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The study of the quality parameters of the tortilla chips products formulated from mixtures of corn flour and legumes

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Abstract: Current study was designed to evaluate chemical composition and quality characteristics of tortilla chips made from corn flour with chickpea flour and red lentil flour in 20%, 50%, 80%, and 100%. The results suggested that moisture, and fat contents and acidity values increased with increasing levels of chickpea and lentil flour. However peroxide value increased with increasing levels of chickpea flour and decreased with increasing levels of lentil flour. Most studies that were carried out on tortilla chips made from corn flour and chickpeas revealed the presence of an unsaturated fatty acid content of up to 90%. Between 17.8 g of unsaturated fatty acids were present in each serving of tortilla chips loaded with chickpeas and samples. These may be monounsaturated or polyunsaturated, with approximately 50% being polyunsaturated. The two most important PUFA acids, linoleic acid and gamma-linoleic acid are essential fatty acids. In terms of the composition profile, the best possible results were achieved by samples that contain a 50% addition. In conclusion, it has been shown that corn flour incorporating chickpea is more nutritious than cornmeal alone. The purpose for which lentils and chickpeas were chosen is due to the high protein content and the entire nutritional composition.

Key words: Proximate composition, tortilla chips, fatty acids, mean relative concentrations, mycotoxins

1. Introduction

The popularity of ready-to-eat (RTE) snacks is increasing among consumers due to their suitability, nutritional value, appealing appearance, and widely acceptable taste (Harper, 1981). The increasing trend of including plant proteins in traditional and new food products has led to increased consumption of cereal-based snacks. Plant proteins have emerged as a promising ecofriendly alternative to corresponding animal food since the water and nitrogen footprints of plant growth are much less as compared to that of livestock farming (Sutton et al., 2018) and are less resource-intensive (Sinak et al., 2022). Some commonly used plant protein sources for human consumption include but not limited to maize, oats, peas, potato, walnut, barley, and wheat (Adenekan et al., 2018). The plant-based snacks global market value was US$44.02 billion in 2021 with an annual growth of up to 6.9% predicted to worth of US$ 80.53 billion by 2030 (Adenekan et al., 2018).

Maize is one of the most widely grown and consumed cereal and produced on an enormous area globally due to its multipurpose use (Erenstein et al., 2018). Global maize production in 2021 was over 1.2 billion metric t. Some maize varieties are preferred for tortillas preparation due to their physical features, such as color and texture (Cardenas et al., 2001). Tortillas are preferably prepared from maize varieties that are more widely utilized due to their color and texture (Gunasekaran et al., 2020). Tortillas are a staple diet for many people and the tortilla manufacturing sector consumes millions of tons of maize annually (Sinaki et al., 2020). Tortillas are often prepared with masa, which has been nixtamalized, or reconstituted nixtamalized maize flour. To nixtamalize maize, grains are soaked or cooked in water and then 1%–2% of $Ca(OH)_2$ per kilogram of maize is added (Chen et al., 2013). Removal of pericarp, partially gelatinizes the maize starch, and increases the calcium contents as much as 400%. Making masa into dough also improves its acceptability due to its altered rheological, physiological, and textural qualities (Erenstein et al., 2020; Cardenas et al., 2001).

The consumer acceptance of snacks is a qualitative one, and it involves sensory, instrumental, and microstructural characterization. Snacks should be evaluated by a panel

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of experienced tasters to provide an objective sensory rating and profile. An instrument's study of a product's texture may provide valuable insight into how people will perceive it (Erenstein et al., 2020; Cardenas et al., 2001; Serena-Saldivar et al., 2012; Pourafshar et al., 2015). The use of instruments to evaluate product textures provides a neutral, quick, and cheap way to assess the qualities of the finished goods (Vargas et al., 2017; Acevedo-Martinez et al., 2021; Bejosano et al., 2016). There is limited information on effects of mixing of various legume flours on the physical properties of snacks containing tortilla are unknown. In this study the effect of varying the proportion of commonly used legumes flour on the quality of tortilla product were studied, with the aim of obtaining new fortified, nutritionally improved products.

Through this study, the identification and finding of new forms of valorization of gluten-free cereals mixed with lentils and chickpeas, for the production of salty snack products "tortilla chips" and the technological progress regarding the production of these products, were pursued.

2. Materials and methods

2.1. Raw materials

The corn flour, chickpea flour, red lentil flour, high free fatty acid sunflower oil, and recrystallized salt used to manufacture the tortilla chips specimens were all sourced from the grocery store. Table 1 shows the seven types of flour combinations that were analyzed.

2.2 Methods used for physical-chemical analysis

2.2.1 Determination of moisture contents

Moisture contents were determined by previously reported method (Shalaby et al., 2018; Vlaic et al; 2019; Fagundes et al., 2018). The formula for calculating the moisture content, WH_2O, in grams per 100 g of the material is as follows: $WH_2O = \left(1 - \frac{m_1}{m_0}\right) \times 100$

Where

m_o - is the mass, in grams, of the test portion (g)

m_1 - is the mass, in grams, of the test portion after drying (g)

2.2.2 Determination of nitrogen and protein contents

The material was mineralized, and nitrogen was measured with Kjeltec®, a 2200 auto distillation apparatus (Ahmad et al., 2018; Accerbi et al., 1999; Ainsworth et al., 2007) based on Kjeldahl principle. In terms of mass fraction or grams per 100 mL, the amount of nitrogen present is equivalent to:

Nitrogen (%) = $\frac{(V0 - V1) * 0{,}0014 * 100}{m} * d$

Total protein content of the dry matter is expressed as a percentage and calculated as:

Total protein = Nitrogen (%)*6.25* $\frac{100}{100 - u}$

V0 – is the volume, in milliliters, of hydrochloric acid or sulfuric acid used in the blank test titration, mL;

V1 – is the volume, in milliliters, of hydrochloric acid or sulfuric acid used in the test portion titration, mL;

m– is the mass, in grams, of the test portion, g;

0, 0014 – is the titer, in moles per liter, of the hydrochloric acid or sulfuric acid; g/mL

u – Moisture of sample, in %;

6.25 – the conversion coefficient of nitrogen into proteins.

