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Evaluation of non-extruded and extruded pecan *(Carya illinoinensis)* shell powder as functional ingredient in bread and wheat tortilla

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Abstract

Pecan shell is a source of dietary fiber and phytochemicals, both necessary in the human diet. Therefore, pecan shell powder could be used as a supplement in the preparation of food products. The present study evaluated the effect of addition of 5, 10 and 15 % (w/w) pecan shell powder, non-extruded (PSN) or extruded (PSE), in the physical properties of bread and wheat tortillas. Breads and tortillas produced with the higher substitution level that did not detriment significantly their physical properties (5% in breads and 10% in tortillas) were characterized in terms of chemical composition, total phenolic content (TPC), radical scavenging activity (RSA) and *in vitro* viability of human-derived liver cancer cells. Breads and tortillas produced with PSE (5% and 10%, respectively) presented the highest total dietary fiber content, TPC and RSA. In addition, extracts obtained from these same treatments, reduced the viability of hepatic cancer cells up to 53 %. Sensory analysis of breads and tortillas supplemented with PSN or PSE showed adequate overall acceptability by consumers. Results obtained in the present study support that the pecan shell powder can be used as functional ingredient in bakery products especially when pretreated by extrusion.

Keywords: pecan shell, by-products, baked products, extrusion, antioxidants

1. Introduction

The demand for functional food is rapidly growing around the world due to the high percentage of people suffering from chronic diseases. This has led to the search for the addition of new ingredients such as fruits, nuts and vegetables by-products with high bioactive compounds like fiber and phenolics (Quirós-Sauceda et al., 2014).

Bread is considered a staple food in most diets around the world. In the other hand wheat tortillas are the second highest selling product in the packaged bread category in North America (Montemayor-Mora et al. 2018). Based on the high consumption of these products, they are considered good products to introduce new ingredients with bioactive compounds. There are some successful works concerning improvement of nutraceutical potential of bread and wheat tortillas by fortification with by-products (Bedrníček et al., 2020; Gawlik-dziki et al., 2015).

The pecan shell (*Carya illinoinensis*) is an important by-product of the food industry, which contains high amounts of polyphenols and consequently, its consumption may lead to benefits on health (Kureck et al., 2018). Previous studies showed that gallic acid, chlorogenic acid, p-hydroxybenzoic acid, epigallocatechin, epicatechin-gallate and tannins are the main phenolic compounds present in pecan shells (Do Prado et al., 2014; Hilbig et al., 2018). Villasante et al., (2020) studied the synergetic effects of pecan shell, roselle flower and red pepper on the quality of beef patties during chilled storage, and found that this mix may prevent lipid oxidation, metmyoglobin formation and microbial contamination. In a previous work, Villasante, (2019) optimized the extrusion conditions of pecan shell, and demonstrated that extrusion at 70 ºC of temperature and 150 rpm of screw speed doubled the concentration of polyphenols and increased radical scavenging activity and soluble dietary fiber compared to the non-extruded pecan shell.

The purpose of the present study was to develop functional bread and wheat tortillas with increased amounts of dietary fiber, total phenolic content and antioxidant activity, by including extruded and non-extruded pecan shell powder in the formulation. The effect of the inclusion at different concentrations of this by-product on the physical features of bread and tortilla, was evaluated. Breads and tortillas produced with the higher substitution level that did not detriment significantly their physical properties were characterized in terms of chemical composition, total phenolic content and antiradical capacity. The effect of bread and tortillas supplemented with pecan shell powder on *in vitro* viability of human-derived liver cancer cells (HepG2) was also assessed. Finally, a sensory analysis was performed to evaluate the overall quality and acceptance of the supplemented products.

2. Materials and methods

2.1. Raw materials

The pecan *(Carya illinoinensis)* shells were obtained in San Pedro, Coahuila, Mexico. The commercial refined all-purpose wheat *(Triticum aestivum L)* flour was purchased from Harina Selecta (Molinera de Mexico SA de CV, Mexico).

2.2. Milling and particle size distribution

The pecan shells were ground using a Wiley knife mill (Arthur Thomas, Philadelphia, PA, USA) equipped with a 2.0 mm screen. Then the product was processed in a Cyclone Mill (Udy Corporation, Model 3010- 014, Collins, CO). The powder obtained was sieved through a US mesh No. 80 in a Rotap (Duratap Model DT 168, Advantech Mfg., New Berlin, WI, USA) and labelled as non-extruded pecan shell powder (PSN).

2.3. Extrusion processing

The PSN was processed in a twin-screw co-rotating extruder (BCTM-30 Bühler, Uzwill, Switzerland), using the same extrusion conditions (70 ºC and 150 rpm) described by Villasante et al. (2019). The resulting extrudates were milled using a Wiley mill (Arthur Thomas, Philadelphia, PA, USA) equipped with a 2.0 mm screen and sieved through a US mesh No. 80. The extruded powder was labelled as PSE (extruded pecan shell powder).

2.4. Rheological properties of the composite flours

The rheological behavior of composite wheat flours substituted with 5 %, 10 % and 15 % w/w of PSN or PSE was studied using the Mixolab (CHOPIN Technologies, France) according to the approved method AACC 54-60.01 and following the standard protocol Chopin+. A control of 100% wheat flour (CWF) was also analyzed. The Mixolab analysis was performed in duplicate.

2.5. Bread production

For bread production, the straight-dough method reported by Serna-Saldivar (2010) was used with slight modifications. The control breads (CNB) were produced using refined wheat flour. The refined wheat flour was replaced with PSN or PSE at 5 %, 10 % and 15 % w/w levels. For breadmaking, the baker's formulation expressed in flour basis was: 6 % w/w cane sugar (HEB de Monterrey, N.L, Mexico), 3.5 % w/w vegetable shortening (Inca, Unilever de Mexico S.A de C.V.; Tultitlán, Edo. de Mexico, Mexico), 2 % w/w dry milk (Nestle de Mexico, Mexico, D.F., Mexico), 2 % w/w salt (La Fina, Sales del Istmo, Coatzacoalcos, Veracruz, Mexico), 1.5 % w/w dry yeast *Saccharomyces cereviceae* (Nevada Roja, Panadis S.A de C.V. Monterrey, N.L., Mexico) and 0.5 % lecithin (Proveedores de Ingeniería Alimentaria S.A. de C.V. Monterrey, N.L., Mexico). Bread was prepared according to the method described by Acosta-Estrada et al. (2014). The water absorption (WA) and mixing time for each treatment were obtained from the Mixolab analysis (*Section 2.4*). After baking, breads were cooled during 30 min at room temperature. Afterwards, bread was cut into slices 15 mm thick. Slices were stored into sealed polyethylene bags for further analysis. Samples were labelled as follows: control bread (CNB) (100 % wheat flour), 5%PNB (with 5 % PSN), 5%PEB (with 5 % PSE), 10%PNB (with 10 % PSN), 10%PEB (with 10 % PSE), 15%PNB (with 15 % PSN) and 15%PEB (with 15 % PSE). Two batches were produced for each treatment.

