Journal of Food and Nutrition Research, 2023, Vol. 11, No. 4, 325-332
Available online at http://pubs.sciepub.com/jfnr/11/4/7
Published by Science and Education Publishing
DOI:10.12691/jfnr-11-4-7

Physicochemical, Mechanical, and Structural Properties of the Femur from Rats Fed with Corn Tortillas (*Zea mays* L.) Prepared with Calcium Sulfate

Ma. Guadalupe Garnica-Romo[1], Diana Cecilia Maya-Cortés[2,3], Juan de Dios Figueroa Cárdenas[4], José Juan Véles-Medina[4], Rafael Zamora-Vega[3], Héctor Eduardo Martínez-Flores[3,*]

[1]Facultad de Ingeniería Civil. Universidad Michoacana de San Nicolás de Hidalgo. Santiago Tapia 403. Col. Centro, C.P. 58000. Morelia, Mich., México
[2]Student of the Programa Institucional de Maestría en Ciencias Biológicas. Universidad Michoacana de San Nicolás de Hidalgo. Morelia, Mich., México
[3]Facultad de Químico Farmacobiología. Universidad Michoacana de San Nicolás de Hidalgo. Tzintzuntzan 173. Col. Matamoros. C.P. 58240. Morelia, Mich., México
[4]Centro de Investigación y de Estudios Avanzados del I.P.N., Unidad Querétaro, Libramiento Norponiente No. 2000, Fracc. Real de Juriquilla, C.P. 76230, Querétaro, Qro., México
*Corresponding author: hector.martinez.flores@umich.mx

Received March 12, 2023; Revised April 17, 2023; Accepted April 26, 2023

Abstract Corn tortillas provide calcium and help maintain a healthy state in the bones of the population. The objective of the study was to evaluate the mechanical and structural properties of rat femurs fed with tortillas made with different processes. The following diets were evaluated: raw corn (RCD), commercial tortillas (COMTD), tortillas processed with the traditional method (TTD) processed with $Ca(OH)_2$, and organic tortillas (ETD) processed with $CaSO_4$. There were no differences in the force to fracture femurs of rats fed TTD (10.66) and ETD (11.27 N), being significantly different from COMTD (5.66 N). Micrographs showed pronounced cavities in femurs of RCD-fed rats and to a lesser extent than those fed COMTD. More compact and healthier bones were shown in femurs of rats fed ETD and TTD. Ecological process provided the same properties of femurs from rats fed with traditional tortillas, with the advantage of being an ecofriendly process.

Keywords: *corn, tortilla, calcium, rat femur*

Cite This Article: Ma. Guadalupe Garnica-Romo, Diana Cecilia Maya-Cortés, Juan de Dios Figueroa Cárdenas, José Juan Véles-Medina, Rafael Zamora-Vega, and Héctor Eduardo Martínez-Flores, "Physicochemical, Mechanical, and Structural Properties of the Femur from Rats Fed with Corn Tortillas (*Zea mays* L.) Prepared with Calcium Sulfate." *Journal of Food and Nutrition Research*, vol. 11, no. 4 (2023): 325-332. doi: 10.12691/jfnr-11-4-7.

1. Introduction

Adequate nutrition throughout life is important to keep bones in a healthy state. Calcium is essential for the development, growth, and maintenance of bone [1]. However, other nutrients like magnesium, phosphorus, vitamin K, vitamin C and vitamin B12 also play an important role in bone development [2]. Magnesium assists in the activation of vitamin D, which helps regulate calcium and phosphate homeostasis to influence the growth and maintenance of bones [3]. A weakened bone can lead to fractures and if it remains constantly in that state, it can lead to osteoporosis. Osteoporosis is a systemic metabolic disease characterized by low bone mass and microstructural destruction of bone tissue, which leads to an increased bone fragility and easy fracture [4]. Osteoporosis is defined as a progressive and systemic skeletal disease characterized by reduced quantity and quality of bones that can lead to osteoporosis, thus increasing incidences of bone fractures [5]. Therefore, it is important to take care of the primary sources of food consumption that can provide the greatest amount of calcium possible. One of the important sources of calcium in countries such as Mexico and Central America, are those foods derived from the processing of corn.

Corn is used as a staple in many regions in the world, or used to produce biofuels, chemical compounds, pseudo-plastics, and other materials [6]. In North America and Latin America there is greater demand for corn products, such as tortillas, nachos, and tacos [7], which are prepared through a process called nixtamalization. Nixtamalization includes the stages of cooking the corn grain with lime and water for 15 to 40 min and then steeping the cooked grain for 12 to 20 h. Then, these corn grains are washed to separate them from the "nejayote" (excess water, lime, nutrients, and pericarp fragments), and then stone-grounded to produce masa. Masa is shaped into small circular discs and cooked on a metallic surface heated by gas combustion until tortillas are obtained [8]. The weight of each tortilla is about 25–30 g. The tortillas can be obtained in a traditional way (artisanal) or industrially. In both cases, the same procedures are followed [9]. Industrially, the dough is dehydrated, grounded, and packaged to be sold in supermarkets or stores that cook tortillas. This product is known as the nixtamalized corn flour. In artisan tortillas, the dough is directly formed into dough discs, cooked, and sold directly to consumers. The nixtamalization is not efficient because it involves: i) high consumption of energy and water, ii) high production of solid residual presence (5-14%, sugars, such as, dietary fiber, proteins, vitamins and minerals), iii) large amounts of water in the nejayote is lost during nixtamalization (this water is not recovered due to the high cost of water treatment plants); iv) nejayote has a highly alkaline pH (up to 12) and can cause scale formation inside drainage pipes [8,10]. For this reason, new alternatives are being sought out to modify the nixtamalization process variables. Some of these methods are called ecological methods, where the calcium source is replaced, to obtain a less polluting nejayote. Studies are being carried out by substituting calcium hydroxide for some other sources such as calcium chloride, calcium carbonate, calcium acetate and calcium sulfate [11-16]. The most significant pH reduction is when calcium sulfate is used. Calcium salts (e.g., sulfate, carbonate, chloride) were used to achieve an incomplete grain dehulling giving product with a higher content of dietary fiber as a result and lower production of polluting substances [17]. It is important to change the source of calcium in nixtamalization to achieve an eco-friendlier process, without affecting the calcium absorption into the corn grains In summary, with the ecological method it is possible to have a slightly acidic pH in the nejayote and therefore does not cause major damage when it is discharged into the pipes or requires a lower cost of the wastewater treatment, there is no detachment of the pericarp of the grains of corn with which it is possible to retain higher contents of soluble and insoluble dietary fiber, and the losses of nutrients are minimized.

