



Nixtamalised flour and tortillas from transgenic maize (*Zea mays* L.) expressing amarantin: Technological and nutritional properties

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ARTICLE INFO

Article history:

Received 23 July 2008

Received in revised form 2 September 2008

Accepted 4 September 2008

Keywords:

Transgenic maize

Amarantin

Nixtamalised flour

Tortillas

ABSTRACT

Nixtamalised flour from transgenic maize (genetically modified maize with the cDNA of amarantin) and its tortillas were evaluated for some technological and nutritional properties and compared to the commercial brand MASECA™. Nixtamalised transgenic maize flour (NTMF) showed higher protein content, total colour difference, pH, water solubility index, essential amino acids content and lower Hunter “L” value, water absorption index, resistant starch and retrograded resistant starch than MASECA™ flour. Tortillas from NTMF had higher protein content (12.64% vs 8.93%, db), essential amino acids content and calculated protein efficiency ratio (C-PER; 2.05 vs 1.04) than tortillas from MASECA™. Tortillas from both nixtamalised transgenic maize and MASECA™ flours showed similar sensory properties (puffing and acceptability). The use of transgenic maize for flour and tortilla preparation may have a positive impact on the nutritional status of people from countries where maize is the basic staple food.

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1. Introduction

The maize kernel contains 7–13% protein (db), but the quality of its proteins is poor because they are deficient in the essential amino acids lysine and tryptophan (Milán-Carrillo et al., 2006). Currently in Mexico, there is a consumption of 800 million tortillas per day, 60% of which are made of instant flour. Thus, because of their economic and basic foodstuff importance, breeding technology in this crop has been the subject of intense efforts resulting in several genotypes with improved agronomic, nutritional and nutraceutical characteristics. In Mexico, researchers at the International Maize and Wheat Improvement Center (CIMMYT) and the National Research Institute for Forestry, Agriculture and Livestock (INIFAP) developed 26 new cultivars and hybrids of quality protein maize (QPM), mainly for tropical and subtropical regions, which are similar in yield and agronomic properties to normal maize, but with an increased content of lysine and tryptophan (INIFAP, 1999).

Through biotechnological approaches, Rascón-Cruz, Sinagawa-García, Osuna-Castro, Bohorova, and Paredes-López (2004) successfully expressed the main seed storage protein of amaranth

(named amarantin) in the kernel of common maize; they obtained a transgenic maize with an increment in total protein (+32%) and the essential amino acids lysine (+18%), isoleucine (+36%) and tryptophan (+22%). Sinagawa-García et al. (2004) reported that recombinant amarantin expressed in maize kernels was successfully digested by simulated gastric fluid treatment; moreover, transgenic maize was unable to induce important levels of specific IgE antibodies in BALB/c mice, concluding that transgenic maize expressing the amarantin protein in the kernel is not an important allergenicity inducer. Nevertheless, additional physicochemical, functional and nutritional evaluations of this transgenic maize and its products (flours, tortillas, etc.) are required to determine its potential use and impact on human nutrition.

The objective of this research was to evaluate some technological and nutritional properties of nixtamalised transgenic maize flour (NTMF) and its tortillas and to compare them to those of the commercial nixtamalised maize flour MASECA™.

2. Materials and methods

2.1. Materials

Seeds of transgenic maize (genetically modified with the cDNA of amarantin) line 1041/1.7k were obtained of T₅ plants grown in

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the greenhouse of the Research and Advanced Studies Center, Campus Guanajuato, National Polytechnic Institute (CINVESTAV-IPN, México).

2.2. Physical characteristics

Thousand-kernel weight was determined by hand-counting 100 whole kernels and multiplying their weight by 10. Test weight (kg/100 l) was determined using a micro scale. A 1 l bowl was filled with grains and weighed with an analytical balance. The test weight was obtained multiplying the weight of the grains by 100. These values were calculated from 10 replicates.

2.3. Proximate composition

The following AOAC (1999) methods were used to determine the proximate composition: drying at 105 °C for 24 h for moisture (method 925.098); incineration at 550 °C for ash (method 923.03); defatting in a Soxhlet apparatus with petroleum ether for crude fat (method 920.39C); acid and alkaline hydrolysis for crude fibre (method 962.09); and micro-Kjeldahl for protein ($N \times 6.25$) (method 960.52). Carbohydrate content was estimated by difference. All determinations were made in triplicate.