2.6. Tortilla production

Control tortilla (CNT) was produced using refined wheat flour. The wheat flour was replaced with PSN or PSE at 5 %, 10 % and 15 % w/w levels. Tortillas were produced according to Montemayor-Mora, et al., (2018) based on the following baker's formulation expressed in flour basis: 13 % w/w vegetable shortening (INCA, ACH Foods Mexico, Mexico), 2 % w/w dry milk (Nestle, Monterrey, NL, Mexico), 2 % w/w double acting baking (Rexal, Productos Mexicanos, Monterrey, NL, Mexico), 0.3 % w/w sodium stearoyl-2-lactylate (TECSA, Monterrey, NL, Mexico), and 0.2 % w/w carboxymethyl cellulose

(PIASA, Monterrey, Mexico). The water absorption (WA) and the treatments produced were: control tortilla (CNT) (100 % wheat flour; 55.95 % WA), 5%PNT (with 5 % PSN; 56.53 % WA), 5%PET (with 5 % PSE; 56.90 % WA), 10%PNT (with 10 % PSN; 57.00 % WA), 10%PET (with 10 % PSE; 57.21 % WA), 15%PNT (with 15 % PSN; 57.47 % WA) and 15%PET (with 15 % PSE; 57.95 % WA). Baked tortillas were cooled at room temperature during 30 min. Then, tortillas were stored into sealed polyethylene bags at room temperature for further analysis. Two batches of 30 tortillas were produced for each treatment.

2.7. Physical properties of bread and tortilla

Bread volume was determined by rapeseed displacement (National Manufacturing Co., Lincoln, NE, USA) according to method 10-05.01 of the AACC. The ΔHeight was determined by measuring the difference between the height of the bread before and after baking. The weight of breads was determined 30 min after baking. Tortillas were characterized in diameter, thickness and weight which were determined in 10 samples as recommended by Montemayor-Mora et al., (2018).

Bread and tortilla color was measured using a Minolta CM-600d Chroma Meter (Konica Minolta Co., Osaka, Japan) with Standard Illuminant D65. The brightness was determined by L^*, red-green by a^*, and yellow-blue by b^*. The chrome (C*) and delta versus control (ΔE) were calculated. Color parameters were obtained from five replicates per batch. Bread color was measured in crumb and crust.

2.8. Bread and tortilla texture analysis

Texture analyses were conducted in a TA.XT2 Texture Analyzer (Stable Micro Systems, Godalming, England). The texture of bread was conducted in slices (15 mm thick), following the methodology described by Buitimea-Cantú et al., (2018). The parameters evaluated were hardness, springiness, cohesiveness, chewiness, and resilience. For tortilla, the texture was evaluated using the Tortilla Burst Rig platform (HDP/TPB) and a 25-mm spherical probe. Tortillas were extended at a test speed of 1 mm/s to a penetration of 40 mm to determine force to break and extensibility (maximum extension before breakage). The subjective rollability test was evaluated according to Montemayor-Mora et al., (2018). All texture analyses were performed in three randomly selected samples of each batch at days 0, 1 and 4 of storage.

2.9. Chemical composition of breads and tortillas

The moisture, ash, crude protein (using 5.81 as conversion factor) and fat contents of breads and tortillas were determined by the official AACC (2000) methods 44-15, 08-01, 46-30 and 30-20, respectively. The soluble (SDF) and insoluble (IDF) dietary fibers were assayed using a commercial kit provided by Megazyme™ (Bray, Ireland) based on the enzymatic-gravimetric AOAC 991.43 method. The total dietary fiber was calculated with the sum of SDF and IDF.

2.10. Preparation of bread and tortilla methanol extracts

Before production of methanol extracts, 100 g of lyophilized sample were defatted with 200 ml of n-hexane. The suspension was stirred for 24 h and filtered with Whatman filter paper No. 1. To guarantee adequate defatting, this procedure was repeated with 100 mL of n-hexan. Lyophilized defatted samples (1g) were mixed with 80 % (v/v) aqueous methanol (10 ml) and stirred for one hour at 900 rpm. The supernatant was recovered after centrifugation (Thermofisher scientific SL 16R, Waltham, MA) at 2500 ×*g* for 15 min at room temperature and used for determination of total phenolic content (TPC) and radical scavenging activity (RSA).

2.11. Determination of Total Phenolic Compounds (TPC)

The TPC was determined in bread and tortilla methanol extracts (Section 2.10) using the methodology reported by Mosca et al., (2018) and the spectrophotometer Fluostar Omega (Paris, France) at 25º C and 750 nm. Results were expressed in mg of gallic acid equivalents (GAE) per g of sample. The analysis was performed in triplicate.

2.12. Determination of Radical scavenging activity (RSA)

The RSA was measured in bread and tortilla methanol extracts (Section 2.10), using the DPPH (1,1-Diphenyl-2-Picrylhydrazyl) and ABTS (2,2'-azino-bis3-ethylbenzothiazoline-6-sulfonic acid) methodologies. DPPH was measured according to the method described by Gallego et al. (2013). Briefly, the methanolic extracts (Section 2.10) reacted with 200 µL of DPPH in methanol and the absorbance was measured at 517 nm after a period of 0 min (A0) and 75 min (A1) using a UV-Vis microplate reader spectrophotometer (Fluostar Omega, Paris, France). The inhibition percentage of radical scavenging activity for each sample was calculated by Equation 1 where A0 is the initial absorbance of DPPH solution and A1 is the absorbance after 75 min. The analysis was performed in triplicate.

$$\%inhibition\ of\ sample = \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \text{ (Equation 1)}$$

ABTS was determined spectrophotometrically, following the Azman et al. (2014) method. Briefly, the methanolic extracts (Section 2.10) were added in 200 µL of solution ABTS (absorbance of 0.75 ± 0.02 at 734 nm, 37 ºC), and then the absorbance was measured at 734 nm after 10 min. Results were expressed as µmol of Trolox equivalents (TE) per g of sample. Each sample was analyzed by triplicate.