Tortilla consumption plays an important role in some countries of Central America and Mexico, due to their contribution to nutrients such as protein, calcium, energy, and dietary fiber. In the United States of America, the preference for the consumption of tortillas has been increasing, because there is a part of the population that seeks to consume foods that guarantee health benefits [18]. In recent years, in North America, Europe and Asia, the tortilla market has been increasing rapidly [17,19]. In the urban areas of Mexico, the daily consumption of tortillas per person was 217.9 g (7.26 tortillas/day) and in urban areas, the daily consumption was 155.4 g (5.18 tortillas/day) [20]. For this sector of the population, the tortilla represents their main source of calcium and is, a key factor in preventing osteopenia and osteoporosis. For the population that regularly consumes tortillas, calcium does not represent a deficiency problem, due to the high content of calcium contained in this food [21]. Corn tortillas are reported to have calcium values between 86.95–283 mg/100 g tortillas [11,22]. Several studies have been carried out in which the positive effects have been demonstrated between the consuming tortillas done by the traditional method of nixtamalization (done with calcium hydroxide) with properties evaluated in bones [21,23,24,25]. Therefore, the objective of the present study was to evaluate the physicochemical, mechanical, and structural properties in femurs of rats fed with diets of tortillas made with calcium sulfate and compare them with diets based on tortillas made with calcium hydroxide.

2. Materials and Methods

2.1. Tortilla

The tortillas were prepared as follows: 1) traditional process of nixtamalization with $Ca(OH)_2$, 2) an ecological method, according to the conditions reported in the patent 289339 [12] using $CaSO_4$, and 3) an industrialized corn flour processed with $Ca(OH)_2$ was obtained from a local market in Morelia, Mich., México. The first stage in preparing tortillas was to prepare the dough or masa from the three processes. To obtain the masa by the traditional nixtamalization method, 3 L of water, 1 kg of corn grains and 100 g of $Ca(OH)_2$ were boiled for 49 min. Subsequently, the nixtamalized corn kernels were steeped for 16 h in the cooking water (nejayote) and washed twice. To obtain the masa by the ecological method, the same procedure was used except for using 100g of $CaSO_4$ instead of $Ca(OH)_2$. To make the tortillas with commercial nixtamalized corn flour, the flour was mixed with water until masa was obtained. From each masa, tortillas were made as follows: portions of 25g of masa were made and molded into flat surfaces about 1 mm thick. They were placed on a metal surface heated to 280°C for 30 secs. The tortillas were turned and allowed to heat up for a further 30 secs and then turned again to heat for another 20 secs. The tortillas obtained were dehydrated in an oven at 45°C and grounded until obtaining a particle size of 250 µ.

2.2. Chemical Composition

The chemical composition analysis of each tortilla and the formulated diets were carried out according to the techniques of the AOAC [26]: moisture, ashes, fat, protein (N x 6.25) and dietary fiber. Carbohydrate content was calculated by difference (100- [moisture + crude protein + fat + ash]).

2.3. Diets, and Biological Assay

Experimental diets based on tortillas were developed along with a control diet. The formulated diets were: Control diet (CD) based on casein, Raw corn-based diet (RCD), Tortilla-based diet using the traditional method of nixtamalization with $Ca(OH)_2$ (TTD), Ecological tortilla-based diet using $CaSO_4$ (ETD), and Tortilla-based diet using commercial corn flour (COMTD). The TTD and ETD diets were made with the same white corn which was used in the RCD diet. In CD, the protein source was casein, and in the experimental diets, the proteins were provided by tortillas or corn. The amount of protein in the tortilla and unprocessed corn diets ranged from 7.27% to 7.77%. The diets were formulated to contain similar amount of lipids (≈ 4%). A mixture of minerals at a concentration of 3.5% was added only to CD. In the other experimental diets, the ashes were provided by tortillas or corn, and was completed until reach 3.5% of mineral adding a mixture of AIN-93G free of calcium. A 1% vitamin mixture AIN-93G were added to all diets, including 0.25 mg of Vitamin D3, corresponding to 400 IU/g, that allows adequate absorption of all the calcium that enters from the diets, in the bones of the rats of all groups. Diet compositions are shown in Table 1.

Male Wistar rats of 22 days old were used in the experiment. The average weight was 55 g. Six animals were used for each of the experimental diets. The environmental conditions were at 22°C and there were light and dark cycles of 12 h. Food and water were provided *ad libitum*. A record was kept of the food consumed and the body weight of the animals during the 28 days of the study. At the end of the experiment, the animals were sacrificed by cervical dislocation, after fasting for 6 h, and the femurs were extracted from each rat, eliminating the tissue adhering to the bone. The femurs were superficially cleaned by impregnating cotton with alcohol solution only to clean possible impurities and stored until use. All animal studies were conducted using appropriate procedures following standard guidelines for animal experiments and carried out under the Mexican official normativity for the production, care, and use of laboratory animals NOM-062-ZOO-1999 [27].