2.2.3. NaCl contents

Salt concentration was calculated by dissolving the sample (1 g) in water (200 mL). An aliquot (10 mL) was then poured into an Erlenmeyer flask and mixed with 5% K2CrO4 (1 mL). Titration volumes were recorded (Ainsworth et al., 2007; Akdogan 1996; Ali, 1996) while the samples were titrated with reaction mixture (0.1 N) until an orange-brown hue was sustained for 30 s. The NaCl percentage was calculated as:

NaCl (%) = (T × 0.00585 × D)/S × 100%

Where S is the weight of the sample in grams, T is the volume of the titration in mL, D is the diluted factor (total volume divided by titration volume), and 1 mm of 0.1 N $AgNO_3$ solution is equal to 0.00585 g of NaCl.

Table 1. Seven types of flour combination.

Sample	Code Sample	Corn flour (%)	Chickpeas flour (%)	Red lentil flour (%)
Tortilla chips corn flour (100%) - reference sample	TCP	100	-	-
Tortilla chips corn flour + chickpeas flour (50% + 50%)	TCN1	50	50	-
Tortilla chips corn flour + chickpeas flour (20% + 80%)	TCN2	20	80	-
Tortilla chips chickpeas flour (100%)	TCN3	-	100	-
Tortilla chips corn flour + red lentil flour (50% + 50%)	TCL1	50	-	50
Tortilla chips corn flour + red lentil flour (20% + 80%)	TCL2	20	-	80
Tortilla chips red lentil flour (100%)	TCL3	-	-	100

2.2.4. Calculating free acidity (FA) using an acid-base titration

Following the Official Methods of Analysis of an EC, the proportion of free fatty acids found in WCO was calculated as the oleic acid content. In a nutshell, 20 g WCO was added to 250 cc wide-mouth Erlenmeyer flasks (Alonso et al., 2000; Altan et al., 2008a) along with 50 cc of an ethanol/ethyl ether solutions (1:1 v/v) and a handful drops of phenolphthalein before being neutralized with 0.1 N KOH that had been standardized with benzoic acid. When a transition to a reddish-brown hue is seen the titration is complete. Two separate sets of calculations were done.

The following formula was used to determine the oil's acidity level in percent:

FA (%) =V × 0.1 N × 0.282m × 100

The following formula was used (Altan et al., 2008c) to determine the oil's acidity level in percent: where V is the volume of KOH used (in milliliter's), N is the normality of KOH (in nanometers), eqOl is the amount of oleic acid (in mill equivalents), and m is the mass of the WCO sample (in milligrams). The FA concentrations varied from 0.15% to 3.77%, with a mean value of 0.94% and a standard deviation of 0.79%.

Acidity is expressed in degrees of acidity; 1° of acidity represents the acidity of 100 g of sample that is neutralized with 1 cm^3 of sodium hydroxide, solution.

$$\text{Acidity} = \frac{V * 0{,}1}{m} * 100$$

In which:

V - Volume of the 0.1 n sodium hydroxide solution used in the titration, in cubic centimeters

M - Mass of the sample corresponding to the volume of filtrate taken for determination, in grams (5 g)

2.2.5 Peroxide concentration measurement

The POV was set to investigate (Alvarez et al., 1990) how palm oil's principal lipid oxidation products change over storage. Titrimetric analysis of POV was conducted in the same way as previously stated. To sum up, 5 g of oil sample was added to a saturate solution of potassium iodide to produce iodine, which was then titrated against a 0.1 N sodium thiosulfate solutions using starch as an indicator. The blank adjusted volumes of sodium thiosulfate required to discolour the solution was multiplied by the concentration of the thiosulfate and the quantity of oil used to get the POV. Point of view was given in units of meq O_2/kg. Oil samples were divided up and placed in the freezer to utilise later as aliquots for quality assurance purposes.

2.3 Chromatographic methods

2.3.1 Determination of fatty acid composition

Fatty acid profile of chips was established by using a gas liquid chromatographic system (model 7820A series, Agilent Technologies, Palo Alto, CA, USA) fitted with a flame ionization detector. A CP-Sil 88 (25 m 0.25 mm 0.20 mm) FAME column was used to determine the fatty acid content of the samples. The oven, detector, and injector temperatures were 210°, 240°, and 230°, respectively. At a splitting rate of 10:1, a sample (2 L) was administered (Alvarez-Martinez et al., 1998). Individual fatty acids were expressed as a proportion of total fatty acids.

2.3.2 Analysis of aroma compounds by the ITEX/GS MS technique

An in-tube extraction method (ITEX) was used to collect aroma volatile components from a sample before analyzing them using a GCMS QP-2010 gas chromatograph-mass spectrometer (Shimadzu Scientific Instruments, Kyoto, Japan). Briefly, the sample (3 g) was placed in a 20 mL headspace vial and incubated at 60 °C for 20 min. The volatile chemicals in the gas phase were absorbed using a fiber injector (ITEX- 2TRAPTXTA, Tenax TA 80/100 mesh) and desorbed straight into the GC-MS injector. The volatile chemicals were separated using a Zebron ZB-5MS (Phenomenex) capillary column, helium as the gas phase, a splitting ratio of 1:5, and a flow rate of 1 mL/min (Anderson et al., 1969). The mass spectra of the standard compounds were identified using the NIST27 and NIST147 mass spectral libraries, and their accuracy was verified using retention indices selected from these two sets of data. For the purposes of determining the total area of peaks (100%) and the comparative areas of the volatile compounds, only the peaks that were recognized in at least one of the three total ion chromatograms were considered.

2.4 ELISA methods

2.4.1 Determination of aflatoxin B1, DON, Fumonisin B1 & B2 and zearalenone

AFB_1, DON, FB1, FB2, and ZEN were extracted as reported already (Phuong et al., 2008; Athar et al., 2006). The mashed sample (25 g) was mixed with 100 mL of methanol: water (80:20), methanol: water (60:40), acetonitrile: water (84:16) and acetonitrile/water (50:50) for AFB_1, DON, ZEN and FB1& FB2 isolation, respectively. Aflatoxins were measured by HPLC (Zorbax SB-Aq column;4.6 × 150 mm, 5 µm; 30 °C) with mobile phase water/acetonitrile/methanol (5:1:1), flow rate 2 mL/min and postcolumn derivatization by Kobra cell and fluorescence detection (excitation & emission wavelength of 360 nm and 440 nm, respectively). Zearalenone was analyzed by HPLC (Hypersil ODS column; 2.1 × 100 mm, 5 µm; 30 °C) using a socratic mobile phase water/acetonitrile (64:36), and fluorescence detection (excitation and emission wavelength of 235 and 460 nm respectively). Fumonisins were first derivatized with naphthalene 2,3-dicarboxaldehyde and then separated by HPLC (Hypersil ODS column; 2.1 × 200 mm, 5 µm) using mobile phase acetonitrile/water/methanol/acetic acid (48/46/5/1) at a flow rate of 0.5 mL/min and fluorescence detection (excitation and emission wavelength of 420 and 500 nm respectively). Control samples were used for quality control of the routine analyses (Badrie et al., 1992; Bakalis et al., 1999).