2.13. Cell viability assays

2.13.1. Preparation of bread and tortilla extracts for cell viability assays

Bread and tortilla lyophilized defatted samples (1 g) were mixed with 80 % (v/v) aqueous methanol (10 mL of). The samples were stirred for one hour at 900 rpm and centrifuged (Thermofisher scientific SL 16R, Waltham, MA) at 2500 ×*g* for 15 min at room temperature. After centrifugation, the pellet was discarded and methanol from the supernatant was evaporated with nitrogen. Bread and tortilla extracts were weighed and resuspended in 2 mL of water. All samples were filtered (DMSO-Safe Acrodisc® Syringe Filter 0.2 µm, 25 mm, sterile) prior to the experiment. Samples were extracted in triplicate.

2.13.2. Cell culture and viability

Human tumor cells (HepG2) derived from liver hepatocarcinoma (ATCC no. HB-8065) were cultured at 37 ºC in 5 % CO_2 in Dulbecco's Modified Eagle's (DMEM) medium supplemented with 100 IU/mL penicillin, 100 mg/mL streptomycin and 10 % (v/v) of fetal bovine serum, using the methodology reported by Gallego et al. (2017). HepG2 cells were seeded in 12-well plates at a density of 2×10^4 cells/well (1 ml per well). After 24 hours, two different concentrations (2 and 10 %, 0.02 and 0.10 ml per well, respectively) of extracts (section 2.13.1) prepared with the following samples were added to the cell culture: CNT, 10%PNT, 10%PET, CNB, 5%PNB and 5%PEB. These treatments were selected considering the best in terms of textural and physical properties (Section 3.2). HepG2 cells incubated with water (2 and 10%) or solvent without extract (2 and 10%) and non-treated cells (CTR), were used as controls. Treated cells and controls were incubated during 48 hours. The cell viability was determined by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as previously described by Gallego et al. (2017). Briefly, 0.63 mM of MTT and 18.4 mM of sodium succinate were added to HepG2 cells and incubated for 3 h at 37º C. After removal of the medium, formazan was resuspended with dimethyl sulfoxide (DMSO) supplemented with 0.57 % CH_3COOH and 10 % sodium dodecyl sulphate. Spectrophotometric determinations were performed at 570 nm in a Cobas Mira S analyzer (Hoffman-La Roche, Basel, Switzerland). The results were expressed as percentage of cell survival relative to non-treated control cells. Measurements were performed in triplicate for each sample.

2.14. Bread and tortilla sensory analysis

A hedonic sensory test was conducted according to Cadioli et al., (2011). The taste panel consisted of 70 untrained panelists, aged from 18 to 60 (70% female and 30% male), involving staff and students from Tecnologico de Monterrey, Campus Monterrey that declared to consume fiber-rich products. Before the sensory analysis test, the panelists were asked to fill out in the answer sheet if they had allergies/intolerances to egg, nuts, soy, gluten and milk and to sign their consent to perform the sensory evaluation. For the sensory analysis, the controls were produced using whole-wheat flour since the aim was to compare the products containing pecan shell with a fiber-rich counterpart, produced with commercial flour. The use of whole-wheat flour prevented biased results due to fiber-rich or no-fiber preferences of consumers. Controls for sensory analysis were labelled as CWT (control tortilla produced with whole-wheat flour) and CWB (control bread produced with whole wheat flour). Participants rated on a five-point hedonic scale, one-day-old tortilla (CWT, 10%PNT and 10%PET) and bread (CWB, 5%PNB and 5%PEB) samples. The evaluated parameters were color, texture, flavor, odor, and overall quality.

2.15. Statistical analysis

The average values and standard deviations were calculated from the data obtained from the samples for each treatment. Significant differences were analyzed by one-way ANOVA and means compared using Turkey´s test using a level of significance of 95 %. All statistical analyses were performed using Minitab-18 Statistical Software.

3. Results and discussion

3.1. Rheology of doughs supplemented with pecan shell powders

Rheological properties of composite flours are summarized in Table 1. CWF had the lowest water absorption (WA) value while flours substituted with 15 % of either PSN or PSE, presented the highest values. The increase of WA could be associated to the presence of hydroxyl groups in the structure of fiber in the pecan shell (Kuchtová et al., 2018). A similar behavior was also observed by other authors after the preparation of wheat dough enriched with fiber-rich by-products as grape skin, apple fiber, lemon and orange fiber (Kohajdová Zlatica, Karovicova Jolana, Jurasová Michaela, 2011; Kuchtová et al., 2018). Increased water absorption has positive effects on the baking industry since it leads to higher yields of baked products. The development time was significantly reduced by the supplementation of pecan shell (Table 1). Flours containing pecan shell presented development times up to 50 % lower compared to CWF. This could be associated with an increase in the dietary fiber content due to wheat flour replacement with pecan shell powders. Increments in total dietary fiber can lead to a larger number of hydroxyl groups available for interaction with water through hydrogen bonding during dough development (Martins et al., 2017). Therefore, the increased presence of hydrophilic moieties due to the addition of pecan shell, may have led to a quicker absorption of water, reducing the dough development time of pecan shell composite flours. During Mixolab analysis, all samples were stable during 8 min-dough mixing.