2.4. Physical Dimension of the Femurs

The femurs were weighed and the physical dimensions such as length (L), mean diameter of the bone (MBD), the diameter of the femoral articulation and pelvis (Dafp), diameter of the femoral articulation and tibia (Daft) were measured using a vernier.

2.5. Determination of Calcium, Magnesium and Phosphorous in Femurs

The femurs of rats were incinerated at 580°C for 6 h, then 1 g of dry sample was weighed and transferred to a 150 mL beaker and 10 mL of concentrated nitric acid was added and digested overnight. Then, it was heated on a hot plate until the red fumes (NO_2) disappeared. The flask was cooled, and a small amount (2-4 mL) of 70% $HClO_4$ was added. The mixture was heated to a small volume by evaporation. The sample was transferred to a 50 mL volumetric flask and made up to volume with deionized water. Then the reading was made in the Atomic Absorption Spectrophotometer (AA) PerkinElmer Model: AAnalyst 200. A wavelength of 422.7 nm was used for calcium and 285.2 nm for magnesium. The phosphorus (P) concentration was carried out by colorimetry according to AOAC [28].

2.6. Force for Fracture, Compression and Modulo of Young Evaluated in Femur

Femoral fracture and compression tests were performed using a TA-XT2 Texturometer (Texture Technologies Corporation. Stable Micro Systems. Surrey, England). Force to fracture femurs: the test was carried out using a flat knife probe 5mm thick. Each femur was placed on a plate cm long, with a hole located at the center of the plate. The compression force was applied at a constant speed of 2 mm/s speed, and a penetration distance of 10 mm, with a cutting force of 0.1 kg. The maximum force required to break the bones was expressed as Newton (N). The fracture force measurement was performed on the six right femurs of each rat in each group. Compression force: to measure the compression force on the four left femurs of each rat in each group, a conical probe with 90° angle was used, which moved at a speed of 1 mm/s on the femur of the animals until making contact, without fracturing it. Young´s modulus or modulus of elasticity (E) behavior of the femurs was calculated using the values obtained from compression force test. Then, Young's Modulus was determined from the load-discharge curve in a relatively low deformation, and according to equations 1 and 2:

$$E = \frac{\sigma}{\varepsilon} \quad (1)$$

$$\varepsilon = \frac{Lo - Lf}{Lo} x100 \quad (2)$$

Where: σ = Normal stress (force/area); ε = Tension; Lo = Initial thickness of the femur; Lf = Final thickness of the femur after compression. The results were expressed as MPa.

2.7. Structural Analysis of the Femurs

For the structural analysis of the bones, a scanning electron microscope with elemental analysis microprobe ESEM XL30 PHILIPS, equipped with an electron beam adjusted to 20 Kv and 50 µA current was used. Two left femurs from each rat fed with each diet group were cut transversely and inserted into an acrylic base using an AcryFix Kit, such that the cross section of the femur was exposed. The acrylic bases containing the femurs were placed on the viewing plate of the equipment, and the femurs were scanned with the scanning electron microscope. The images obtained were digitized and processed with image analysis software (IMAGE J).

2.8. Statistical Analysis

All analysis corresponding to the chemical composition of flours and diets were conducted in triplicate. Physicochemical characterization tests on the bone material were done in sixth femurs of each group. The data obtained was subjected to by analysis of variance (ANOVA). Significant differences between means were determined by Tukey's test with a significance level of α = 0.05. The results of the biological assays were obtained from the six rats in each group were analyzed using the Analysis of Variance (ANOVA), with a level of significance of α = 0.05. When within subject effects for different diets were significant, differences between diets were explored using the Tukey–Kramer test and a = 0.05. Statistical analyses were carried out using JMP version 6.0 software, SAS, 2005 [29].

Table 1. Chemical composition of experimental diets (Dry basis).

Component (%)	CD	RCD	COMTD	TTD	ETD
Carbohydrates	62.26 ±1.03^{c}	74.34±0.98^{b}	80.77±1.65^{a}	80.22± 1.13^{a}	73.51±0.92^{b}
Insoluble Fiber	5.06±0.08^{c}	8.79±0.10^{b}	3.90±0.07^{d}	4.05±0.09^{d}	9.35±0.21^{a}
Soluble Fiber	1.06±0.02^{c}	1.57±0.01^{b}	0.83±0.01^{d}	0.93±0.03^{d}	1.70±0.56^{a}
Protein	20.36±1.13^{a}	7.62±0.20^{b}	7.10±0.23^{c}	7.27±0.30^{c}	7.77±0.33^{b}
Fat	7.10±0.06^{a}	4.19±0.09^{b}	3.92±0.04^{b}	4.03±0.07^{b}	4.17±0.01^{b}
Ash	4.16±0.09^{a}	3.49±0.04^{b}	3.48±0.07^{b}	3.50±0.05^{b}	3.50±0.02^{b}
Ca (mg/100g)	499.8±25^{a}	7.1±0.35^{e}	86.9±3.47^{d}	162.9±9.40^{b}	105.4±3.16c
P (mg/100g)	318.2±7.95^{e}	530.0±22^{b}	430.0±14^{c}	610.0±19^{a}	350.0±9.8^{d}
Ratio C/P	1.57	0.01	0.20	0.26	0.30
Mg (mg/100g)	50.6±1.01^{c}	122.1±2.07^{b}	219.1±7.68^{a}	145.4±1.81^{b}	121.4±3.64^{b}
Kcal/100 g	394	366	387	386	363

Values are mean of three replicates ± standard deviation. Means followed by different letter within a row have a statistically significant difference ($P < 0.05$). Ca = Calcium. P: phosphorus. Mg: magnesium. CD: control diet. RCD: raw corn diet. COMTD: Commercial tortilla diet. TTD: traditional tortilla diet. ETD: ecological tortilla diet.