2.4. Production of nixtamalised transgenic maize flour (NTMF)

Nixtamalised flour was prepared as recommended by Milán-Carrillo, Gutiérrez-Dorado, Cuevas-Rodríguez, Garzón-Tiznado, and Reyes-Moreno (2004). A 100 g lot of transgenic maize kernels was placed into a perforated (36 holes/cm²) nylon bag (17.0 cm × 12.5 cm) and cooked for 31 min in a lime solution [5.4 g of Ca(OH)₂/L of distilled water] at 85 °C using a 1:3 (w/v) ratio of grain to cooking medium, followed by a steep time of 8.1 h; nixtamalisation was finished by draining the cooking liquor (nejayote). Nixtamal (alkaline-cooked maize kernels) was washed with running tap water for 40 s and drained between paper towels. Wet nixtamal was dried at 55 °C for 12 h in a forced air oven and then cooled at room temperature for 30 min in a desiccator. Finally, dried nixtamal was milled (UD Cyclone Sample Mill, UD Corp. Boulder, CO, USA) to pass through a 80-US mesh (0.180 mm) screen, and packed in plastic bags. Nixtamalised transgenic maize flour (NTMF) was stored at 4 °C until it was used for further analysis.

2.5. Tortilla preparation/tortilla puffing

Tortillas were prepared by mixing 500 g of nixtamalised transgenic maize flour (NTMF) with water until an adequate consistency for producing tortillas was achieved. The fresh masa (30 g per tortilla) was rounded and shaped into the form of a flat disc using a manual machine. The masa discs were baked on a hot griddle at 290 ± 10 °C for 27 s on one side, followed by 30 s on the other side, and then turned them back on the first side until they expanded (puffing). Puffing of tortillas was evaluated observing the percentage of the total surface that puffed, using scores of 1–3; where 1 = little or not puffing (0–30%), 2 = medium puffing (30–70%), and 3 = complete puffing (70–100%). Five determinations were made for each treatment (Milán-Carrillo et al., 2006).

2.6. Sensory evaluation

Sensory tests were done by using a simple combination from two different experiments. Tortillas were made from either NTMF or from MASECA™ and evaluated 30 min after their preparation at room temperature (25 °C). A panel of 30 untrained judges (both sexes) was selected from students, faculty and staff (18–35 years

old) of the Faculty of Biological and Chemical Sciences of the Autonomous University of Sinaloa, Mexico. The screening and selection of the panellists was based on the participant interest, taste and odour acuity, and ability to understand the test procedures, besides that they all declared to enjoy eating tortillas and to consume them in a regular basis. The sensory evaluations were conducted in a room with controlled conditions of temperature (25 °C) and humidity (50–60%) and using day-light fluorescent lights. The panellists were seated in individual booths and the samples were presented monadically in random order. The judges were asked to rinse their mouths with water between samples. Samples were rated on a six points hedonic (0, dislike extremely; 5, like extremely) for one attribute: acceptability. Sensory tests were repeated three times on different days (Milán-Carrillo et al., 2006).

2.7. Water activity (a_w)

This parameter was determined in 5 g flour samples tempered at 25 °C, using a Hygrometer Aqua Lab Model CX-2 (Decagon Devices Inc, Pullman, WA, USA), which was previously calibrated with a potassium chloride saturated solution ($a_w = 0.841$ at 25 °C). Readings were taken after leaving the sample for 1 h to attain headspace equilibrium.

2.8. Total colour difference (ΔE)

The surface colour of the samples was measured using a Minolta colour-difference meter Model CR-210 (Minolta LTD, Osaka, Japan). The parameters L (0 = black, 100 = white), a (+ value = red, – value = green) and b (+ value = yellow, – value = blue) were recorded. The L , a , and b values of a white standard (std) tile used as reference were 97.63, 0.78 and –2.85, respectively. ΔE was calculated as $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$, where $\Delta L = L_{std} - L_{sample}$, $\Delta a = a_{std} - a_{sample}$, $\Delta b = b_{std} - b_{sample}$.

2.9. pH

This parameter was measured with a calibrated pH metre. Ten grams of each sample were suspended in 100 ml of boiled distilled water. The slurry was shaken (1500 rpm, 25 °C, 20 min) using an orbital shaker (Cole Parmer Model 21704-10, Cole Parmer International, Vernon Hills, IL, USA) (Gutiérrez-Dorado et al., in press).

2.10. Water absorption index (WAI) and water solubility index (WSI)

The methodology described by Anderson, Conway, Pfeifer, and Griffin (1969) was used. Each flour sample (2.5 g) was suspended in 30 ml of distilled water in a tared 60 ml centrifuge tube. The suspension was homogenised with a glass rod for 1 min at 25 °C, and centrifuged (3000g, 25 °C, 10 min). The supernatant was separated and placed into a tared evaporating dish. The WAI was calculated from the weight of the remaining gel and expressed as g gel/g solids (db). The WSI, expressed as g solids/g original solids, was calculated from the weight of dry solids recovered by evaporating the supernatant at 110 °C for 12 h.