The C2 parameter increased significantly due to the addition of PSN and PSE in comparison with CWF. Kuchtová et al. (2018) studied the effects of flour replacement with fiber-rich grape by-products on the rheological properties of composite doughs and found a similar behavior when grape seeds were added. Authors attributed the higher C2 values to a reduced weakening of the dough and gluten network during the increase of temperature in the Mixolab analysis. Rosell et al. (2010) mentioned that the higher C2 values are the result of some impediments in the protein unfolding. The higher fiber content of composite flours containing different levels of pecan shell could have impaired gluten unfolding, leading to lower protein weakening, better holding of the dough structure and consequently, higher C2 values. The addition of PSN and PSE significantly increased the C3 values compared to

CWF (Table 1). C3 measures the maximum torque during the heating stage, which in turn could be related to the starch gelatinization. A reduction in C3 in flour containing pecan shell was expected due to reduced starch content. However, previous reports have also found higher C3 values when wheat flour is substituted with cellulose fiber (Lauková et al., 2017). The addition of fiber could contribute to dough consistency during heating, leading to higher C3 values. In general, C4 was higher in samples supplemented with pecan shell (extruded and non-extruded). This result suggests that hot gels produced with flour supplemented with pecan shell had increased consistency. However, differences observed in C3 and C4 with increasing levels of PSN and PSE indicated lower cooking stability. C5 is related to starch retrogradation. Lower values are desirable in baked products since starch retrogradation is related with staling. The composite flour with 15 % PSE had the lowest C5 value among samples. We previously reported that extrusion of pecan shell causes an increment in soluble dietary fiber content (Villasante, et al., 2019). High contents of soluble fiber may keep the dough system hydrated and hence, delay retrogradation (Ma et al., 2019). Therefore, the lower C5 value found in the 15 % PSE, was attributed to the higher content of soluble dietary fiber in the supplemented flour.

3.2. Physical properties of bread and tortilla

Table 2 shows the physical properties of bread. The ΔHeight and specific volume of 5%PNB and 5%PEB were not significantly different than CNB. However, breads with 10 % and 15 % of PSN or PSE presented significantly lower specific volumes. Supplementation of wheat flour with fiber-rich materials such as the pecan shell (Villasante, et al., 2019), causes a dilution of starch and gluten, decreasing the loaf volume (Lafarga et al., 2019). Ma (2019) suggested that high fiber content could limit the expansion during fermentation. Similar results were also observed for bread supplemented with fiber-rich by-products from the flaxseed industry and fenugreek gum (Roberts et al., 2012). The weight of bread was not significantly affected by the addition of pecan shell.

Color lightness (L^*) and yellowness (b^*) of bread crumb decreased with increasing levels of PSN and PSE while redness (a^*) increased (Table 2). Changes in the color of the bread crumb were associated to the pigments of pecan shell such as tannin compounds (Kureck et al., 2018). Compared to CNB, the crust presented lower *L*, a** and *b** values when pecan shell was added to bread. Also the bread crust presented lower *L** values compared to the crumb counterparts. This was related to brown color formation during caramelization and Maillard reactions occurring in baking. Lower *L*, a** and *b** values in the crust of pecan shell breads were consistent with previous reports. Martins et al., (2017) studied the fortification of wheat bread with agroindustry by-products and also found that the addition of 7 % elderberry extract caused a decrease in *L*, a** and *b** of the crust compared to the control. Martins et al., (2017) also attributed these changes to Maillard reactions. The chrome (C*) values of crumb and crust of supplemented breads were significantly different. Interestingly, the ΔE* was higher in the samples containing PSE compared to the counterparts with PSN. The differences in color among PSE and PSN breads, could be associated to changes in the pigments of pecan shell during thermoextrusion processing (Villasante, et al., 2019).

For tortilla, the addition of shell powder did not produce significant effects on diameter, thickness and weight in comparison to the control (Table 2). The *L** and *b** values decreased in tortillas containing PSN and PSE, while *a** increased. Changes in color, were also associated to the pigments present in PSN and PSE powders.

3.3. Textural parameters of bread

Results obtained from the textural analysis of bread slices from different treatments are shown in Table 3. At Day 0, CNB, 5%PNB, 5%PEB and 10%PNB presented lower hardness compared to 10%PEB, 15%PNB and 15%PEB breads. The same trend was observed at day 1. Interestingly, at day 4, 5%PEB showed the lowest hardness among all breads. The springiness was not significantly affected by the addition of PSN and PSE. Breads showed average springiness values ranging from 0.96 to 0.98. The bread cohesiveness was significantly affected by the addition of PSN or PSE (Table 3). At day 0, 5%PNB, 5%PEB and 10%PEB did not show significant differences compared to CNB, while the other breads presented a reduced cohesiveness. At day 1, only breads containing 5 % and 10 % PSE were comparable to CNB in cohesiveness. However, at day 4, all breads containing pecan shell presented lower cohesiveness compared to CNB. The chewiness of breads containing pecan shell were not significantly different to CNB at day 0 and day 1. Among breads containing pecan shell, treatments 5%PNB, 5%PEB and 10%PNB had lower chewiness values at day 0. At day 4, breads

5%PNB and 10%PNB had lower chewiness compared to the CNB. According to Wang et al. (2020) a lower chewiness is associated with better tasting properties. The effect of pecan shell on textural parameters could be partially explained by the high fiber content previously reported in this matrix (Villasante et al., 2019). Fiber could avoid water loss during storage, and may also be interacting with starch to delay starch retrogradation.

In conclusion, the addition of 5% PSN or PSE, did not significantly affect the hardness. The values obtained for these breads were similar to those found in CNB at the different days. Furthermore, samples with 5% PSN or PSE did not present significant differences in springiness compared to CNB. Moreover, cohesiveness and chewiness of 5%PNB and 5%PEB at day 0, were not different than CNB. Based on such textural results and the physical properties of supplemented breads (Section 3.2), further characterizations were only performed in 5%PNB and 5%PEB, using CNB as control.

3.4. Textural parameters of tortilla

When tortilla texture is characterized, higher breaking force values and longer distance indicate a stronger and more stretchable tortilla (Liu et al., 2017). At day 0, 10%PET presented a higher breaking force compared to the control (CNT), while the rest of the treatments did not show significant differences (Table 3). In day 1, the breaking force was significantly lower in 10%PNT and 15%PNT compared to CNT while the rest did not present significant differences. Interestingly, at day 4, 10%PET exhibited higher breaking force compared to CNT. In general, tortilla extensibility was reduced by the addition of pecan shell powders. Compared with CNT, only 5%PNT and 10%PET did not show significant differences in extensibility at day 0, while the rest of the samples presented lower values. At day 1, only 10%PET was not significantly different than CNT in extensibility, whereas at day 4, tortillas containing 5% pecan shell (PSN or PSE) and 10%PSE presented higher elasticity than CNT. Within the same sample, the breaking force and extensibility values were significantly reduced during storage. When the subjective rollability was measured, no significant differences were found among tortillas containing pecan shell compared to CNT. In general, within the same sample, reductions in rollability values were observed up to day 4.

