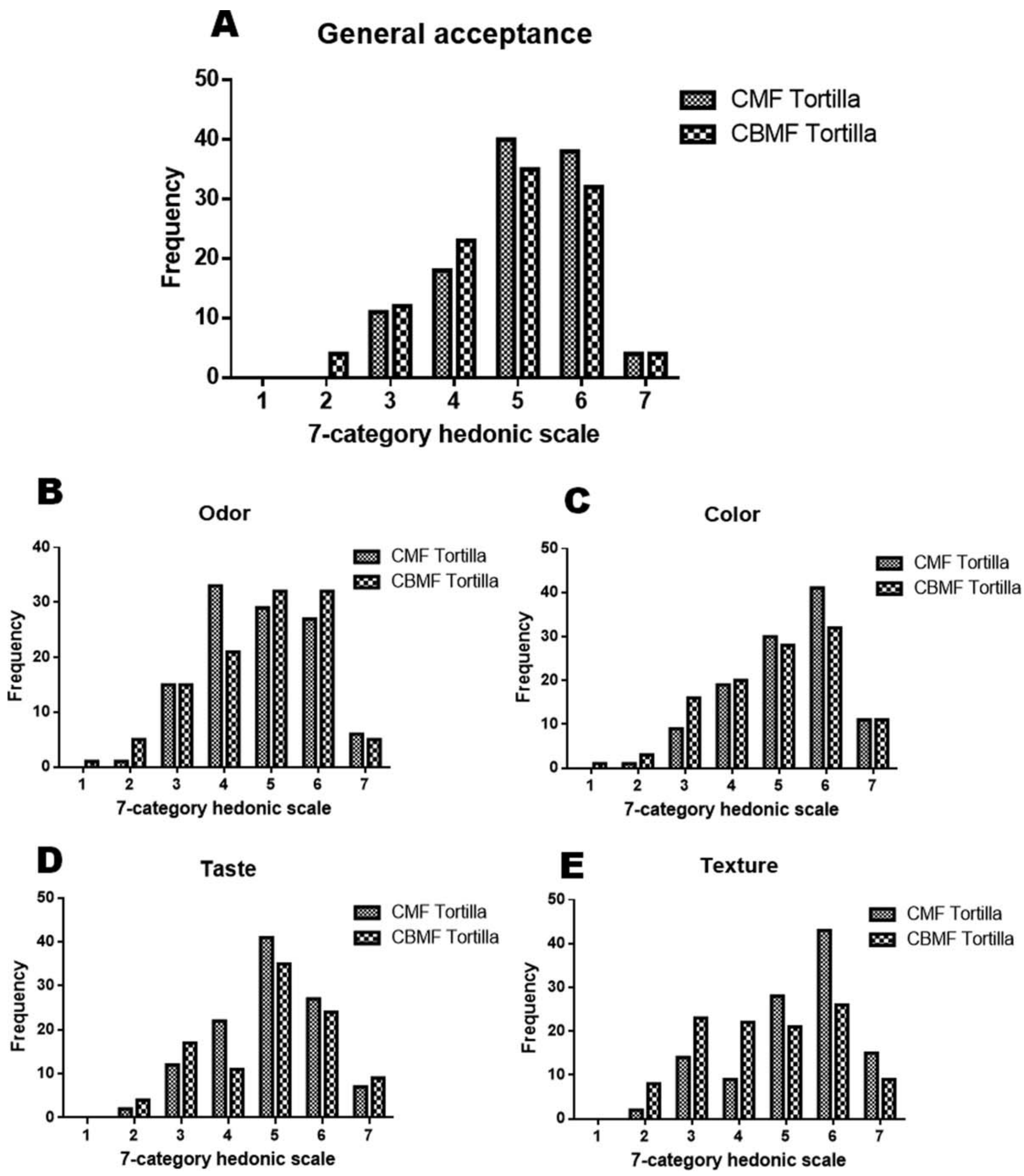

FIG. 4. FREQUENCY BAR PLOTS OF CONSUMER ACCEPTANCE REGARDING. (A) GENERAL ACCEPTANCE, (B) ODOR, (C) COLOR, (D) TASTE AND (E) TEXTURE. THE CONSUMERS WERE ASKED TO INDICATE HIS/HER DEGREE OF LIKING/DISLIKING USING A 7 CATEGORY HEDONIC SCALE (1 – DISLIKE EXTREMELY TO 7 – LIKE EXTREMELY).