2.11. Resistant starch (RS) and retrograded resistant starch (RRS)

Resistant starch was measured using the method described by Goñi, García-Díaz, Mañas, and Saura-Calixto (1996). The flour sample (100 mg) was mixed with 10 ml of a 0.2 M KCl–HCl solution (pH 1.5) and treated with 20 mg of pepsin for 60 min at 40 °C. The sample was mixed with 9 ml of 0.1 M Tris–maleate buffer (pH 6.9) and digested with 40 mg of α -amylase for 16 h at 37 °C. The residue collected by centrifugation (3000g, 15 min) was dispersed with 6 ml of 2 M KOH, mixed with 5.5 ml of 2 M HCl and

3 ml of 0.4 M acetate buffer (pH 4.75) and then digested with 80 µl of amyloglucosidase (A-9913, Sigma Chemical Co, St Louis, MO, USA) for 45 min at 60 °C in a shaking water bath. After centrifugation (3000g, 15 min), the amount of glucose released in the supernatant was measured spectrophotometrically (510 nm) using the peridochrom oxidase/peroxidase reagent (GOD-PAP, Ref. 676543, Boehringer, Ingelheim, Germany). The conversion factor from glucose to starch was 0.9.

Retrograded resistant starch was measured using the method described by Saura-Calixto, Goñi, Bravo, and Mañas (1993), which determines RS from insoluble dietary fibre. The sample (100 mg) was mixed with 10 ml of 0.08 M phosphate buffer (pH 6.9) and digested with 10 µl of thermo-stable α -amylase (Sigma A-3306) for 35 min at 100 °C. The pH of the sample was adjusted to 7.5 and then treated with 5 mg of a protease for 35 min at 60 °C. After adjusting the pH to 4.5, the sample was digested again with 60 µl of amyloglucosidase (Sigma A-9913) for 30 min at 60 °C. The insoluble dietary fibre was obtained after several steps of rinsing and centrifugation. The RS in the insoluble residue was dispersed with 6 ml of 2 M KOH and determined exactly as described in the previous paragraph.

2.12. In vitro protein digestibility (IVPD)

The method proposed by Hsu, Vavak, Saterlee, and Miller (1977) was used to determine IVPD. A multi-enzyme system, consisting of a mixture of porcine pancreatic trypsin type IX, bovine pancreatic chymotrypsin type II and porcine intestinal peptidase grade III (Sigma), was used. Flour samples and distilled water were used to prepare 50 ml of an aqueous protein suspension (6.25 g of protein/l) with pH adjusted to 8.0, while stirring in a water bath at 37 °C. The multi-enzyme solution was maintained in an ice bath and adjusted to pH 8.0. Five millilitres aliquots of the multi-enzyme solution were added with stirring to the protein suspension at 37 °C. The rapid pH drop was recorded automatically over a 10 min period using a pH metre. IVPD was calculated from the equation $IVPD = 210.46 - 18.10X$, where X = pH after 10 min.

2.13. Available lysine

Available lysine was determined as recommended by Hurrell, Lerman, and Carpenter (1979) using acid orange 12. This dye binds to lysine residues of proteins, which can be precipitated at low pH. In this procedure, equal amounts (0.5 g) of the same sample are treated in two different ways and defined as reading A and B. For reading A, the sample was mixed with 40 ml of orange acid solution [1.3626 g dye/l in phosphate buffer (0.2 M oxalic acid, 0.025 M potassium phosphate monobasic, 1 M acetic acid, pH 1.25)]. Reading B corresponds to a propionylation; the sample was mixed with 0.2 ml of propionic anhydride and 2 ml of phosphate buffer and the mixture was shaken at 400 rpm for 1 h at 25 °C with an orbital shaker (Cole Parmer Model 21704-10, Cole Parmer International, Vernon Hills, IL, USA). Fifteen millilitres aliquots from each reading were centrifuged (5000g, 25 °C, 15 min) and each supernatant was diluted 1:100 with distilled water. The absorbance was measured at 475 nm with a Spectronic 21D spectrophotometer model 1146 (Milton Roy, Ivyland, PA, USA); a standard curve for lysine was constructed. The amount of available lysine was obtained by subtracting reading B from reading A. All determinations were made in triplicate.