3. Results and Discussion

The tortilla is considered a staple in the diet of Mexico and Central America. In recent years, alternatives have been sought to optimize the traditional process, such as minimizing polluting effluents for the environment, ensuring that the final product has an ideal nutritional value and without affecting the organoleptic characteristics of the final product. In this work, an ecological nixtamalization alternative is presented that, apart from the ecological advantages, also has the same efficiency in terms of intake and use of calcium evaluated in the bone of rats fed with diets formulated with different nixtamalization processes.

3.1. Biological Assay

The food consumption and the amount of calcium ingested during the 28 days of the study are shown in Table 2. There was no significant difference ($P<0.05$) between the food consumed by the rodents fed the different diets. A significant difference ($P<0.05$) was observed in calcium intake between all diets, with the highest intake in rodents that consumed the TTD with the value of 483 mg calcium, followed by ETD, COMTD, and RCD, presenting the values of 362 mg, 233 mg, and 21 mg of calcium, respectively. The highest calcium intake was obtained in TTD compared to ETD, even though there was no significant difference in food consumption ($p<0.05$), however, because tortillas processed by the traditional method contained a higher amount of calcium, significantly different compared to tortillas processed with the ecological method. It is also noteworthy that the control group had the highest calcium intake, being 2055 mg, however, since it was a control diet with all the ingredients that the rodent needs and the response variables will always be greater than those of the other experimental diets.

3.2. Physical and Chemical Characteristics of the Femurs

The data of the physical dimensions of the femurs are presented in Table 3. The rats fed with ETD and TTD had heavyweight femurs, showing no significant differences between them ($P<0.05$). ETD presented the values of 0.20 g and TTD showed 0.19 g. The weight of the bones of the rats fed with COMTD presented a lower weight value (0.15 g) and lastly the rats fed with RCD (0.11 g). The group fed with CD registered the highest weight (0.32 g), being statistically different ($P<0.05$) from the other groups. Gómez-Aldapa et al. [22] reported a value of 0.13 g of femur in rats fed with nixtamalized tortillas. For the femur length parameter, no significant difference ($P<0.05$) was observed between the diets containing tortillas, oscillating in a range from 2.50 to 2.57 cm. The values for this parameter coincided with the report by Martínez-Flores et al. [24] whose values displayed 2.50 cm for tortillas and corn flour. The thickness of the femur was also evaluated. There is no significant difference ($P <0.05$) between the values obtained from the femurs of the rats who were fed the tortilla-based diets, the ETD and TTD diets caused an increase in thickness as compared to the RCD diets, of the order of 33%. The femurs of the rats fed with CD showed the highest values in the dimensions of the bones. Regarding bone weight, the femurs of the rats fed TTD and ETD presented 62.5% and 59.37%, respectively, which was more weight in relation to the values obtained for those fed with CD. If we take the thickness of the femurs of the rats fed with CD as a reference, the femurs of the rats fed with TTD and ETD reach 68.75% of the normal thickness. This is because the tortilla is considered a good source of Ca, since in México it provides 49.1% of the recommended daily requirements [30]. In general, the physical dimensions of the bones of the rats fed TTD and ETD showed the best results compared to the commercial tortilla. This may be due to the higher calcium content and to a better calcium/phosphorus ratio in ingested in both treatments as compared to RCD and COMTD. On the other hand, even when TTD provided more calcium than ETD, the values obtained in calcium concentration were similar. One explanation is that the ETD calcium/phosphorus ratio was 0.30, slightly better than the TTD value of 0.26. At a higher intake of calcium than phosphorus, and maintaining a positive relationship between calcium/phosphorus, a higher content of minerals in bone is observed [31], and therefore greater bone strengthening.

Table 2. Food and calcium consumed by rodents during 28 days

Diets	Feed intake (g)	Calcium intake (g)
CD	411.06±13.15[a]	2055.20±[a]
RCD	354.26±7.79[b]	21.56±0.62[e]
COMTD	338.45±6.43[b]	233.80±6.78[d]
TTD	335.49±13.75[b]	483.56±19.34[b]
ETD	345.52±6.56[b]	362.04±8.14[c]

Values are mean of six replicates ± standard deviation. Means followed by different letter within a column have a statistically significant difference ($P < 0.05$). CD: Control diet (DC), RCD: Raw.

Table 3. Physical dimensions, mineral content, fracture strength and compressibility tests of the femurs of rats fed with the experimental diets

	CD	RCD	COMTD	TTD	ETD
Weight (g)	0.32±0.02[a]	0.11±0.01[d]	0.15±0.02[c]	0.19±0.01[b]	0.20±0.01[b]
Length (cm)	2.97±0.11[a]	2.49±0.10[b]	2.50±0.04[b]	2.50±0.05[b]	2.57±0.08[b]
Thickness (cm)	0.34±0.1[a]	0.25±0.02[c]	0.29±0.01[b]	0.29±.01[b]	0.29±0.01[b]
Mean diameter (cm)	0.74± 0.03[a]	0.62±0.01[b]	0.65±0.02[b]	0.65±0.04[b]	0.67±0.01[b]
Cortical bone thickness (mm)	0.48±0.04[a]	0.22±0.02[c]	0.30±0.01[b]	0.33±0.02[b]	0.33±0.01[b]
Calcium (mg/g)	248.67±9.81[a]	34.81±0.95[e]	115.20±4.32[d]	157.49±4.34[b]	149.42±3.13[c]
Phosphorus (mg/g)	149.97±6.72[a]	56.24±0.21[d]	59.38±2.43[d]	84.50±3.14[b]	79.46±2.21[c]
Calcium/Phosphorus ratio	1.66	1.61	1.94	1.86	1.88
Magnesium (mg/g)	4.57±0.17[c]	0.17±0.007[e]	5.43±0.20[a]	4.84±0.19[b]	3.92±0.18[d]
Fracture force (N)	17.74±1.97[a]	5.66±0.62[d]	8.93±0.54[c]	10.66±1.02[b]	11.27±0.90[b]
Young's modulus (MPa)	626.34±28.49[a]	71.78±3.24 [d]	136.36±7.09[c]	438.41±22.35[b]	446.55±21.08[b]

Values with different letters in the same row have a statistically significant difference (P<0.05), n=6. CD: Control diet (DC), RCD: Raw corn diet, COMTD: Commercial tortilla diet, TTD: Traditional tortilla diet, and ETD: Ecological tortilla diet.