dislike, 2 = Strong dislike, 3 = Slight dislike, 4 = not like, not dislike, 5 = Slight like, 6 = Strong like and 7 = Extremely like. In Fig. 4B it can be observed that regarding odor, the major proportion correspond to 4, 5 and 6, going from not like, not dislike, to strong like. Color evaluation showed that the major proportion corresponded to option 4: not like, not dislike (Fig. 4C). According to consumer's comments, tortillas are preferred in white color, therefore, the light brown color in CBMF tortillas were not totally accepted. Although it was not performed in this work, it could be corrected with a bleaching agent addition. The major proportion of taste and texture evaluation pointed to options 5 and 6, indicating that CBMF tortillas flavor and texture (Fig. 4D,E) were accepted in general terms. Moreover, the results of global acceptability (Fig. 4A) indicated that majority of consumers selected option 5: slight like.

CONCLUSION

To the best of our knowledge, this is the first report of a common bean added maize tortilla with enriched nutraceutical characteristics and good acceptability by consumers. The addition of common bean flour to maize tortillas resulted in a general improvement regarding their nutraceutical and nutritional composition. An increase of tortillas puffing, as well as an increase in the concentration of protein, soluble fiber, resistant starch, stachyose, total flavonoids content and antioxidant capacity was observed. Furthermore, a reduction of lipids content and calories was found. In the other hand, the most typical physicochemical properties of tortillas were not affected with the addition of common bean flour. The results suggest that our formulation provide a benefit to tortillas without affecting their organoleptic properties. Further studies will confirm the biological potential of the bean/maize tortilla in *in vivo* models.

ACKNOWLEDGMENTS

Authors D.T. and D.L. were supported by a scholarship from the Consejo Nacional de Ciencia y Tecnologia CONACyT-Mexico. This work was financially supported by the Consejo Nacional de Ciencia y Tecnologia (CONACYT)/Fondos Mixtos Queretaro Grant: QRO-2011-CO2-175184, Mexico.

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

REFERENCES

ADOM, K.F. and LIU, R.H. 2002. Antioxidant activity in grains. J. Agric. Food Chem. *50*, 6182–6187.

AMERICAN ASSOCIATION OF CEREAL CHEMISTS (AACC). 2000. In *Approved Methods of American Association of Cereal Chemists*, Method 22-08, Method 61-02, Method 76-21, 10th ed., AACC, St. Paul, MN.

ANDERSON, R.A. 1982. Water abosrption and solubility and amylograph characteristics of roll-cooked small grain products. Cereal Chem. *59*, 265–269.

APARICIO-FERNÁNDEZ, X., MANZO-BONILLA, L. and LOARCA-PIÑA, G. 2005. Comparison of antimutagenic activity of phenolic compounds in newly harvested and stored common beans Phaseolus vulgaris against aflatoxin B1. J. Food Sci. *70*, 73–78.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC). 2002. *Official Methods of Analysis Methods 920.87, 920.39, 923.03, 991.43*, 17th ed., AOAC, Arlington, VA.

BEDOYA, S. and ROONEY, L.W. 1984. Characteristics of U.S. and Mexican instant maize flours for tortilla and snack preparation. Cereal Food World *29*, 732–735.

BELLO-PÉREZ, L.A. and PAREDES-LÓPEZ, O. 2009. Starches of some food crops, changes during processing and their nutraceutical potential. Food Eng. Rev. *1*, 50– 65.

BELLO-PÉREZ, L.A., FLORES-SILVA, P.C., AGAMA-ACEVEDO, E., FIGUEROA-CÁRDENAS, J.D., LÓPEZ- VALENZUELA, J.A. and CAMPANELLA, O.H. 2014. Effect of the nixtamalization with calcium carbonate on the indigestible carbohydrate content and the starch digestibility of corn tortilla. J. Cereal Sci. *60*, 421–425.

CAMPOS-VEGA, R., GARCÍA-GASCA, T., GUEVARA-GONZALEZ, R., RAMOS-GOMEZ, M., OOMAH, B.D. and LOARCA-PIÑA, G. 2012. Human gut flora-fermented nondigestible fraction from cooked bean (*Phaseolus vulgaris* L.) modifies protein expression associated with apoptosis, cell cycle arrest, and proliferation in human adenocarcinoma colon cancer cells. J. Agric. Food Chem. *60*, 12443–12450.