2.14. Amino acid analysis

Total amino acid composition was determined using the method described by López-Cervantes, Sánchez-Machado, and Rosas-Rodríguez (2006) with some modifications. Fifty milligrams of

flour were mixed with 10 ml of 6 M HCl and incubated for 24 h at 100 °C. The hydrolysed sample was filtered and the extract diluted 200 times with milliQ water. A 300 µl aliquot of the extract was dried and derivatized with 300 µl of 9-fluorenylmethyl-chloroformate (FMOC). A 20 µl aliquot was analysed using an analytical scale (4.6 mm × 250 mm) SGE Hypersil ODS C18 column (SGE, Dandenong, Australia) kept at 38 °C and connected to an HPLC system (GBC, Dandenong, Australia) equipped with a fluorescence detector LC 5100. The mobile phases used were as follows: (A) 30 mM ammonium phosphate (pH 6.5) in 15:85 (v/v) methanol/water; (B) 15:85 (v/v) methanol/water; and (C) 90:10 (v/v) acetonitrile/water. The flow rate was 1.2 ml/min and the gradient programme used was as reported in Table 1 by López-Cervantes et al. (2006). Fluorescence detection was at 270 and 316 nm for excitation and emission, respectively. A calibration curve was constructed using a mix of standard amino acids.

Tryptophan levels were determined using an alkaline hydrolysis. Twenty five milligrams of sample were mixed with 3 ml of 4.2 M NaOH and incubated in sealed tubes (N_2 atmosphere) at 120 °C for 4 h. After hydrolysis, the sample was adjusted to pH 9, washed with borate buffer (pH 9), vacuum filtered and then diluted to 50 ml with borate buffer. After centrifugation, the supernatant was filtered (0.45 µm) and then a 20 µl aliquot was analysed as described above. Tryptophan was detected at 280 nm with an ultraviolet detector.

2.15. Chemical score (CS)

The chemical score is a measure of protein quality based on the amino acid composition. It was calculated dividing the content of the limiting essential amino acid in the sample by the content of the same amino acid in the standard amino acid reference mixture. The value was calculated using the FAO amino acid scoring pattern for pre-school children (FAO/WHO, 1991).

$$CS = (\text{Content of the most limiting EAA/REAR}) \times 100$$

Table 1

Proximate composition and physicochemical, functional and nutritional properties of nixtamalised transgenic maize flour

Property ^{a,b}	NTMF ^c	MASECA ^m
<i>Proximate composition (% db)</i>		
Protein	12.73 ^a	8.98 ^b
Lipids	4.61 ^b	5.10 ^a
Ashes	1.63 ^b	1.75 ^a
Crude fibre	1.82 ^a	1.50 ^b
Carbohydrates	79.21 ^b	82.67 ^a
<i>Physicochemical</i>		
Colour		
Hunter "L" value	88.70 ^b	89.91 ^a
ΔE	13.55 ^a	12.13 ^b
a_w	0.40 ^b	0.50 ^a
pH	7.01 ^a	6.61 ^b
<i>Functional</i>		
WAI (g gel/g solids, db)	2.90 ^b	3.39 ^a
WSI (g solids/g original solids, db)	5.02 ^a	3.97 ^b
<i>Nutritional</i>		
Starch (% db)		
Resistant starch	1.2 ^b	1.6 ^a
Retrograded resistant starch	1.0 ^b	1.5 ^a
In vitro protein digestibility (%)	74.98 ^a	73.65
C-PER ^d	2.24 ^a	1.22 ^b

^a Means with the same letter in the same row are not significantly different (Duncan, $p \leq 0.05$).

^b a_w = water activity; ΔE = total colour difference; WAI = water absorption index; WSI = water solubility index.

^c NTMF = nixtamalised transgenic maize flour.

^d Calculated protein efficiency ratio.

Where CS is the chemical score; EAA is the essential amino acid and REAAR is the recommended essential amino acid requirement.

2.16. Calculated protein efficiency ratio (C-PER)

C-PER was calculated as described by Satterlee, Marshall, and Tennyson (1979) and summarised by the AOAC (1999). This procedure is based on using the IVPD and the essential amino acid (EAA) composition of the two different samples (nixtamalised transgenic and commercial MASECA™ flours) and tortillas from these flours.

2.17. Statistical analysis

The results were analysed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test comparisons among means with a significance level of 5%.

3. Results and discussion

3.1. Physical characteristics of transgenic maize kernels

The transgenic maize grain had 0.98 cm in length, 0.72 cm in width and 0.45 cm in thickness. The thousand-kernel weight and test weight of transgenic maize were 284 g and 77.68 kg/100 l, respectively. Physical dimensions of the grains are indicative of merchantable quality, handling and storage of foods; moreover, it is generally recognised that the physical characteristics are important factors influencing the nixtamalisation process and product characteristics (Rooney & Suhendro, 1999). Bockholt and Rooney (1987) recommended an "ideal kernel" for nixtamalisation with a thousand-kernel weight larger than 300 g and a test weight of 77.2 kg/100 l. The transgenic maize used in this study had smaller thousand-kernel weight and similar test weight than the "ideal kernel"; however, it showed good nixtamalisation characteristics and was suitable for producing nixtamalised transgenic maize flour (NTMF) and its tortillas.