The 10%PET induced the highest values of breaking force, extensibility and subjective rollability along the 4 days of storage. This indicated that 10 % substitution with pecan shell, especially when it is extruded, would result in adequate textural properties. Previous reports have found that the presence of fiber slows down the staling of tortilla during storage by limiting gelatinization and retrogradation of starch (Liu et al., 2017; Santos et al., 2008). Therefore, based on the results obtained on the texture and the physical properties, further characterization was only performed in CNT, 10%PNT and 10%PET.

3.5. Chemical composition of breads (5%PNB and 5%PEB) and tortillas (10%PNT and 10%PET)

The moisture and starch contents were significantly reduced in tortillas and breads supplemented with PSN or PSE (Table 4). This indicated that flour supplemented with pecan shell retained lower amounts of water during baking, compared to wheat flour. On the other hand, the starch content was reduced due to a dilution effect caused by substitution of wheat flour with pecan shell powders. The protein and fat contents were not significantly different among bread samples but were slightly reduced in tortillas containing pecan shell. These results could be a consequence of the low fat and protein contents of pecan shell (Villasante et al., 2019).The ash content was not affected by the addition of pecan shell to bread or tortilla (Table 4).

Total dietary fiber increased significantly with the addition of PSN or PSE to bread and tortillas. Compared to the control (CNB), 5%PNB and 5%PEB contained 43 % and 120 % more soluble dietary fiber and 2.9- and 3.3-fold more insoluble dietary fiber, respectively. Furthermore, in 10%PNT and 10%PET, the soluble dietary fiber increased 44 % and 120 %, while the insoluble dietary fiber increased 2.9- and 3.2-fold compared to the control (CNT), respectively. This was consistent with the lower development times found in composite flours containing PSN or PSE (Section 3.1). According to the European Parliament and the Council of the European Union (2006), bread labelled as “source of fiber” or “high in fiber” must contain at least 6 g of total fiber per 100 g of bread. Breads and tortillas enriched with PSN or PSE (5% in bread and 10% in tortilla) fulfilled with this requirement.

3.5.1. Total phenolic compounds (TPC) and radical scavenging activity (RSA)

TPC and RSA (DPPH and ABTS) increased in breads and tortillas supplemented with PSN or PSE (Table 4). According to De la Rosa et al. (2014) the pecan shell contains more than 60 mg GAE/g. Also, Hilbig et al., (2018) found the presence of more than 40 different phenolic compounds in pecan shell extracts, including catequins, gallic acid and chlorogenic acid. The high amount of phenolic compounds in pecan shell extracts could explain the increment of TPC and RSA in the supplemented products. Interestingly, when TPC of samples containing PSN or PSE were compared, no significant differences were found, however, the DPPH was significantly higher in bread and tortilla produced with extruded pecan shell (PSE). In this regard, Villasante, et al. (2019) demonstrated that the extrusion process of pecan shell increment its radical scavenging activity. Previous reports have studied the nature of interactions existing between different groups of phenolic compounds and dietary fiber. In a study where 5 % raw mango peel powder was added to whole wheat bread, the DPPH (% inhibition) triplicated, from 21.51 % to 68.54 %, compared to the control (without mango peel) (Pathak et al., 2017). On the other hand, according to Barišić et al., (2020), the addition of cocoa shell to chocolate results in reduced contents of TPC, total flavonoid, and individual compounds. The reduction or increment of TPC and antioxidant activity depend on the type of fiber (insoluble and soluble), the type and molecular weight of polyphenols present, physicochemical interactions and complexation between the polyphenols with fiber, proteins or starch molecules (Barišić et al., 2020).

3.6. Viability-reducing activity of bread and tortilla extracts against human cancer cell lines

The remarkable presence of phenolic and antioxidant compounds in pecan shell prompted to assay viability of human tumor-derived cells in the presence of extracts from bread and tortilla supplemented with pecan shell. To this end, in the present study the cell viability-reducing activity of pecan shell extracts was addressed in HepG2 cells. The HepG2 cell line derives from human hepatocellular carcinoma and is widely used for evaluating cytotoxicity and antiproliferative assays.

The cytotoxicity of bread and tortilla containing PSN or PSE was evaluated in the cancer cell line HepG2, by means of the MTT assay (Fig. 1). In bread, the addition of 5%PSN promoted similar results compared to the control (CNB), while the lowest cell viability value among bread samples was found in 5%PEB. Compared to CTR, addition of 10 % (aqueous extract in cell culture) of 5%PEB extract inhibited almost 50 % of cell proliferation. Palafox et al. (2019) characterized the *in vitro* chemoprotective potential of an aqueous extract of bread enriched with green coffee extract in HepG2 cells. After incubation for 24 h, they found that the cell number decreased by approximately 50 %, possibly as a result of phenolic compounds such as chlorogenic acid present on green coffee. Moreover, extracts produced with tortillas containing 10%PSE and applied at a 10% level (aqueous extract in cell culture) inhibited more than 50 % of the growth of HepG2 cells after 48 h (Fig 1). A protective anticancer role of wheat tortilla has never been studied. Results shown in Fig. 1 were consistent with the findings presented in Table 4. This indicated that the increased TPC and radical scavenging activity found in samples supplemented with extruded pecan shell powder, could be associated with the lower viability observed in HepG2 cells treated with these products. Hitherto, little information is available regarding the cytotoxicity of nut shells in cancer cell lines. Nevertheless, previous studies have also showed positive results when chestnut shell extracts were tested in different cancer cell lines, including HepG2 (Cacciola et al., 2019; Jung et al., 2015; Sorice et al., 2016). Therefore, the results found herein open the possibility to consider inclusion of pecan shell as a promising ingredient to develop new functional foods using nut by-products.