2.4.2 Determination of gluten content – Veratox test for gliadin R5

Sample material (1.0 g) was combined with the extraction additive in a tube (Fisher Scientific, Pittsburgh, PA, USA) (Wang et al., 2021). Ethanol (10 mL; 60%) was added and stirred for 10 min. Each sample was diluted to 1:50 with phosphate buffered saline (PBS, Sigma, MO, USA) by adding 4.9 mL to the tube containing 100 L of the top layer of the extract after centrifugation for 10 min at 2500 g. Two to three h following dilutions, they were tested. Heat-treated samples (0.25% of the total mass) were placed in a tube, and 2.5 mL of the solution was added. A 30-s homogenization and 10-min incubation at 50 °C followed. After waiting three min, ethanol (7.5 mL; 80%) was added, as well as the mixture was shaken for an additional hour. The material was centrifuged for ten min at 2500 g after extraction. The sample was diluted 1:12.5 with PBS (2.3 mL) by adding it to 200 L of the top layer. After injecting about 150 L of blanks, standards, and samples into the red marked mixing well and transferring 100 L of each aliquot into the specific antibodies well, the red marked mixing well was withdrawn from the plate for 10 min of incubation. After removing the standard and sample solution from the antibody-coated well, the wells were washed with a wash buffer five times. Initially, 100 L of the conjugated was added to the well, and after 10 min, the plate was incubated and the conjugate was rinsed away five times (Wang et al., 2021). After incubating the well with 100 µL of substrate for 10 min, absorbance at 650 nm was measured using a micro plate ELISA reader (VersaMaxTM, Molecular Device, CA, USA).

2.5 Microbiological determinations

2.5.1 Horizontal method for the enumeration of yeasts and molds

Bengal Rose media (VWR Chemicals) was melted and chilled before being used for counting mold and yeast on Petri plates. Dilutions of 102, 103, and 104 of 0.1 mL were infected by surface spreading through a sterilized glass spreader. After five days at 25 °C oven, the plates were counted for their colony count and the final count was calculated using equation.

(UFC/g) N = $\frac{\Sigma c}{V(n_1 + n_2)d}$

In which:

Σc - The sum of the colonies counted on the plates chosen from two successive dilutions;

n1 - the number of colonies counted from the first dilution;

n2 - the number of colonies counted from the second dilution;

d - Primary dilution obtained (d = 0.1 for dilution 10-1 and d = 0.01 for dilution 10–2);

V - Volume of inoculum, in milliliters, applied to each plate.

2.5.2. Determination of Enterobacteriaceae - plate casting method ISO 21525-2

The sample (10 g) was sectioned by sterile scissors. Each sample contained 90 mL saline. A stomacher-type homogenizer was used for 3 min and the filtered substance was transferred to a sterile container for the first dilution (10–1). Inoculum/dilution (1 mL) was transferred to two sterile Petri plates. The VRBG medium (15 mL) was poured over the inoculum and homogenized. After solidification, the plates were flipped and incubated at 37 °C for 24 h. The pink-red-purple, halo-less colonies was counted. One colony from each plate was examined for oxidase reactivity. The oxidase-negative colonies were cultured for 24 h at 37 °C on glucose agar (Azevedo et al., 2003). Confirmed typical colony counts per dish were used to determine the quantity of Enterobacteriaceae in milliliter or per gram of the test material (Table 2).

2.6 The recipe for the preparation of tortilla chips

The recipe for the preparation of tortilla chips is shown step by step in Figure 1.

The tortilla chips formulations are described in Table 3. The flours used in this study are raw flours, not heat treated.

Tortilla chips recipe used flour of different grain size, according to the below Table 4.

2.7. Formulation of tortilla chips

2.7.1 Dough preparation

Automatic powder dispensers dosed the flour (Azevedo et al., 2003). Pump dispensers were used to treat the dough

Table 2. Criteria regarding the microbiological and hygiene standards stipulated in the Official Standards of Romania, Part I, No. 27/2011.

Food category	Yeasts and molds		Enterobacteriaceae	
	m	M	m	M
Derivatives from cereals (wheat flakes, corn, rice, expanded products)	10 cfu/g	100 cfu/g	1 cfu/g	5 cfu/g