3.2. Chemical composition, physicochemical and nutritional properties of nixtamalised transgenic maize flour (NTMF)

Table 1 shows the proximate composition and some physicochemical and nutritional properties of NTMF and commercial nixtamalised maize flour MASECA™. NTMF had higher ($p < 0.05$) protein content than MASECA™ (12.73% vs 8.98%, db). Gómez, Rooney, Waniska, and Pflugfelder (1987) reported that protein content of nixtamalised common maize flours is similar to that of raw corn (7–12%, db). Others reported protein contents of Mexican nixtamalised maize flours between 8.5% and 10.27% (db) (Bedolla & Rooney, 1984; Flores-Farías et al., 2000). Gutiérrez-Dorado et al. (in press) reported a protein content of 10.1% (db) for nixtamalised QPM flour. The highest protein in NTMF is due to the overexpression of the amarantin gene in the transgenic maize (Rascón-Cruz et al., 2004).

NTMF had lower ($p < 0.05$) lipid and ash contents, and higher ($p < 0.05$) crude fibre content than MASECA™ (Table 1). These results are in agreement with those reported by Gutiérrez-Dorado et al. (in press), Flores-Farías et al. (2000) for nixtamalised QPM flour and Mexican nixtamalised maize flours.

NTMF had lower ($p < 0.05$) Hunter "L" value (88.70 vs 89.91) and higher ($p < 0.05$) ΔE (13.55 vs 12.13) than MASECA™ (Table 1). Lime affects nixtamalised maize flours colour even if they are produced from white kernels; a high concentration of lime leads to a yellowish end product (Gutiérrez-Dorado et al., in press). Serna-Saldívar (1996) reported that some commercial nixtamalised maize flours for making tortillas are added with bleaching agents,

which may explain the slightly higher whiteness of MASECA. Bedolla and Rooney (1984) reported that nixtamalised maize flours with Hunter "L" values similar or higher than 82 are suitable for making tortillas.

The range of water activity values found (0.40–0.50) for NTMF and MASECA™ (Table 1) corresponded to those values where development of enzymatic activities, growth of microorganisms and chemical reactions occur very slowly, meaning a long shelf-life.

The pH values for NTMF and MASECA™ were 7.01 and 6.61, respectively (Table 1). Bedolla and Rooney (1984) reported that tortillas made from commercial nixtamalised maize flours from Mexico and USA with a pH between 7.1 and 7.2 had the "traditional alkaline flavour" preferred by the consumers. Flores-Farías et al. (2000) found that the pH of Mexican nixtamalised maize flours varied from 6.2 to 6.9. Gutiérrez-Dorado et al. (in press) reported a pH of 7.2 for nixtamalised quality protein maize flour.

NTMF had a lower ($p < 0.05$) water absorption index (WAI) than MASECA™ (2.90 vs 3.39 g gel/g solids, db) (Table 1). These WAI values are in the range (2.5–3.8 g gel/g solids, db) reported by other researchers for nixtamalised common maize flours (Flores-Farías et al., 2000; Gómez et al., 1987). Gutiérrez-Dorado et al. (in press) found a WAI of 2.4 g gel/g solids (db) for nixtamalised QPM flour. Campas-Baypoli, Rosas-Burgos, Torres-Chávez, Ramírez-Wong, and Serna-Saldívar (1999) indicated that after excessive heating, which might occur during nixtamalisation, starch granules lose their structure and integrity resulting in a gelatinized paste with higher WAI. Bedolla and Rooney (1984) reported that the WAI in nixtamalised maize flours depends on the protein content, pH, enzyme susceptible starch, and particle size. Furthermore, this property is related to the presence of natural gums from hydrolysis of the pericarp or additives (Flores-Farías et al., 2000). Water-soluble gums are added to the commercial nixtamalised maize flours to improve the water-binding capacity, which helps to retain the flexibility of tortillas during storage (Flores-Farías et al., 2000). The presence of additives (gums) could explain the higher WAI value of MASECA™ than NTMF.