3.7. Preference sensory analysis

Results of hedonic tests performed in breads and tortillas produced with whole wheat flour (controls) and supplemented with pecan shell powders, are shown in Figure 2. Overall, no significant differences were observed in texture and acceptability parameters among bread samples. Color of control bread was scored higher compared to breads containing pecan shell powders. This was probably caused by the browner color of these samples which was provided by the pecan shell. Ali et al. (2018) obtained similar results in the production of bread with the addition of black gram flour. The flavor of bread is one of the most important parameters influencing its acceptance by consumers (Sirbu et al., 2015). In this regard, bread samples with PSN or PSE had better flavor scores compared to the control. When tortilla was evaluated, the addition of PSN or PSE did not affect the scores in texture but color had lower scores. Results obtained for flavor and acceptability were better for control tortillas (CWT) and 10%PET compared to scores obtained for 10%PNT. In summary, general acceptability of bread was not significantly affected by addition of PSN or PSE while tortilla with 10%PSN had the lowest general acceptance.

4. Conclusions

Pecan nut shell can be used as ingredient in bread and wheat flour tortilla. The incorporation of either non-extruded or extruded pecan shell powder in bread and tortilla formulations affected their quality characteristics. However, the extruded pecan shell powder promoted higher antioxidant activity and soluble dietary fiber in the supplemented products. Therefore, inclusion of extruded pecan shell powder can improve bioactivity in foods while valorizing an agro-industry by-product. Our findings showed that it is possible to incorporate up to 5% and 10% of extruded pecan shell powder to bread and tortilla formulations, respectively, to enrich health benefits while maintaining satisfactory sensory properties and textural properties during storage. Since this study was performed in pilot-plant scale it results promising for implementation in extended scales. Further studies to evaluate the shelf life of resulting products and potential replacement in other baked goods are needed.

Conflict of interest: All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Mixolab parameters of composite flours containing pecan shell powder (non-extruded and extruded) and wheat flour

Parameter	CWF	5%PSN	5%PSE	10%PSN	10%PSE	15%PSN	15%PSE
Water absorption (14% basis)	58.5±0.80[d]	59.5±0.00[c]	59.5±0.01[c]	60.0±0.02[bc]	60.0±0.00[bc]	60.5±0.02[ab]	61.0± 0.03[a]
Dough development time (min)	2.96±0.39[a]	1.51±0.04[b]	1.50±0.01[b]	1.48±0.15[b]	1.45±1.04[b]	1.39±0.02[b]	1.37±0.02[b]
C2 (protein weakening, Nm)	0.51±0.02[e]	0.59±0.02[d]	0.63±0.01[d]	0.76±0.02[c]	0.84±0.01[ab]	0.78±0.01[bc]	0.86±0.14[a]
C3(starch gelatinization, Nm)	2.02±0.01[d]	2.16±0.03[c]	2.14±0.00[c]	2.22±0.01[b]	2.19±0.00[bc]	2.28±0.00[a]	2.24±0.00[ab]
C4 (hot gel stability, Nm)	1.92±0.44[b]	2.03±0.02[a]	2.04±0.00[a]	2.07±0.00[a]	2.05±0.02[a]	2.02±0.05[a]	1.99±0.02[ab]
C5 (starch retrogradation in the cooling phase, Nm)	3.91±0.02[ab]	3.84±0.02[abc]	3.77±0.00[abc]	3.94±0.03[a]	3.72±0.07[bc]	3.74±0.11[abc]	3.65±0.01[c]

Data are means ± standard deviations of 2 replicates. Values with the same letter in a row do not differ significantly according to ANOVA (Tukey's test) at $P < 0.05$.

CWF: 100% wheat flour, 5%PSN 95% wheat flour with 5% non-extruded pecan shell powder, 5%PSE: 95% wheat flour with 5% extruded pecan shell powder, 10%PSN: 90% wheat flour with 10% non-extruded pecan shell powder, 10%PSE: 90% wheat flour with 10% extruded pecan shell powder, 15%PSN: 85% wheat flour with 15% non-extruded pecan shell powder, 15%PSE: 85% wheat flour with 15% extruded pecan shell powder.

Table 2. Physical properties of breads and tortillas produced with composite flours containing pecan shell powder (non-extruded and extruded) and wheat flour