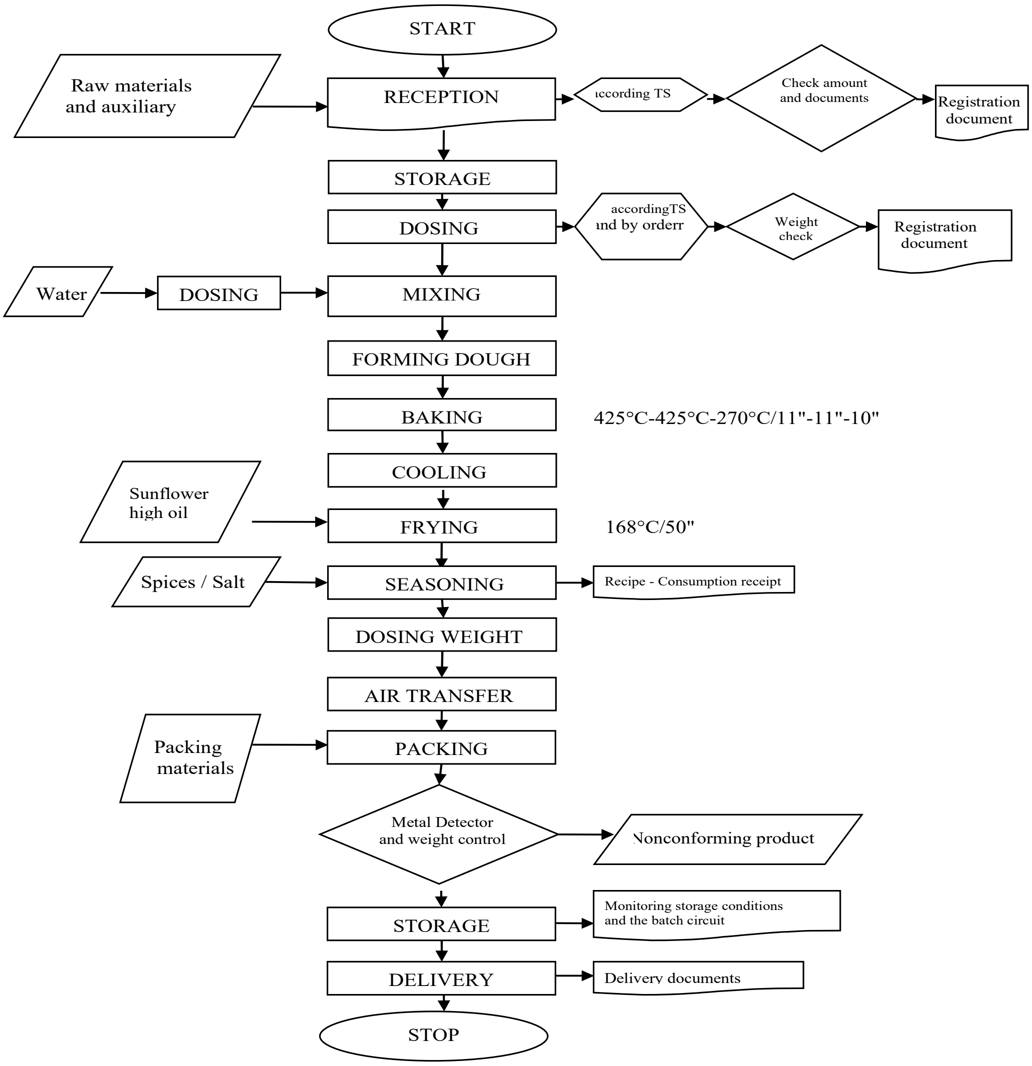

Figure 1. Flow chart of formulation of tortilla chips.

with water according to the formula for tortilla chips. The water temperature was 26—28 ˚C. The stainless-steel mixer equipped with a high-torque drive having a hydraulic mixer bowl was used for, 2–4 min depending upon the flour used. The dough was then deposited on rolling drums so the tortilla chip mold could carve off the triangle form. To get 6/6/5 cm chips,mechanically driven drums were used to get baked chips of 1.9 g/piece (Hassanien et al., 2014).

2.7.2 Cooking

Belts transfer uncooked chips into the oven. Uncut chips were fed into the oven via the top-level belt, and then moved to the center belt by the overturning conveyor belt. The bottom belt transported tortillas to the next machine. All levels independently regulated temperature. When the dough entered the oven, it was 49%–52% humid (Farca et al., 2015; Berto et al., 2015).

2.7.3 Cooling

Tortilla pieces release heat and moisture while cooling. Migration of vapors from middle to outer layers uniformly distributes moisture in the chip bulk. Increasing conveyor belt speed improves tortilla spacing but lowers chilling time. The belt was run slowly to help achieve chips moisture equilibrium (Farca et al., 2015; Berto et al., 2015).

2.7.4 Frying

The paddles immersed the product in 168 °C/50s high-oleic sunflower oil hot oil and conveyed it to the belt (Mohammad Reza et al., 2012).

Table 3. The recipe for making tortilla chips with different flour additions.

Sample	Sample cod	Flour quantity			Water quantity (l)	Mixing time	Baking time	Frying time	Weight of 1pcs tortilla
		Corn flour (%)	Chickpea flour (%)	Red lentil flour (%)					
Tortilla chips corn flour 100% -reference sample	TCP	100	-	-	62	4 min	11”–425 °C 11”–425 °C 10”–270 °C	50”–168 °C	1.9 g
Tortilla chips corn flour + chickpeas flour (50% + 50%)	TCN1	50	50	-	48	3 min	11”–425 °C 11”–425 °C 10”–270 °C	50”–168 °C	1.9 g
Tortilla chips corn flour + chickpeas flour (80% + 20%)	TCN2	20	80	-	48	2 min	11”–425 °C 11”–425 °C 10”–270 °C	50”–168 °C	1.9 g
Tortilla chips chickpeas flour (100%)	TCN3	-	100	-	48	2 min	11”–425 °C 11”–425 °C 10”–270 °C	50”–168 °C	1.9 g
Tortilla chips corn flour + red lentil flour (50% + 50%)	TCL1	50	-	50	50	3 min	11”–425 °C 11”–425 °C 10” - 270 °C	50”–168 °C	1.9 g
Tortilla chips corn flour + red lentil flour (80% + 20%)	TCL2	20	-	80	50	2 min	11”–425 °C 11”–425 °C 10”–270 °C	50”–168 °C	1.9 g
Tortilla chips red lentil flour (100%)	TCL3	-	-	100	50	2 min	11”–425 °C 11”–425 °C 10”–270 °C	50”–168 °C	1.9 g

Tortilla chips recipe used flour of different grain size, according to Table 4 given below.

Table 4. Tortilla chips recipe.

Flour granulation (%)	1000µm = max.2%
	710 µm = 0%–15%
	500 µm = 5%–10%
	355µm = 5%–15%
	250 µm = 15%–20%

2.7.5 Seasoning

In this step, a revolving drum ensured consistent spice dispersion. Spices and tastes were sprayed onto chips using an electromagnetic field (Slot et al., 2015).

2.7.6 Weighing and packing

Chips were transported into the measuring cups by elevator. The tortilla chips were wrapped in airtight bags to prevent spoilage after being cooled to room temperature. A loss of crispness might occur if the packing process is delayed for fried foods since they are particularly hygroscopic (they rapidly absorb and hold moisture). Tortilla chips when transferred into packaging machine contained roughly 1.5% moisture. The chips were weighed and placed in a sealed bag by the machine.

2.8 Statistical analysis

Statistics 8.1 was used for statistical evaluation utilizing a complete randomized design. A least significant difference test (5% probability) was used to compare treatments. A measure of the degree to which two or more variables are associated with one another is what is meant by the term “correlation”. When two variables are correlated, a change in the size of one variable relates to a change in the size of the other variable, either in the same direction (positive correlation) or in the opposite direction (negative correlation). In the context of a linear connection between two continuous variables, the word correlation is used, and its value is often stated as a Pearson product-moment correlation.