MASECA™ had lower ($p < 0.05$) water solubility index (WSI) than NTMF (3.97 vs 5.02 g solids/g original solids, db) (Table 1). These values agree with the WSI range (4.4–7.2 g solids/g original solids, db) previously reported by other researchers (Flores-Farías et al., 2000; Gómez et al., 1987) for nixtamalised maize flours. Flores-Farías et al. (2000) reported that the WSI increases as the WAI decreases; our results showed that MASECA™ flour had the highest WAI value and the lowest WSI value, while the opposite was observed for NTMF. The WSI is a property that reflects the quantity of soluble solids in water, indicating the degree of cooking of the flours (Flores-Farías et al., 2000). Gómez et al. (1987) reported a little increment in WSI during the elaboration of nixtamalised maize flours, which implies that little solubilisation of starch, protein, or fibre components occur; however, some corn solubles are lost by leaching into the cooking water.

NTMF showed lower ($p < 0.05$) resistant starch (RS) content than MASECA™ (1.02% vs 1.6%, db) (Table 1). According to Saura-Calixto et al. (1993) the heat treatments of the grain during the nixtamalisation process may have promoted the interaction of starch with other components (proteins, lipids or itself) making it less accessible to enzyme hydrolysis. Milán-Carrillo et al. (2006) reported an increase of RS during the production of instant flours from quality protein maize by nixtamalisation. A high RS value could be important to elaborate maize products with a low caloric content that help to maintain a healthy intestine (Méndez-Montelvo et al., 2005). Non absorbed starch in the small intestine is fermented by the microflora of the large intestine, producing short chain fatty acids that have been associated with various health benefits (Lorraine, 2002). The physiological importance of RS has been also investigated in relation to a reduction of the glycemic

and insulinemic responses to a food, as well as hypocholesterolemic and protective effects against colorectal cancer (Asp, Van-Amelsvoort, & Hautvast, 1996). The RS called “resistant starch type III” or “retrograded resistant starch (RRS)” is formed during thermal processing of starch-rich foods (García-Alonso et al., 1999) and is composed mainly of retrograded amylose. This type of starch was not detected in raw transgenic maize (data not shown) while NTMF and MASECA™ had RRS contents of 1.0% and 1.5% (db), respectively (Table 1).

The *in vitro* protein digestibility values of proteins from NTMF and MASECA™ were 74.98% and 73.65%, respectively (Table 1). Gutiérrez-Dorado et al. (in press) reported an *in vitro* protein digestibility of 74.28% for nixtamalised quality protein maize flour. Wolzak, Bressani, and Gómez-Brenes (1981) reported that the response of different types of proteins to the multienzyme assay is different. They found highly significant correlations between *in vivo* and *in vitro* estimates for unprocessed vegetables samples, but this was not the case when these samples were thermally processed.

NTMF had higher ($p < 0.05$) calculated protein efficiency ratio (C-PER) than MASECA™ (2.24 vs 1.22) (Table 1). C-PER is an *in vitro* technique that relates the essential amino acid (EAA) scores of the sample to those of the reference protein casein. To obtain these EAA scores, each EAA is expressed as percentage of the FAO recommendations and corrected by the *in vitro* digestibility. The higher C-PER value of NTMF than MASECA™ can be attributed mainly to the larger essential amino acid content of NTMF (Table 2). The highest essential amino acid content in NTMF is due to the expression of the amarantin gene in the transgenic maize (Rascón-Cruz et al., 2004). Gutiérrez-Dorado et al. (in press) reported a C-PER of 2.08 and 1.68 for nixtamalised and raw quality protein maize flours, respectively. They attributed this effect to an increase in the rate of release of most of the essential amino acids due to nixtamalisation.

3.3. Essential amino acid content and chemical score of tortillas from nixtamalised transgenic maize flour

The essential amino acid (EAA) contents of tortillas from nixtamalised transgenic maize flour (NTMF) were higher ($p < 0.05$) than those of tortillas from MASECA™ (Table 3), which is in agreement with the better nutritional value of transgenic maize. The chemical score (CS) for proteins of tortillas from NTMF and MASECA™ were calculated based on the amino acid recommendations for

Table 3

Essential amino acids content and chemical score of tortillas from nixtamalised transgenic maize flour

EAA content ^{a,b} (g EAA/100 g protein)	Tortillas from NTMF ^c	Tortillas from MASECA™	EAA requirements (2–5 years) ^d
Histidine	4.21 ^a	2.52 ^b	1.9
Isoleucine	3.89 ^a	2.78 ^b	2.8
Leucine	17.5 ^a	13.05 ^b	6.6
Lysine ^e	4.12 ^a	2.24 ^b	5.8
Met + Cys	4.94 ^a	3.46 ^b	2.5
Phe + Tyr	11.12 ^a	7.61 ^b	6.3
Threonine	5.40 ^a	2.71 ^b	3.4
Tryptophan	0.95 ^a	0.55 ^b	1.1
Valine	6.32 ^a	4.19 ^b	3.5
Chemical score ^d	71	39	
Limitant EAA	Lysine	Lysine	

^a Means with the same letter in the same row are not significantly different (Duncan, $p \leq 0.05$).