Bread	**CNB**	**5%PNB**	**5%PEB**	**10%PNB**	**10%PEB**	**15%PNB**	**15%PEB**
∆Height (mm)	2.6±0.0^{a}	2.7±0.2^{a}	2.4±0.1^{a}	1.4±0.0^{b}	1.1±0.1^{bc}	1.3±0.2^{bc}	0.9±0.1^{c}
Specific volume (cm^3)	798±11^{a}	760±26^{ab}	800±14^{a}	668±19^{bcd}	677±24^{bc}	566±3^{d}	623±54^{cd}
Weight (g)	142±2^{a}	142±3^{a}	140±2^{a}	144±3^{a}	142±2^{a}	145±1^{a}	144±1^{a}
Crumb color							
*L**	76.6±0.6^{a}	59.3±1.5^{b}	55.9±1.0^{c}	45.2±0.4^{d}	42.8±0.3^{e}	38.4±0.5^{f}	36.8±0.2^{g}
*a**	0.1±0.2^{g}	6.5±0.3^{f}	7.5±0.3^{e}	8.3±0.1^{d}	9.7±0.2^{c}	8.9±0.4^{b}	11.1±0.1^{a}
*b**	15.8±1.1^{a}	11.8±0.2^{e}	12.9±0.5^{c}	11.7±1.1^{f}	13.1±0.5^{b}	11.4±0.2^{g}	12.6±0.4^{d}
C*	15.8±1.5^{c}	13.5±0.3^{g}	15.0±0.5^{d}	14.4±1.6^{f}	16.3±0.5^{b}	14.4±0.8^{e}	16.8±0.1^{a}
∆E*		18.9±1.6^{f}	22.1±0.6^{e}	32.7±0.4^{d}	35.2±0.2^{c}	39.4±0.7^{b}	41.4±0.2^{a}
Crust color							
*L**	42.2±0.2^{a}	37.3±0.1^{b}	33.5±0.1^{g}	36.8±0.2^{c}	35.5±1.1^{e}	36.7±0.2^{d}	34.1±0.1^{f}
*a**	11.3±1.0^{a}	7.8±0.3^{d}	8.0±0.4^{c}	5.5±0.3^{g}	8.9±0.5^{b}	5.8±1.4^{f}	6.8±0.2^{e}
*b**	16.9±0.9^{a}	16.3±0.5^{b}	10.7±1.3^{e}	10.1±0.9^{f}	12.9±0.5^{c}	9.8±0.9^{g}	11.7±0.9^{d}
C*	20.3±0.6^{a}	18.1±1.0^{b}	13.4±0.3^{e}	11.6±0.6^{f}	15.7±0.0^{c}	11.42±2.31^{g}	13.5±0.3^{d}
∆E*		6.1±0.6^{f}	11.1±0.5^{a}	10.4±0.4^{d}	8.1±0.5^{e}	10.5±0.5^{c}	10.6±0.5^{b}
Tortilla	**CNT**	**5%PNT**	**5%PET**	**10%PNT**	**10%PET**	**15%PNT**	**15%PET**
Diameter (cm)	13.5±0.5^{ab}	12.8±0.2^{ab}	12.2±0.0^{b}	12.9±0.6^{ab}	13.5±0.1^{a}	13.2±0.4^{ab}	13.4±0.0^{ab}
Thickness (cm)	2.9±0.3^{a}	3.0±0.3^{a}	2.9±0.1^{a}	3.2±0.3^{a}	3.2±0.1^{a}	3.2±0.0^{a}	3.2±0.3^{a}
Weight (g)	31.4±0.0^{a}	31.6±0.0^{a}	31.7±0.3^{a}	31.4±0.0^{a}	31.3±0.1^{a}	31.3±0.3^{a}	31.1±0.1^{a}
Color							
*L**	81.8±1.7^{a}	54.9±1.3^{b}	54.5±4.3^{bc}	50.5±0.6^{bc}	49.5±1.1^{bc}	47.7±0.2^{bc}	47.0±0.6^{c}
*a**	0.7±0.2^{c}	7.0±0.4^{b}	7.5±0.6^{ab}	8.1±0.3^{ab}	8.6±0.2^{a}	8.2±0.4^{ab}	8.7±0.0^{a}
*b**	17.7±0.9^{a}	11.5±1.0^{b}	11.0±0.2^{b}	11.9±1.2^{b}	12.1±0.5^{b}	10.9±0.2^{b}	10.8±0.5^{b}
C*	17.7±0.9^{a}	13.5±1.0^{b}	13.3±0.2^{b}	14.4±1.1^{b}	14.9±0.2^{ab}	13.7±0.4^{b}	13.8±0.4^{b}
∆E*		28.2±0.3^{b}	28.9±2.8^{b}	32.5±1.1^{ab}	33.7±0.6^{ab}	35.5±1.6^{a}	36.3±2.1^{a}

Data are means ± standard deviations of at least 2 replicates. Values with the same letter in a row do not differ significantly according to ANOVA (Tukey's test) at $P < 0.05$. CNB or CNT: Bread or Tortilla with 100% wheat flour, 5%PNB or PNT: Bread or Tortilla with 95% wheat flour and 5% non-extruded pecan shell powder, 5%PEB and PET: Bread and Tortilla with 95% wheat flour and 5% extruded pecan shell powder, 10%PNB and PNT: Bread and Tortilla with 90% wheat flour and 10% non-extruded pecan shell powder, 10%PEB and PET: Bread and Tortilla with 90% wheat flour and 10% extruded pecan shell powder, 15%PNB and PNT: Bread and Tortilla with 85% wheat flour and 15% non-extruded pecan shell powder, 15%PEB and PET: Bread and Tortilla with 85% wheat flour and 15% extruded pecan shell powder.

Table 3. Textural analysis of breads and tortillas produced with composite flours containing pecan shell powder (non-extruded and extruded) and wheat flour during four days of storage.

Bread traits	**Hardness (N)**			**Cohesiveness**			**Chewiness (N)**		
	Day 0	Day 1	Day 4	Day 0	Day 1	Day 4	Day 0	Day 1	Day 4
CNB	8.6±0.7^{cdB}	10.6±0.9^{dAB}	21.0±3.2^{bcdA}	0.60±0.10^{aA}	0.50±0.04^{abA}	0.50±0.01^{aA}	5.5±0.9^{abB}	5.3 ± 0.6^{abB}	9.7 ± 1.4^{aA}
5%PNB	9.1±1.0^{cdB}	13.0±1.3^{bdAB}	20.2±2.1^{cdA}	0.50±0.10^{abA}	0.40±0.10^{cA}	0.40±0.01^{bcA}	4.7±0.3^{bB}	4.9±0.6^{bB}	7.2±0.9^{bcA}
5%PEB	8.1±1.1^{dB}	12.2±1.0^{cdAB}	17.5±1.1^{dA}	0.60±0.10^{aA}	0.50±0.01^{abA}	0.40±0.01^{bcA}	5.1±0.6^{bB}	5.6±0.4^{abB}	9.8±1.4^{aA}
10% PNB	10.7±1.0^{bcB}	12.9±1.2$^{b\text{-}dAB}$	27.3±4.0^{abA}	0.50±0.10^{bA}	0.40±0.01^{bcA}	0.30±0.01^{bcA}	5.0±0.7^{bA}	5.0±0.8^{abA}	5.3±0.5^{cA}
10%PEB	11.9±1.5^{abA}	14.4±2.1$^{a\text{-}cA}$	27.3±3.2^{abA}	0.50±0.01^{abA}	0.40±0.10$^{a\text{-}cA}$	0.30±0.01^{bcA}	5.9±0.5^{abB}	5.9±0.5^{abB}	8.1±1.0^{abA}
15% PNB	14.6±1.4^{aA}	17.0±0.8^{aA}	26.9±4.3^{abA}	0.50±0.01^{bA}	0.40±0.01^{cA}	0.40±0.01^{bcA}	6.9±0.9^{aB}	6.2±0.5^{aB}	9.0±1.3^{abA}
15%PEB	12.8±1.5^{abA}	15.2±2.5^{abA}	28.6±4.1^{aA}	0.50±0.10^{bA}	0.40±0.01^{cA}	0.30±0.01^{cA}	5.8±0.4^{abB}	5.50±0.9^{abB}	7.9±0.9^{abA}