3. Results and discussion

3.1 Physical and chemical determinations

Table 5 shows the physico-chemical characteristics of the seven tortilla chips prepared from the mixture of corn flour with chickpea flour and red lentil flour in 20%, 50%, 80%, and 100% respectively. The reference tortilla chips contained 100% corn flour. The results suggested that moisture, and fat contents and acidity values increased with increasing levels of chickpea and lentil flour. However peroxide value decreased increased with increasing levels of chickpea flour and decreased with increasing levels of lentil flour (Guy RCE et al., 2003). The moisture contents decreased during the study year as well as with a corresponding increase of corn flour contents in the chips (Alonso-Miravalles et al., 2019).

The same decreasing trend was reported (Vogelsang-O'Dwyer et al., 2020) earlier for tortilla chips containing lentil and maize. Nutritionists recommend (Onwulata et al., 2001; Ghumman et al., 2015) the vegetable proteins for obtaining essential amino acids in a balanced diet. Addition of chickpea and lentil flour makes protein-deficient corn flour a protein-rich food. Tortilla chips with red lentil flour have a significant amount of high-quality protein, showing an increase from 4.25% from the control sample, to 22.32%, and 22.79% for corn flour and with 80% and 100% red lentil flour, respectively.

The reported differences among our study and previous studies may be due to the different variety of corn used in tortilla chips. Slightly higher protein contents for tortilla with 50% addition of chickpeas flour and 100% lentil flour were observed, i.e. 13. 97% and 22.79% as compared to previously reported of 13.38%. However, control sample in previous study had 4.25% protein content, with an increase of 9.36% by addition of chickpea flour, while in current study the increase of protein with 50% addition of chickpea is 13.79%. Decreased lipid contents from control sample (17. 6%) to the tortilla chips having 50% chickpea flour (15.85%) were observed. Same trend was reported by Morten et al. (2007). Increase of up to 2.11% and 1.02% with addition of chickpea flour and red lentil to the corn flour was observed.

The salt contents showed small, statistically nonsignificant differences. The salt content in tortilla with chickpea and red lentil flour mixture (DGF-Einheits methode et al., 2014) showed a slight increase of 1.35% and 1.17%, respectively as compared to control (1.12%). This increase is due to the relatively high mineral substances like NaCl in chickpea flour and it is greater than reported previously, up to 1.30% for corn flour samples, compared to the control sample.

Table 5. Physical and chemical determinations.

Sample	Sample Code	Moisture Content	Fat Content	Protein content	NaCl (g/100g)	Acidity (degrees /100 g)	Peroxid value (meq/kg)
flour (100%) - reference sample	TCP	2.43 ± 0.08^{AB}	17.6 ± 0.81^{AB}	4.25 ± 0.98^{C}	1.12 ± 0.66^{C}	0.18 ± 0.44^{C}	0.58 ± 0.69^{D}
Tortilla chips corn flour + chickpeas flour (50% + 50%)	TCN1	1.48 ± 0.63^{C}	15.85 ± 0.11^{C}	13.97 ± 0.81^{B}	1.35 ± 0.95^{A}	0.25 ± 0.59^{BC}	0.78 ± 0.77^{A}
Tortilla chips corn flour + chickpeas flour (20% + 80%)	TCN2	2.40 ± 0.37^{AB}	17.12 ± 0.14^{AB}	18.98 ± 0.84^{AB}	1.30 ± 0.97^{AB}	0.30 ± 0.32^{B}	0.70 ± 0.11^{BC}
Tortilla chips chickpeas flour (100%)	TCN3	2.47 ± 0.14^{AB}	17.97 ± 0.13^{A}	22.32 ± 1.03^{A}	1.28 ± 0.77^{AB}	0.35 ± 0.18^{AB}	0.72 ± 0.44^{B}
Tortilla chips corn flour + red lentil flour (50% + 50%)	TCL1	1.99 ± 0.18^{B}	15.28 ± 0.03^{C}	21.63 ± 0.96^{AB}	1.17 ± 0.66^{BC}	0.30 ± 0.25^{B}	0.60 ± 0.33^{CD}
Tortilla chips corn flour + red lentil flour (20% + 80%)	TCL2	2.14 ± 0.03^{B}	16.33 ± 0.05^{BC}	22.32 ± 0.48^{A}	1.25 ± 0.55^{B}	0.37 ± 0.31^{AB}	0.65 ± 0.48^{C}
Tortilla chips red lentil flour (100%)	TCL3	2.77 ± 0.73^{A}	17.03 ± 0.26^{B}	22.79 ± 0.18^{A}	1.30 ± 0.04^{AB}	0.39 ± 0.99^{A}	0.67 ± 0.22^{BC}
LSD 5%		0.38	0.92	6.33	0.08	0.07	0.05

Identical superscripts letters within columns indicate no significant difference ($p > 0.05$); * PM-control sample; Tortilla chips corn flour 100%-corn flour with 50% chickpea; corn flour with 100% chickpea flour; corn flour with 80%chickpea flour-corn flour with 50% red lentil-corn flour with 80% red lentil flour; corn flour with 100% red lentil flour.

The acidity increased from 0.25% for 50% chickpea flour to 0.39% for 100% lentil flour. The chickpea addition to corn flour, leads to an increase of 0.2 compared to control samples. These results are less than previously reported whereby an increase of 0.37% was observed by addition of the same percentage.

Higher peroxide values are reported than those obtained. These differences may be due to the different variety of chickpea and corn (Östbring et al., 2019).

The results shown in Figure 2 on Pearson correlations show a positive significance for PM with humidity. In the case of raw proteins, a positive correlation was identified with increased corn flour, regardless of variety. The same positive correlation was observed in the case of lipids, protein, fiber, acidity, NaCl, and energy value, for the samples with 50% addition of chickpea flour.

3.2 Chromatographic determinations of the tortilla chips

Table 6 shows eighteen saturated and unsaturated fatty acids in the tortilla chips. The saturated fatty acids (SFA) were palmitic acid, stearic acid, myristic acid, margaric acid, and arachidic acid while linoleic acid, oleic acid, gamma-linolenic acid, and palmitoleic acid were major unsaturated fatty acids (UFA). The highest values were shown by oleic acid. A decrease was observed with the addition of red lentil flour. There was a very significant positive correlation between the control sample and myristic, palmitic, oleic, arachidic, and palmitoleic acid, but a significantly negative correlation with the other three fatty acids. Addition of chickpea flour to the sample positively influenced (5% significant) the linoleic acid, and negatively for five other fatty acids. Similarly, 20% positive influences are found for oleic and gamma-linolenic acids. Significant positive correlations were observed in the case of samples with 20% addition for stearic, gamma-linoleic, and palmitoleic acid (Schlegel et al., 2020: Wang et al., 2001).