^b EAA = essential amino acid.

^c NTMF = nixtamalised transgenic maize flour.

^d FAO/WHO (1991).

^e Determined according to Hurrel et al. (1979).

pre-school children (Table 3). The CS value was higher ($p < 0.05$) in tortillas from NTMF than in tortillas from MASECA™ (71% vs 39%) (Table 3). When compared to the amino acid scoring pattern for pre-school children (FAO/WHO, 1991), lysine was the limiting amino acid in tortillas from both flours. The proteins of tortillas from NTMF had deficiencies in lysine and tryptophan, although they had better CS than proteins of tortillas from MASECA™. Gutiérrez-Dorado et al. (in press) reported a CS value of 69% for tortillas from nixtamalised QPM flour, which is close to that obtained for tortillas from NTMF. These authors also reported that QPM tortillas had deficiencies in lysine and tryptophan, although the limiting amino acid was tryptophan whose content was significantly lower than that of tortillas from NTMF (0.76 vs 0.95 g/100 g protein). Even though CS is the most theoretically sound and commonly used chemical method, it does not take into account the bioavailability of amino acids or protein digestibility.

3.4. Protein content and physicochemical, nutritional and sensorial properties of tortillas made from NTMF

Tortillas from NTMF had higher ($p < 0.05$) crude protein content than tortillas from MASECA™ (12.64% vs 8.93%, db) (Table 4) reflecting the higher protein content of the transgenic maize used.

The tortillas from NTMF had a higher whiteness than those from MASECA™, as indicated by their higher ($p < 0.05$) Hunter “L” value (84.66 vs 80.77) and lower ($p < 0.05$) ΔE (17.5 vs 21.43) (Table 4). In both cases, the conversion of flour into tortilla decreased ($p < 0.05$) the Hunter “L” value and increased ($p < 0.05$) ΔE (Tables 1 and 4). However, this effect was more pronounced in MASECA™ than NTMF. Bedolla and Rooney (1984) reported that a dramatic decrease in whiteness and an increase in yellowness occur when the nixtamalised maize flours are processed into tortillas. They also reported that tortilla colour is a better quality index than the colour of the instant flour from which they are prepared. The tortillas made from NTMF and MASECA™ showed pH values of 7.05 and 6.78, respectively. Bedolla and Rooney (1984) reported that tortillas with pH between 7.1 and 7.2 retain better the characteristic flavour of the final product with acceptable shelf life.

The resistant starch (RS) content of tortillas from NTMF and MASECA™ were 2.4% and 3.2% (db), respectively; these tortillas showed retrograded resistant starch (RRS) contents of 1.63% and 1.57% (db), respectively (Table 4). The RS and RRS contents were higher ($p < 0.05$) in tortillas than flours (Tables 1 and 4). The increase in RS content is due mainly to the formation of RRS during

Table 2

Essential amino acids content and chemical score of nixtamalised transgenic maize flour

EAA content ^{a,b} (g EAA/100 g protein)	NTMF ^c	MASECA™	EAA requirements (2–5 years) ^d
Histidine	4.20 ^a	2.57 ^b	1.9
Isoleucine	3.88 ^a	2.88 ^b	2.8
Leucine	17.82 ^a	13.9 ^b	6.6
Lysine ^e	4.12 ^a	2.29 ^b	5.8
Met + cys	4.94 ^a	3.51 ^b	2.5
Phe + tyr	11.2 ^a	7.79 ^b	6.3
Threonine	5.41 ^a	3.03 ^b	3.4
Tryptophan	0.96 ^a	0.56 ^b	1.1
Valine	6.38 ^a	4.3 ^b	3.5
Chemical score (%) ^d	71	39	
Limitant EAA	Lysine	Lysine	

^a Means with the same letter in the same row are not significantly different (Duncan, $p \leq 0.05$).

^b EAA = essential amino acid.

^c NTMF = nixtamalised transgenic maize flour.

^d FAO/WHO (1991).

^e Determined according to Hurrel et al. (1979).