3.3. Minerals in Femurs

The concentration of Ca, P and Mg in the femurs were determined (Table 3). There was a significant difference between all the diets (P<0.05). The highest value of calcium was presented in rats fed with CD. At the level of diets with tortillas, it was observed that the highest value of calcium was TTD with 157 mg/g, followed by ETD with 149 mg/g, not showing a significant difference among treatments, with a difference of only 5%. The value obtained with the RCD presented a low value of calcium 34.81 mg/g. Regarding the content of P in the femur, there was a significant difference (P<0.05) between all the diets, presenting the highest values in those who were fed with TTD and ETD. The Ca/P ratio was also determined. A ratio of 1 or higher is recommended since lower values can cause osteoporosis [31]. Only the diet with RCD had values lower than this relationship. The Ca/P ratio found in the femurs of rats fed ETD and TTD presented the highest values with 1.88 and 1.88, respectively. Although the femurs of rats fed with RCD and COMTD had excellent ratios Ca/P, the content of calcium and phosphorus were very low. At the Mg level, all diets have a good content of magnesium, with exception of femur of rats fed with RCD (0.17 mg/g). A deficiency in Mg intake contributes to the loss of bone mineral density, and this can contribute to the appearance of osteoporosis since magnesium helps in the formation of crystals and bone cells as well as influences the secretion and activity of the parathyroid hormone [32].

3.4. Compression Force

The force necessary to break the femur transversely was determined (Table 3). The force required to fracture the femurs did not present significant differences between the values obtained with the rodents fed the DET and DTT, being 11.27 N and 10.66 N, respectively. The main objective of this study was covered since its purpose was that by substituting the calcium source (calcium sulfate) of the ecological method, it will not affect the calcium absorption properties compared to the traditional method (calcium hydroxide). The ETD value presented 49.7% more resistance to fracture of the femurs compared to RCD. Therefore, it is shown that the traditional nixtamalization process provided a significant amount of calcium. The CD presented the best value, being 17.74 N, the force required to fracture the bones. And it was 36.47% better than the value of those provided by ETD. It is worth mentioning that generally a single food does not provide all the calcium required per day by the population. In Mexico, the average consumption per person per year is more than 80 kg [33], which means that at least 219 g of tortillas are consumed per day per person, which the organic tortilla would provide 231 mg Ca/day and the traditional tortilla 334 mg/day of Ca/day. Regarding the biomechanical behavior of the bone, the collagen fibers provide flexibility and resistance to tension while the mineral component (representing 30 to 40%) gives it hardness, rigidity, and resistance to compression. Bone has a viscoelastic behavior, since its mechanical response changes as a function of the applied strain rate and its mechanical properties depend on its density, where trabecular bone is very porous and cortical bone is dense. When a compressive load is applied and the strain is measured, a curve is generated, such as is shown in Figure 1. The first slope of the displacement force curve or Zone I corresponds to the absorption of compression by the articular cartilage and soft tissues [34]. The second slope or Zone II demonstrates the loading of the bone, and the slope recorded in this region measures the stiffness of the bone. The peak force or maximum force measurement in a sample indicates the inflection produced by the appearance of the bone fracture.

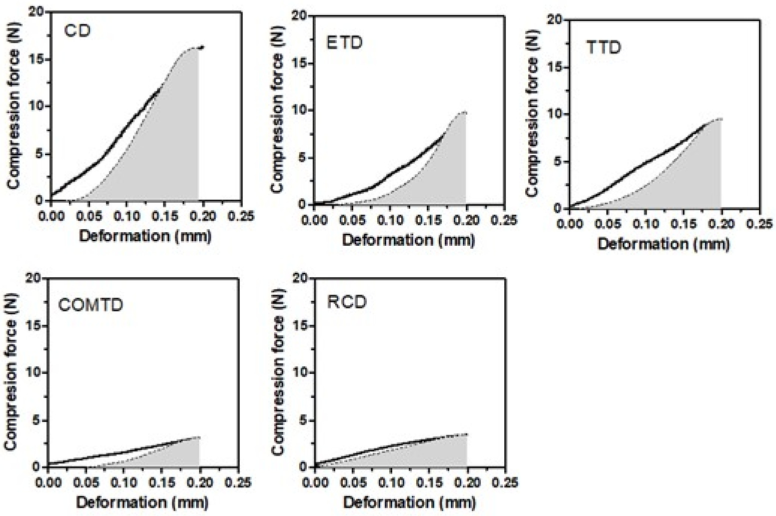

Figure 1. Compression force curves measured in femurs of rats fed the experimental diets. The solid line is the loading force, and the dashed line is the unloading force. The gray area under the curve is the elastic work and the white area is the plastic work behavior of the bone material. CD: control diet. RCD: raw corn diet. COMTD: commercial tortilla diet. TTD: traditional tortilla diet. ETD: ecological tortilla diet

With the XY graphs obtained from the compression test (Figure 1), the Modulus of Elasticity parameter was calculated, which is defined as the ratio between the increase in stress and the change corresponding to the unitary strain (Young's modulus), and it is a constant independent of the effort if it does not exceed a maximum value called the elastic limit. This relationship is identifiable in the force-deformation curve (Figure 1), from the origin to the elastic limit since the slope of the segment of this graph is the modulus of elasticity of the material. The highest value of force was obtained for the DC data (626 MPa), being statistically different in relation to the other diets; the values for DE (446 MPa) and DT (438 MPa) registered the highest values and did not present differences between them. The value found for the rats fed COMTD turned out to be 69% lower than those found for ETD and TTD (Figure 1).