Previous studies (Schlegel et al., 2020; Wang et al., 2001; Arendt et al., 2020) on tortilla chips containing chickpea reported the presence of unsaturated fatty acids up to 90%. It is well-known that consumption of SFAs increases incidence of coronary heart disease, metabolic syndromes and changes in the intestinal microbiota, while the consumption of UFSs has health improving effects. The total quantity of UFAs in the tortilla chips with chickpea was 17.8 g. Linoleic acid (18:2, n-6) and gamma-linoleic acid (18:3, n-6), the main PUFAs, are essential fatty acids (EFAs) as they cannot be synthesized by the human body and must be assimilated from the diet. Tortilla chips having chickpea flour are an adequate source of EFAs. Saturated FAs contents in tortilla chips are less than 30% (Doğan et al., 2016) of all FAs and their quantity decreased with the addition of red lentil flour. Figure 3 presents confidence intervals of fatty acid contents.

In Table 7 mean relative concentrations (% from total peak areas) of volatile compounds analyzed by HS-ITEX/GC-MS technique are shown. Aroma is one of the key quality indicators for tortilla chips and plays a main role in overall flavor and consumer acceptance. Volatile compounds determine the aroma, odor and flavor of tortilla chips. The content and composition of tortilla chips depends upon composition. Usually alcohols, ketones, aldehydes, acids and other volatiles constitute the aroma of tortilla chips. Aldehydes are the dominant aromatic compounds in aroma that form and contribute to the characteristic flavor of tortilla chips. Aldehydes were the chief component of volatiles. Nonanal, benzene acetaldehyde and octanal were the key aldehydes and greatly influence the aroma due to their abundance. Ketones and

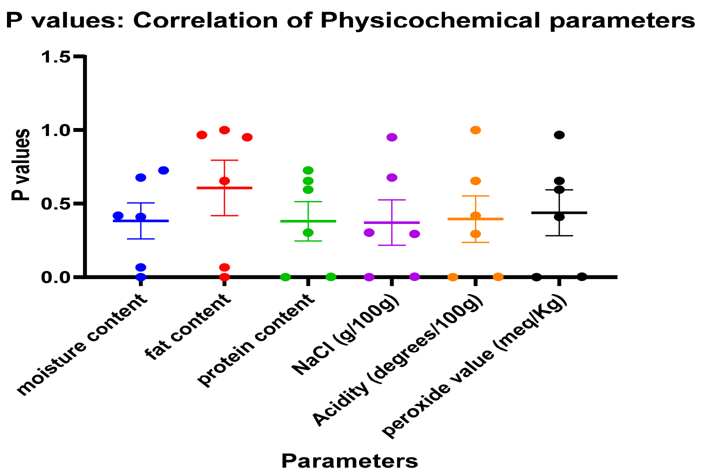

Figure 2. Pearson correlation between physical and chemical parameters.

Table 6. Fatty acid contents (g/100 g) of tortilla chips.

Fatty acid (g/100g)	Tortilla Chips corn flour (100%) - reference sample	Tortilla Chips corn flour+ chickpea flour (50% + 50%)	Tortilla Chips corn flour+ red lentil flour (50% + 50%)	LDS 5%
	TCP	TCN1	TCL1	
Total monounsaturated fatty acid (MUFA) composition	13.7 ± 0.13[b]	15.1 ± 0.20[a]	13.1 ± 0.48[b]	0.92
Total polyunsaturated fatty acid (PUFA) composition	1.5 ± 0.44[b]	2.7 ± 0.57[a]	1.5 ± 0.82[b]	0.63
Total Omega 3- fatty acids	<0.1 ± 0.11[a]	0.1 ± 0.13[a]	0.1 ± 0.55[a]	ns
Total Omega 6 - fatty acids	1.5 ± 0.27[b]	2.6 ± 0.38[a]	1.5 ± 0.61[b]	0.56
Total Omega 9 - fatty acids	13.5 ± 0.33[b]	14.9 ± 0.56[a]	12.9 ± 0.32[b]	1.01
C14:0 myristic acid	0.1 ± 0.92[a]	<0.1 ± 0.63[a]	<0.1 ± 0.72a	ns
C16:0 palmitic acid	1.0 ± 0.22[a]	1.0 ± 0.11[a]	0.7 ± 0.04[b]	0.15
C18:0 stearic acid	0.6 ± 0.45[a]	0.50 ± 0.36[b]	0.40 ± 0.11[c]	0.09
C18:1n9 oleic acid	13.4 ± 0.18[b]	14.8 ± 0.14[a]	12.9± 0.03[b]	0.83
C18:1n7 vaccenic acid	0.1 ± 0.24[b]	0.20 ± 0.81[a]	0.10 ± 0.74[b]	0.05
C18:1 total amount	13.6 ± 0.07[b]	15.0 ± 0.16[a]	13.0 ± 0.13[b]	0.88
C18:2 total amount	1.5 ± 0.80[b]	2.6 ± 0.61a	1.5 ± 0.38[b]	0.55
C18:2n6 linoleic acid (LA)	1.5 ± 0.44[b]	2.6 ± 0.96a	1.5 ± 0.57[b]	0.56
C20:0 arachidic acid	0.1 ± 0.97[a]	0.1 ± 0.54a	<0.1 ± 0.25[a]	ns
C22:0 behenic acid	0.2 ± 0.59[a]	0.2 ± 0.36[a]	0.1 ± 0.31[b]	0.05
C24:0 lignoceric acid	0.1 ± 0.13[a]	0.1 ± 0.16[a]	0.10 ± 0.89[a]	ns
Total saturated fatty acids (SAFA)	1.9 ± 0.26[a]	1.7 ± 0.51[a]	1.4 ± 0.92[b]	0.22
Total trans fatty acids	<0.1 ± 0.83[b]	<0.1 ± 0.13[b]	<0.20 ± 0.18[a]	0.05