Table 4

Protein content and physicochemical, functional, nutritional and sensorial properties of tortillas from nixtamalised transgenic maize flour

Property ^{a,b}	Tortilla from	
	NTMF ^c	MASECA [™]
<i>Chemical composition (% db)</i>		
Protein	12.64 ^a	8.93 ^b
<i>Physicochemical</i>		
Colour		
Hunter "L" value	84.66 ^a	80.77 ^b
ΔE	17.5 ^b	21.43 ^a
pH	7.05 ^a	6.78 ^b
<i>Nutritional</i>		
Starch (% db)		
Resistant starch	2.4 ^b	3.2 ^a
Retrograded resistant starch	1.63 ^a	1.57 ^a
<i>In vitro</i> protein digestibility	77.36 ^a	76.80 ^a
C-PER ^d	2.05 ^a	1.04 ^b
<i>Sensorial</i>		
Puffing	3.0 ^a	3.0 ^a
Acceptability	4.0 ^a	4.03 ^a

^a Means with the same letter in the same row are not significantly different (Duncan, $p \leq 0.05$).

^b ΔE = total colour difference.

^c NTMF = nixtamalised transgenic maize flour.

^d Calculated protein efficiency ratio.

cooking, cooling, storage, and drying of tortillas (Gutiérrez-Dorado et al., in press). Agama-Acevedo et al. (2004) evaluated tortillas made from several commercial nixtamalised maize flours and found RS and RRS contents of 1.20–3.79% (db) and 1.29–2.84% (db), respectively. Flours with high RS content could produce tortillas with low glycemic index, given that the RS content increases when the tortilla is elaborated and stored (Rendón-Villalobos, Bello-Pérez, Osorio-Díaz, Tovar, & Paredes-López, 2002).

The *in vitro* protein digestibility (IVPD) of tortillas from NTMF and MASECA[™] were 77.36% and 76.80%, respectively (Table 4); this parameter increased ($p < 0.05$) when the flour was converted to tortilla (Tables 1 and 4). However, these *in vitro* values could differ from the *in vivo* values due to the poor correlation that has been reported between them in thermally processed vegetable samples (Wolzak et al., 1981). Gutiérrez-Dorado et al. (in press) found a higher IVPD value in tortillas from nixtamalised QPM flour than its corresponding flour, while the apparent *in vivo* protein digestibility was similar in both flour and tortillas. Other reports had indicated that *in vitro* and *in vivo* protein digestibility stays the same or decreases slightly during the baking of the masa to make tortillas (Mora-Avilés et al., 2007; Serna-Saldívar et al., 1988).

The C-PER of tortillas from NTMF was higher ($p < 0.05$) than that of tortillas from MASECA[™] flour (2.05 vs 1.04) (Table 4), which is in agreement with the results obtained for the flours. Gutiérrez-Dorado et al. (in press) reported a C-PER value of 1.85 for tortillas from nixtamalised QPM flour.

Tortillas made from NTMF and MASECA[™] showed similar ($p > 0.05$) puffing values (Table 4). This characteristic is very important in tortillas because it indicates whether the masa from which they are made had an adequate cooking grade or an inadequate proportion of time exposed to heat in both sides of the tortilla during the baking process. Tortillas made from NTMF and MASECA[™] had similar ($p > 0.05$) acceptability during the sensory evaluation. The panellists appreciated that the tortillas from NTMF and MASECA[™] had similar colour, aroma, taste and rollability, attributes that were considered for the evaluation of the acceptability of this product. The fact that the tortillas from NTMF had similar sensory properties to those from commercial flours such as MASECA[™] is important to take advantage of the high nutritional quality

of the transgenic maize, contributing to improve the nutritional status of people that consume tortillas as their main source of energy and protein.

4. Conclusions

Compared to the commercial nixtamalised maize flour MASECA[™], nixtamalised transgenic maize flour showed higher ($p < 0.05$) protein content, total colour difference, water solubility index, essential amino acids content and lower ($p < 0.05$) Hunter "L" value, water absorption index, resistant starch and retrograded resistant starch. Tortillas from nixtamalised transgenic flour showed higher ($p < 0.05$) protein content and essential amino acids content than those from MASECA[™] flour. Flour and tortillas from transgenic maize showed higher ($p < 0.05$) value of the nutritional indicator calculated protein efficiency ratio. The improved nutritional properties of NTMF and its tortillas with respect to MASECA[™] flour and its tortillas can be explained by the higher protein content and better protein quality of the transgenic maize expressing the amarantin gene. Tortillas made from both NTMF and MASECA[™] flours had similar ($p > 0.05$) sensorial properties. The use of transgenic maize for flour and tortilla preparation may have a positive impact on the nutritional status of people from countries where maize is the basic staple food.

Acknowledgements

This research was supported by grants from the Consejo Nacional de Ciencia y Tecnología (CONACyT – México, Project 44,665), Consejo Estatal de Ciencia y Tecnología (CECyT – Sinaloa), and the Programa de Fomento y Apoyo a Proyectos de Investigación (PROFAP) of the Universidad Autónoma de Sinaloa.

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