CARDADOR-MARTÍNEZ, A., LOARCA-PIÑA, G. and OOMAH, B.D. 2002. Antioxidant activity in common beans (*Phaseolus vulgaris* L.). J. Agric. Food Chem. *50*, 6975–6980.

CARDONA, F., ANDRÉS-LACUEVA, C., TULIPANI, S., TINAHONES, F.J. and QUEIPO-ORTUÑO, M.I. 2013. Benefits of polyphenols on gut microbiota and implications in human health. J. Nutr. Biochem. *24*, 1415–1422.

DESHPANDE, S.S. and CHERYAN, M. 1985. Determination of phenolic compounds of dry beans using vainillin, redox and precipitation assays. J. Food Sci. *52*, 332–334.

DIAZ-BATALLA, L., WIDHOLM, J.M., CASTAÑO-TOSTADO, E. and PAREDES-LÓPEZ, O. 2006. Chemical components with health implications in wild and cultivated Mexican common bean seeds (*Phaseolus vulgaris* L.). J. Agric. Food Chem. *54*, 2045–2052.

DU, S.K., JIANG, H., AI, Y. and JANE, J.L. 2014. Physicochemical properties and digestibility of common bean (*Phaseolus vulgaris* L.) starches. Carbohydr. Polym. *108*, 200–205.

FARHAT, M.B., LANDOULSI, A., CHAOUCH-HAMADA, R., SOTOMAYOR, J.A. and JORDÁN, M.J. 2013. Characterization and quantification of phenolic compounds and antioxidant properties of *Salvia* species growing in different habitats. Ind. Crop. Prod. *49*, 904–914.

FIGUEROA, J.D., ACERO, G.M.G., VASCO, M.N., LOZANO, G.A., FLORES, A.L. and GONZÁLEZ, H.J. 2001. Fortificación y evaluación de tortillas de nixtamal. ALAN *51*, 293–302.

FLORES-FARÍAS, R., MARTÍNEZ-BUSTOS, F., SALINAS-MORENO, Y., CHANG, Y.K., HERNÁNDEZ, J.G. and RÍOS, E. 2000. Physicochemical and rheological characteristics of commercial nixtamalised Mexican maize flours for tortillas. J. Sci. Food Agric. *80*, 657–664.

GRAJALES-GARCÍA, E.M., OSORIO-DÍAZ, P., GOÑI, I., HERVERT-HERNÁNDEZ, D., GUZMÁN-MALDONADO, S.H. and BELLO-PÉREZ, L. 2012. Chemical composition, starch digestibility and antioxidant capacity of tortilla made with a blend of quality protein maize and black bean. Int. J. Mol. Sci. *13*, 286–301.

GÜLÇIN, İ. 2012. Antioxidant activity of food constituents: An overview. Arch. Toxicol. *86*, 345–391.

HERVERT-HERNÁNDEZ, D., GARCÍA, O.P., ROSADO, J.L. and GOÑI, I. 2011. The contribution of fruits and vegetables to dietary intake of polyphenols and antioxidant capacity in a Mexican rural diet: Importance of fruit and vegetable variety. Food Res. Int. *44*, 1182–1189.

KUMAR, M., HEMALATHA, R., KUMAR, R., NAGPAL, R., DEVRAJ, J.P., VERMA, V., BEHARE, P., MAL, G. and SINGH, B. 2013. Epigenetics, probiotic metabolites and colon cancer prevention: An overview of progress, opportunities and challenges. Med. Epigenet. *1*, 60–69. DOI:10.1159/000354719.

LECUONA-VILLANUEVA, A., BETANCUR-ANCONA, D.A., CHEL-GUERRERO, L.A. and CASTELLANOS-RUELAS, A.F. 2012. Protein fortification of corn tortillas: Effects on physicochemical characteristics, nutritional value and acceptance. Food Nutr. Sci. *3*, 1658–1663.

LOARCA-PIÑA, G., MENDOZA, S., RAMOS-GÓMEZ, M. and REYNOSO, R. 2010. Antioxidant, antimutagenic, and antidiabetic activities of edible leaves from Cnidoscolus chayamansa Mc. Vaugh. J. Food Sci. *75*, H68–H72.

LUNA VITAL, D.A., GONZÁLEZ DE MEJÍA, E., DIA, V.P. and LOARCA-PIÑA, G. 2014. Peptides in common bean fractions inhibit human colorectal cancer cells. Food Chem. *157*, 347–355.