Animals fed with ETD and TTD presented higher compression values than animals fed with RCD (Figure 1). The maximum value was presented by the ETD, with an increase in those who were fed with RCD (75%). With the XY graphs obtained from the compression test, the Modulus of Elasticity parameter can be calculated, which is shown in Figure 1. The modulus of elasticity is defined as the ratio between the increase in stress and the change corresponding to the unit strain. If the stress is a tension or a compression, the modulus is called Young's modulus (E) and has the same value for a tension as for compression. It is a constant independent of the effort if it does not exceed a maximum value called elastic limit. It was observed that the maximum value of Young's modulus in the experimental diets was for ETD, 446.55 MPa, with an increase of 84% with respect to the value obtained from RCD. Young's modulus (E) can be considered as a magnitude of stiffness. The higher the value of this constant, the greater the stiffness of the material. Regarding the biomechanical behavior of the bone, it can be mentioned that the collagen fibers are those that provide flexibility and resistance to tension, while the mineral parts give it hardness, rigidity, and resistance to compression. These analyses show evidence regarding ETD. Despite containing 5.12% less calcium than TTD, it did not present a significant difference ($P<0.05$) in terms of the thickness dimensions in the cortical bone of the femurs analyzed, both being 0.33 mm thick. The highest Young's modulus was observed for the group fed CD with 626.34 MPa, which was significantly different from the rest.

3.5. Scanning Electron Microscopy (SEM) Measured in Femur of Rats

SEM analysis of cortical thickness of the femurs of the rats fed with the different diets (Figure 2) resulted in the following findings: CD obtained an average of 0.48mm, TTD and ETD did not register any difference, both being 0.33mm. This is relevant for the comparison of the bioavailability of the Ca source proposed in this work. The modification of the Ca source within the nixtamalization process does not reduce the bioavailability of this mineral in the food matrix. On the other hand, the femurs of the rats fed with COMTD and RCD registered values of 0.30mm and 0.28mm, respectively.

This parameter is correlated with the inorganic/organic proportions of the tissue, where the inorganic phase represents 70% of the weight in the material. This confers the hardness and resistance to the bone [25]. Additional information was obtained from the study by SEM. The following findings in micrographs were found: the RCD group shows that the micrograph (50 µm) presented abnormal bone resorption zones on the surface of the cut. These anomalies in bones observed in this work are associated as intracortical osteopenia, which is characterized by the production of longitudinal cortical striations or radiolucent areas. Microscopy images for RCD indicate, that Ca from the bone was removed to cover the normal serum Ca concentration, and normal losses in feces and urine by the animal. Regarding the micrograph of the group fed with CD, normal bone formation and resorption sites were observed. For those fed with TTD and ETD, some resorption sites were observed and more formation sites. COMTD indicated more widely distributed resorption sites and few formation sites on the micrograph.