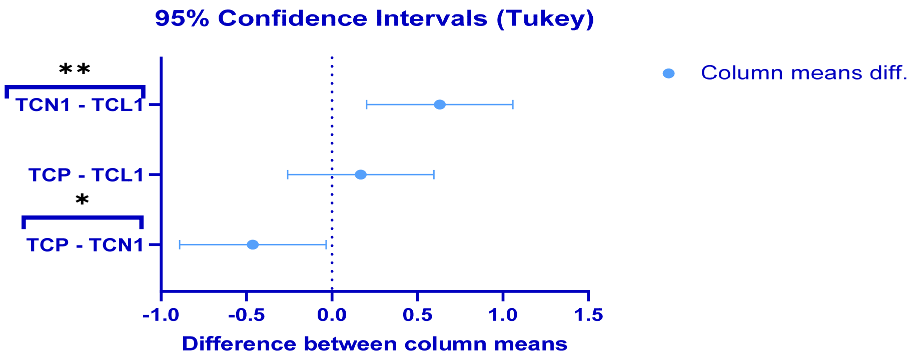

Figure 3. Confidence intervals of fatty acid contents.

furans were the second and third most abundant volatiles respectively in this study. Ketones have a floral and fruity sweet flavor (Odeyemi et al., 2018). 1-hexanol followed by 1-pentanol were key alcohols. Our results are different from previous studies due to different raw material used for tortilla chips (El-Shayeb et al., 2017; Buttery and Ling, 1995). Tortilla chips with chickpea showed maximum volatile contents. The advantages of HS-SPME-GC/MS for the analysis of volatile compounds in corn flour are well-known; however, it may be challenging to optimize the method when numerous factors are involved. Hence, multivariate optimization techniques may be required to lessen the intricacy of method optimization. Figure 3 shows 95% confidence intervals (CI) for different types

Table 7. Mean relative concentrations (% from total peak areas) of volatile compounds analyzed by HS-ITEX/GC-MS.

Volatile compound	Odor perception	Tortilla Chips corn flour (100%) - reference sample TCP	Tortilla Chips corn flour+ chickpeas flour (50% + 50%) TCN1	Tortilla Chips chickpeas flour (100%) TCN3	Tortilla Chips corn flour+ red lentil flour (50% + 50%) TCL1	Tortilla Chips red lentil flour (100%) TCL3
Alcohols						
1-Pentanol	Fruit			5.051	5.067	
1-Hexanol	green, fat		9.376	9.25	9.218	9.249
Totals			9.376	14.301	14.285	9.249
Aldehydes						
Hexanal	green, fat	6.211	6.208	6.213	6.208	6.206
Heptanal	fat, citrus, rancid	10.688	10.696	10.694	10.697	10.612
Benzaldehyde	almond, burnt sugar	13.427	13.41	13.4		13.399
Benzeneacetaldehyde	almond, burnt sugar	17.136	17.121			
Octanal	fat, soap, lemon, green		15.382	15.375	15.384	15.376
Nonanal	must, flower, almond		19.8	19.803	19.795	19.8
Totals		47.462	82.617	65.485	52.084	65.393
Ketones						
2-Heptanone	Soap	10.123	10.113	10.111	10.11	10.112
Acetophenone	must, flower, almond	10.123	18.064		18.065	18.06
Totals		20.246	28.177	10.111	28.175	28.172
Furans						
Furan, 2-pentyl-	green bean, butter	14.742	14.736	14.733	14.736	14.728
Others						
Disulfide, dimethyl	onion, cabbage, putrid	4.269	4.27	4.27	4.265	4,264
Dimethyl trisulfide	sulfur, fish, cabbage	13.615	13.616	13.613	13.611	13.615
Pyrazine, 2,5-dimethyl-	popcorn		11.257		11.197	11.197
Limonene	Citrice, mentă			16.438		
Benzoic Acid	Urine		21.894		21.934	21.893
Totals		17.884	51.037	34.321	51.007	4310.705

of fatty acids. The CI for TCN1 and TCL1 are highly significant ($p > 0.01$) ranging from –0.1 to 0.0 while CI for TCP and TCL1 was nonsignificant. Same was true for CI between TCN1 and TCL1.

3.3 Microbiological evaluation of tortilla chips

Five types of tortilla chips with various combinations of corn flour, chickpea flour and lentil flour did not show any quantifiable quantity of aflatoxins as mentioned in Table 8, mycotoxins, zearalenone and DON (deoxynivalenol) as mentioned in Table 9, and fumonisin contents as mentioned in Table 10.

Remaining mycotoxins do cross-react. Some mycotoxins have high cross-reaction rate with their metabolites. Although antibodies can specifically recognize and bind to antigens, however, antibodies face recognition problems for compounds with similar chemical structure. The cross-reactivity assay indicates the low cross-reaction rate of antibodies revealing desirable specificity of the detection method (Steel et al., 2012). Some commercial test kits also demonstrate high cross.

Microbial determination of Fumonisin B2 and B1B2 in Tortilla chips is shown in Figure 4.

The number of yeasts and molds, as well as Enterobacteriaceae, was measured during the entire shelf life of the products. The yeasts and molds in the tortilla samples showed a decreasing tendency with the addition of chickpea flour (Pico et al., 2015). They had the same path, in some cases statistically insignificant, with the

Table 8. Determination of mycotoxins, zearalenone and DON (μg/kg).

Sample	Sample code	Microbiological determinations	
		Zearalenone (μg/kg)	DON (μg/kg)
Tortilla chips corn flour (100%) - reference sample	TCP	5^{b}	74^{ab}
Tortilla chips corn flour + chickpeas flour (50% + 50%)	TCN1	4^{b}	23^{c}
Tortilla chips corn flour + chickpeas flour (20% + 80%)	TCN2	5^{b}	47^{b}
Tortilla chips chickpeas flour (100%)	TCN3	0^{c}	91^{a}
Tortilla chips corn flour + red lentil flour (50% + 50%)	TCL1	6^{ab}	65^{b}
Tortilla chips corn flour + red lentil flour (20% + 80%)	TCL2	7^{ab}	83^{ab}
Tortilla chips red lentil flour (100%)	TCL3	8^{a}	47^{b}
LSD 5%		2.44	22.29

Identical superscripts letters within rows indicate non-significant difference (p > 0.05); * PM-control sample; Tortilla chips corn flour 100%-corn flour with 50% chickpea; corn flour with 100% chickpea flour; corn flour with 80%chickpea flour-corn flour with 50% red lentil-corn flour with 80% red lentil flour; corn flour with 100% red lentil flour.