MAHAN, A.L., MURRAY, S.C., CROSBY, K. and SCOTT, M.P. 2014. Characterization of quality protein maize germplasm for amino acid profiles and endosperm opacity. Crop Sci. *54*, 863–872.

MALOVANY, D., PACYNIAK, B., HANACEK, A. and CLARK, M.O. 2004. Tortillas: A new American staple. Cereal Food World *49*, 186–187.

MAURICIO, R.E., FIGUEROA, J.D., TAHA, S., REYES, M.L., RINCÓN, F. and MENDOZA, A. 2004. Characterization of maize accessions by grain and tortilla quality traits. Rev. Fitotec. Mex. *27*, 213–222.

MÉNERA-LÓPEZ, I., GAYTÁN-MARTÍNEZ, M., REYES-VEGA, M.L., MORALES-SÁNCHEZ, E. and FIGUEROA, J.D.C. 2013. Physicochemical properties and quality assessment of corn flour processed by a continuous ohmic heating system and traditional nixtamalization. CyTA-J. Food *11*, 8–14.

MORA-AVILÉS, A., LEMUS-FLORES, B., MIRANDA-LÓPEZ, R., HERNÁNDEZ-LÓPEZ, D., PONS-HERNÁNDEZ, J.L., ACOSTA-GALLEGOS, J.A. and GUZMÁN-MALDONADO, S.H. 2007. Effects of common bean enrichment on nutritional quality of tortillas produced from nixtamalized regular and quality protein maize flours. J. Sci. Food Agric. *87*, 880–886.

OOMAH, D., CARDADOR-MARTÍNEZ, A. and LOARCA-PIÑA, G. 2005. Phenolics and antioxidative activities in common beans (*Phaseolus vulgaris* L.). J. Sci. Food Agric. *85*, 935–942.

PAREDES-LÓPEZ, O., GUEVARA, F. and BELLO-PÉREZ, L.A. 2008. La nixtamalización y el valor del maíz. Ciencias *92*, 60–70.

RAMÍREZ-JIMÉNEZ, A.K., REYNOSO-CAMACHO, R., MENDOZA-DÍAZ, S. and LOARCA-PIÑA, G. 2014. Functional and technological potential of dehydrated *Phaseolus vulgaris* L. flours. Food Chem. *161*, 254–260.

SAURA-CALIXTO, F., GOÑI, I., BRAVO, L. and MAÑAS, E. 1993. Resistant starch in foods: Modified method for dietary fiber residues. J. Food Sci. *58*, 642–643.

SÁYAGO-AYERDI, S.G., TOVAR, J., OSORIO-DIAZ, P., PAREDES-LÓPEZ, O. and BELLO-PÉREZ, L.A. 2005. In vitro starch digestibility and predicted glycemic index of corn tortilla, black beans, and tortilla-bean mixture: Effect of cold storage. J. Agric. Food Chem. *53*, 1281–1285.

SERPEN, A., GÖKMEN, V. and FOGLIANO, V. 2012. Total antioxidant capacities of raw and cooked meats. Meat Sci. *90*, 60–65.

SHIGA, T.M., LAJOLO, F.M. and FILISETTI, T.M.C.C. 2003. Cell wall polysaccharides of common beans (*Phaseolus vulgaris* L.). Cienc. Tecnol. Aliment, Campinas *23*, 141–148.

SINGH, N., RICHA, B., RHYTHM, G., MUKTI, G. and JAGHMOHAN, S. 2009. Physico- chemical, thermal and pasting properties of fractions obtained during three successive reduction milling of different corn types. Food Chem. *113*, 71–77.

SINGLETON, V.L. and ROSSI, J.A.J.R. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. *16*, 144–158.

SLAVIN, J. 2013. Fiber and prebiotics: Mechanisms and health beneftis. Nutrients *5*, 1417–1435.

TOPPING, D.L., ANTHONY, M.F. and BIRD, F. 2003. Resistant starch as prebiotic and synbiotic: State of the art. Proc. Nutr. Soc. *62*, 171–176.

TOVAR, J., SÁYAGO-AYERDI, S.G., PEÑALVER, C., PAREDES-LÓPEZ, O. and BELLO-PEREZ, L.A. 2003. In vitro starch hydrolysis index and predicted glycemic index of corn tortilla, black beans (Phaseolus vulgaris L.), and Mexican “taco”. Cereal Chem. *80*, 533–535.