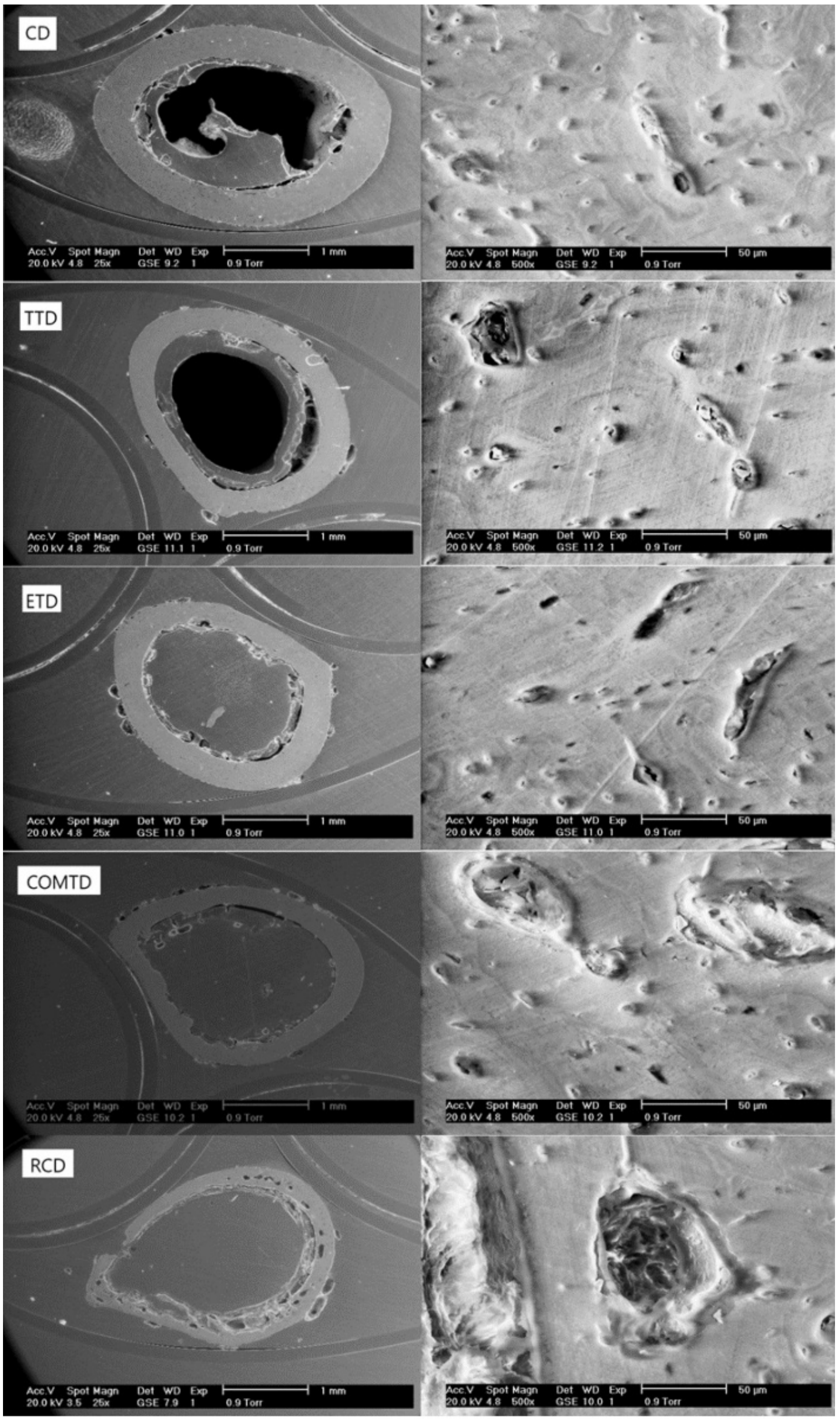

Figure 2. Cross-sectional scanning electron microscopy images of rat bone tissue from rats fed the experimental diets. CD: control diet. RCD: raw corn diet. COMTD: Commercial tortilla diet. TTD: traditional tortilla diet. ETD: ecological tortilla diet

4. Conclusions

There were no significant differences in the physical properties and in the force required to fracture the femurs between the animals fed with tortillas from ecological method as compared to the traditional nixtamalization process to produce tortillas. It is important to note that the tortillas made with the ecological method allowed a lower intake of calcium in the food consumed by the rats. However, it presented high bioavailability since it displayed similar characteristics on the rats fed with the traditional tortilla.

Acknowledgements

Diana C. Maya-Cortés gratefully acknowledges the support of Consejo Nacional de Ciencia y Tecnología de México - CONACyT in the form of a scholarship received for MSc studies.

Declaration of Interest Statement

The authors have no conflicts of interest to declare.

References

[1] Ciosek, Z., Kot, K., Kosik-Bogacka, D., Łanocha-Arendarczyk, N., and Rotter, I., "The effects of calcium, magnesium, phosphorus, fluoride, and lead on bone tissue," *Biomolecules*, 11, 506. 2021.

[2] Rudrapal, M., and Chetia, D. "Calcium and vitamin D through diet for healthy bones,", *Everyman´s Science*, 52, 190-195. 2017.

[3] Uwitonze, A.M., and Razzaque, M.S. "Role of Magnesium in Vitamin D Activation and Function," *The Journal of the American Ostheopatic Association*, 118, 3, 181-189. 2018.

[4] Li, L., Rao, S., Cheng, Y., Zhuo, X., Deng, C., Xu, N., Zhang, H., Yang, L., "Microbial osteoporosis: The interplay between the gut microbiota and bones via host metabolism and immunity," *Microbiology Open*, 8, e810. 2019.

[5] Chang, H-C., Hsieh, C-F., Lin, Y-C., Tantoh, D. M., Kung, Y-Y., Lin, M-C., Liaw, Y-C., and Liaw, Y-P., "Coffee consumption might reduce the risk of osteopenia/osteoporosis in premenopausal Taiwanese Women," *Journal of Food and Nutrition Research*, 5, 789-793. 2017.

[6] García-Lara, S., and Serna-Saldivar S.O., "Corn History and Culture. In S.O. Serna-Saldivar (Ed.), Corn Chemistry and Technology, third edition. AACC International. Elsevier Inc., 1-18. 2019.

[7] Báez-Aguilar, Á.M., Arámbula-Villa, G., Prinyawiwatkul, W., López-Espíndola, M., Ramírez-Rivera, E. J., Contreras-Oliva, A., and Herrera-Corredora, J.A. "Effect of calcium hydroxide mixed with preservatives on physicochemical characteristics and sensory shelf-life of corn tortilla," *Journal of the Science of Food and Agriculture*, 102(2), 688-695. 2022.

[8] Martínez-Flores, H.E., Martínez-Bustos, F., Figueroa J.D.C., and González-Hernández, J., "Studies and biological assays in corn tortillas made from fresh masa prepared by extrusion and nixtamalization processes," *Journal of Food Science*, 67, 1196-1199. 2002a.

[9] Méndez-Lagunas, L.L., Cruz-Gracida, M., Barriada-Bernal, L.G., and Rodríguez-Méndez, L.I., "Profile of phenolic acids, antioxidant activity and total phenolic compounds during blue corn tortilla processing and its bioaccessibility," *Journal of Food Science and Technology*, 57, 4688-4696. 2020.

[10] Ramírez-Araujo, H., Gaytán-Martínez, M., & Reyes-Vega, M. L. (2019). Alternative technologies to the traditional nixtamalization process: Review. Trends Food Science & Technology, 85, 34-43.

[11] Maya-Cortés, D.C., Figueroa-Cárdenas, J.D., Garnica-Romo, M.G., Cuevas-Villanueva, R.A., Cortés-Martínez, R., Véles-Medina, J. J., and Martínez-Flores, H.E., "Whole-grain corn tortilla prepared using an ecological nixtamalization process and its impact on the nutritional value," *International Journal of Food Science and Technology*, 45, 23-28. 2010.

[12] Figueroa, J.D.C., Rodríguez-Chong, A., and Véles-Medina, J.J. Proceso ecológico de nixtamalización para la producción de harinas, masa y tortillas integrales. 15 de agosto de 2011. Patente Mexicana Número 289339. 2011.

[13] Campechano, C.E.M., Figueroa, J.D.C., Arámbula, V.G., Martínez, F.H.E., Jiménez, S.J.S., and Luna, B.J.G., "New ecological nixtamalisation process for tortilla production and its impact on the chemical properties of whole corn flour and wastewater effluents," *International Journal of Food Science and Technology*, 47, 564-571. 2012.