Table 9. Determination of mycotoxins, zearalenone and DON (μg/kg).

Sample	Sample code	Microbiological determinations	
		Zearalenone (μg/kg)	DON (μg/kg)
Tortilla chips corn flour (100%) - reference sample	TCP	5^{b}	74^{ab}
Tortilla chips corn flour+ chickpeas flour (50% + 50%)	TCN1	4^{b}	23^{c}
Tortilla chips corn flour+ chickpeas flour (20% + 80%)	TCN2	5^{b}	47^{b}
Tortilla chips chickpeas flour (100%)	TCN3	0^{c}	91^{a}
Tortilla chips corn flour+ red lentil flour (50% + 50%)	TCL1	6^{ab}	65^{b}
Tortilla chips corn flour+ red lentil flour (20% + 80%)	TCL2	7^{ab}	83^{ab}
Tortilla chips red lentil flour (100%)	TCL3	8^{a}	47^{b}
LSD 5%		2.44	22.29

Identical superscripts letters within columns indicate no significant difference (p > 0.05); * PM-control sample; Tortilla chips corn flour 100%-corn flour with 50% chickpea; corn flour with 100% chickpea flour; corn flour with 80% chickpea flour-corn flour with 50% red lentil-corn flour with 80% red lentil flour; corn flour with 100% red lentil flour

short-term storage. This decrease during storage was due to the low moisture content of the tortilla samples (Table 11). This conclusion was also supported by Pearson's correlation between humidity and microbiological content, as all correlations are positive. The inhibition of yeasts, molds and the development of gram-positive and negative bacteria were also due to the presence of lactic acid in bread or other fermented products.

4. Conclusions

This study demonstrated that the incorporation of chickpea flour into corn flour tortilla chips enhances the nutritional qualities of tortilla, which is particularly relevant due to consumer demand for snack foods especially corn flour tortilla for human consumption. The maize variety affects nutritional characteristics of tortilla. This combination of chickpea and lentil flour to corn flour resulted in tortilla

Table 10. Fumonisin determination µg/kg.

Sample	Sample code	Microbiological determinations		
		Fumonisin contents (µg/kg)		
		B1	B2	Sum of fumonisin B1B2
Tortilla chips corn flour (100%) - reference sample	TCP	190	<100	190
Tortilla chips corn flour + chickpeas flour (50% + 50%)	TCN1	180	<100	180
Tortilla chips corn flour+ chickpeas flour (20% + 80%)	TCN2	180	<100	180
Tortilla chips chickpeas flour (100%)	TCN3	-	-	-
Tortilla chips corn flour + red lentil flour (50%+50%)	TCL1	180	<100	180
Tortilla chips corn flour + red lentil flour (20%+80%)	TCL2	180	<100	180
Tortilla chips red lentil flour (100%)	TCL3	-	-	-

Identical superscript letters within rows indicate non-significant difference ($p > 0.05$); * PM-control sample; Tortilla chips corn flour 100%-corn flour with 50% chickpea; corn flour with 100% chickpea flour; corn flour with 80% chickpea flour-corn flour with 50% red lentil-corn flour with 80% red lentil flour; corn flour with 100% red lentil flour.

Table 11. Microbiological parameters (total combined yeasts and molds and Enterobacteriaceae (ufc/g) of corn flour samples.

Sample	Sample code	Microbiological determinations	
		number of yeasts and molds	Enterobacteriaceae
Tortilla chips corn flour (100%) - reference sample	TCP	13^{ab}	3^{ab}
Tortilla chips corn flour + chickpeas flour (50% + 50%)	TCN1	7^{b}	3^{ab}
Tortilla chips corn flour + chickpeas flour (20% + 80%)	TCN2	7^{b}	2^{b}
Tortilla chips chickpeas flour (100%)	TCN3	7^{b}	1^{b}
Tortilla chips corn flour + red lentil flour (50% + 50%)	TCL1	10^{b}	4^{a}
Tortilla chips corn flour + red lentil flour (20% + 80%)	TCL2	14^{a}	4^{a}
Tortilla chips red lentil flour (100%)	TCL3	14^{a}	3^{ab}
LSD 5%		3.22	1.05

Identical superscripts letters within columns indicate no significant difference ($p > 0.05$); * PM-control sample; Tortilla chips corn flour 100%-corn flour with 50% chickpea; corn flour with 100% chickpea flour; corn flour with 80% chickpea flour-corn flour with 50% red lentil-corn flour with 80% red lentil flour; corn flour with 100% red lentil flour.

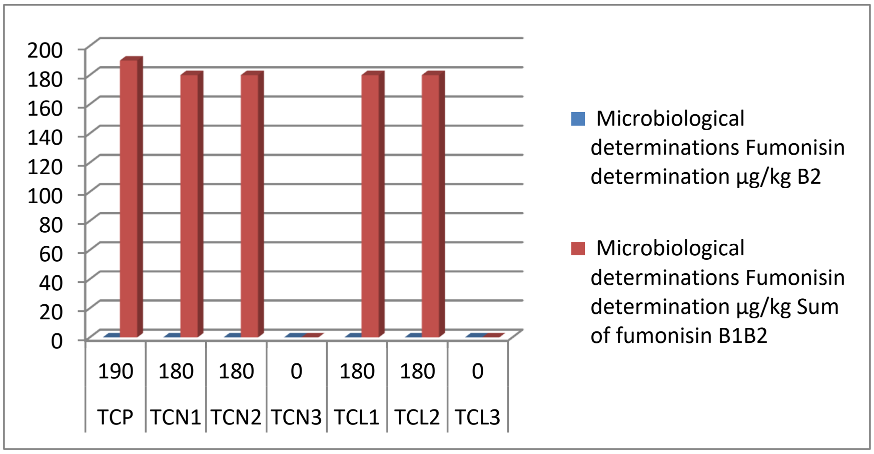

Figure 4. Microbial determination of Fumonisin B2 and B1B2 in tortilla chips.

having good quantity of proteins, essential amino acids, and unsaturated fatty acids while having little to no impact on the end product's rheological qualities. These findings indicate the importance of including legume flour in tortilla products that are consumed on a regular basis. This combination of legumes flour and cereals like maize can expand the variety of available tortilla recipes producing new nutritious products.

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