[14] Bello-Pérez, L.A., Flores-Silva, P.C., Agama-Acevedo, E., Figueroa-Cárdenas, J.D., López-Valenzuela, J.A., and Campanella, O.H., "Effect of the nixtamalization with calcium carbonate on the indigestible carbohydrate content and starch digestibility of corn tortilla," *Journal of Cereal Science*, 60, 421-425. 2014.

[15] Santiago-Ramos, D., Figueroa-Cárdenas, J.D., Véles-Medina, J.J., and Mariscal-Moreno, R.M., "Changes in the thermal and structural properties of maize starch during nixtamalization and tortilla-making processes as affected by grain hardness," *Journal of Cereal Science*, 74, 72-78. 2017.

[16] Mariscal-Moreno, R. M., Figueroa Cárdenas J. D., Santiago-Ramos, D., Rayas-Duarte, P., Veles-Medina, J. J., & Martínez-Flores, H. E. (2017). Nixtamalization process affects resistant starch formation and glycemic index of tamales. Journal of Food Science, 82, 1110-1115.

[17] Escalante-Aburto, A., Mariscal-Moreno, R.M., Santiago-Ramos, D., and Ponce-García, N., "An update of different nixtamalization technologies, and its effects on chemical composition and nutritional value of corn tortillas," *Food Reviews International*, 36, 456-498. 2020.

[18] Colín-Chávez, C., Virgen-Ortiz, J.J., Serrano-Rubio, L.E., Martínez-Tellez, M.A., and Astier, M., "Comparison of nutritional properties and bioactive compounds between industrial and artisan fresh tortillas from maize landraces," *Current Research in Food Science*, 3, 189-194. 2020.

[19] Guzmán-Soria, D., Taboada-González, P., Aguilar-Virgen, Q., Baltierra-Trejo, E., and Marquez-Benavides, L., "Environmental impact of corn tortilla production: A case study," *Applied Sciences*, 9, 4852. 2019.

[20] Ministry of Economy. Department of Basic Industries. Analysis of the corn-tortilla value chain: current situation and local competition factors. Mexico City, Mexico, 31. 2012.

[21] Rosado, J.L., Díaz, M., Rosas, A., Griffit, I., and García, O.P., "Calcium absorption from corn tortilla is relatively high and is dependent upon calcium content and liming in Mexican women," *The Journal of Nutrition*, 135, 2578-2581. 2005.

[22] Gómez-Aldapa, C., Martínez-Bustos, F., Figueroa, C.J.D., and Ordorica, F.C.A., "A comparison of the quality of whole corn tortillas made from instant corn flours by traditional or extrusion processing," *International Journal of Food Science and Technology*, 34, 391-399. 1999.

[23] Serna-Saldivar, S.O., Rooney, L.W., and Greene, L.W., "Effects of lime treatment on the bioavailability of calcium in diets of tortillas and beans: bone and plasma composition in rats," *Cereal Chemistry*, 69, 565-570. 1992.

[24] Martínez-Flores, H.E., Figueroa, J.D.C., Martínez-Bustos, F., Gonzalez-Hernandez, J., Rodríguez García, M.E., Baños López, A.M.L., and Garnica-Romo, M.G., "Physical properties and composition of femurs of rat fed with diets based on corn tortillas made from different processes," *International Journal of Food Sciences and Nutrition*, 53, 155-162. 2002b.

[25] Vannucci, L., Fossi, C., Quattrini, S., Guasti, L., Pampaloni, B., Gronchi, G., Giusti, F., Romagnoli, C., Cianferotti, L., Marcucci, G., and Brandi, M.L., "Calcium intake in bone health: A focus on calcium-rich mineral waters," *Nutrients*, 10, 1930. 2018.

[26] AOAC. (1990). Official Methods of Analysis. 15th. Ed. Association of Official Analytical Chemistry. Arlington, Virginia, U.S.A,

[27] NOM-062-ZOO-1999. Especificaciones Técnicas para la Producción, Cuidado y Uso de los Animales de Laboratorio; Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación: Mexico City, Mexico, 1999.

[28] AOAC (2000) Official methods of analysis of AOAC international, 17th edn. AOAC International, Md., USA.

[29] SAS Institute Inc. (2005). JMP 6.0 software.

[30] Figueroa C., Acero G., Lozano G., Flores A., and González H., "Fortificación y evaluación de tortillas de nixtamal," *Archivos Latinoamericanos de Nutrición*, 51, 293-302. 2001.

[31] Teegarden, D., Lyle, R., McCabe, G., McCabe, L., Proulx, W., Michon, K., Knight, A., Conrad, J., and Weaver, C., "Dietary calcium, protein, and phosphorus are related to bone mineral density and content in young women," *The American Journal of Clinical Nutrition*, 68, 749-754. 1998.

[32] Castiglioni, S., Cazzaniga, A., Albisetti, W., and Maier, J.A.M., "Magnesium and osteoporosis: Current state of knowledge and future research directions," *Nutrients*, 5, 3022-3033. 2013.

[33] Žilić, S., Nikolić, V., Mogol, B.A., Hamzalıoğlu, A., Taş, N.G., Kocadağlı, T., Simić, M., and Gökmen, V., "Acrylamide in corn-based thermally processed foods; A review," *Journal of Agricultural and Food Chemistry*, 70 (14), 4165-4181.

[34] Hamer, A.J., Strachan, J.R., Black, M.M., Ibbotson, C.J., Stockley, I., Elson, R.A., "Biomechanical properties of cortical allograft bone using a new method of bone strength measurement. A comparison of fresh, fresh-frozen and irradiated bone," *The Bone and Joint Journal*, 78-B(3):363-368. 1996.