Tortilla traits	**Breaking force (g)**			**Extensibility (mm)**			**Subjective rollability**		
	Day 0	Day 1	Day 4	Day 0	Day 1	Day 4	Day 0	Day 1	Day 4
CNT	1045±100^{bA}	791±73^{abB}	570±70^{bcC}	26.0±1.4^{aA}	16.5±1.8^{aB}	10.2±1.0^{cB}	5.0±0.0^{aA}	4.9±0.1^{aA}	3.9±0.1^{aB}
5%PNT	1132±119^{abA}	894±96^{aB}	665±85^{abC}	24.1±2.0$^{a\text{-}cA}$	12.8±1.3^{bB}	12.2±1.4^{aB}	5.0±0.0^{aA}	4.6±0.1^{aA}	3.5±0.4^{aA}
5%PET	1040±126^{bA}	814±85^{aB}	571±74^{bcC}	20.1±1.6^{dA}	13.1±1.2^{bAB}	11.8±1.2^{abB}	5.0±0.0^{aA}	4.9±0.0^{aA}	3.3±0.4^{aB}
10%PNT	1004±119^{bA}	580±100^{cB}	424±43^{dC}	21.4±1.8^{cdA}	12.9±1.3^{bB}	10.2±0.4^{bcB}	5.0±0.0^{aA}	4.4±0.0^{aAB}	4.1±0.3^{aB}
10%PET	1239±128^{aA}	829±132^{aB}	734±83^{aC}	24.4±2.8^{abA}	14.7±1.8^{abA}	12.4±1.1^{aC}	5.0±0.0^{aA}	4.7±0.0^{aA}	4.2±0.1^{aB}
15%PNT	990±167^{bA}	607±84^{cB}	472±63^{cdC}	20.9±1.7^{dA}	13.2±1.0^{bB}	9.3±0.7^{cB}	5.0±0.0^{aA}	4.3±0.6^{aA}	3.6±0.6^{aA}
15%PET	1022±80^{bA}	651±118^{bcB}	457±43^{dC}	22.7±2.0$^{b\text{-}dA}$	13.9±1.2^{bAB}	9.8±1.1^{cB}	5.0±0.0^{aA}	4.1±0.1^{aA}	3.4±0.3^{aA}

Data are means ± standard deviations of 6 replicates. Values with the same letter in a column do not differ significantly according to ANOVA (Tukey's test) at $P < 0.05$. The letters on the top right corner of each value indicate significantly different values between samples (lower case) and the same sample between days (capital letters), according to ANOVA (Tukey's test) at $P < 0.05$. CNB or CNT: Bread or Tortilla with 100% wheat flour, 5%PNB or PNT: Bread or Tortilla with 95% wheat flour and 5% non-extruded pecan shell powder, 5%PEB or PET: Bread or Tortilla with 95% wheat flour and 5% extruded pecan shell powder, 10%PNB or PNT: Bread or Tortilla with 90% wheat flour and 10% non-extruded pecan shell powder, 10%PEB or PET: Bread or Tortilla with 90% wheat flour and 10% extruded pecan shell powder, 15%PNB or PNT: Bread or Tortilla with 85% wheat flour and 15% non-extruded pecan shell powder, 15%PEB or PET: Bread or Tortilla with 85% wheat flour and 15% extruded pecan shell powder

Table 4. Chemical composition, Total Phenolic Compounds (TPC) and Radical Scavenging Activity (RSA) (DPPH and ABTS*) of breads and tortillas produced with composite flours containing pecan shell powder (non-extruded and extruded) and wheat flour

Parameter	CNB	5%PNB	5%PEB	CNT	10%PNT	10%PET
Moisture (%)	32.2±0.0^{a}	30.4±0.0^{b}	29.9±0.0^{c}	33.8±0.0^{A}	32.3±0.3^{B}	32.3±0.0^{B}
Protein (%)	14.5±0.3^{a}	13.2±1.5^{a}	12.7±0.4^{a}	12.7±0.0^{A}	12.4±0.1^{B}	12.1±0.1^{C}
Fat (%)	4.2±0.2^{a}	4.4±0.4^{a}	4.3±0.9^{a}	13.2±0.0^{A}	12.6±0.0^{C}	13.0±0.0^{B}
Ash (%)	1.0±0.0^{a}	1.04.0±0.0^{a}	1.1±0.0^{a}	2.0±0.0^{A}	2.1±0.1^{A}	1.9±0.1^{A}
Total Carbohydrate (%)	51.9±0.1^{a}	50.9±0.5^{a}	52.1±0.3^{a}	58.7±0.0^{B}	60.6±0.1^{A}	60.6±0.0^{A}
Available Carbohydrate (%)	44.4±0.1^{a}	41.7±0.4^{b}	40.9±0.3^{b}	52.7±0.1^{A}	46.9±0.2^{B}	44.6±0.0^{C}
Starch (%)	64.3±0.0^{a}	59.7±0.0^{b}	57.9±0.0^{b}	59.0±0.3^{A}	49.6±0.2^{B}	49.3±0.0^{B}
Total dietary fiber (%)	3.7±0.1^{c}	9.3±0.1^{b}	11.1±0.2^{a}	5.6±0.2^{C}	13.7±0.6^{B}	16.0±0.0^{A}
Soluble dietary fiber (%)	0.9±0.0^{c}	1.3±0.0^{b}	2.0±0.1^{a}	1.9±0.0^{C}	2.6±0.1^{B}	4.0±0.0^{A}
Insoluble dietary fiber (%)	2.8±0.0^{c}	8.0±0.1^{b}	9.2±0.3^{a}	3.8±0.2^{B}	11.1±0.5^{A}	12.0±0.0^{A}
TPC (mg GAE/g)	3.8±0.0^{b}	4.5±0.1^{a}	4.0±0.2^{a}	2.5±0.2^{B}	3.7±0.1^{A}	3.8±0.1^{A}
DPPH (%)	25.2±0.2^{c}	3579.0±0.4^{b}	50.0±0.8^{a}	15.3±0.0^{C}	20.5±0.2^{B}	30.1±0.1^{A}
ABTS^{+} (µmols TE /100 g)	478.1±0.1^{b}	501.1±0.1^{a}	518.2±0.4^{a}	266.0±0.1^{B}	288.2±0.1^{A}	305.3±0.2^{A}

Data are means ± standard deviations of at least 4 replicates. Values with the same letter within the same product (bread or tortilla) and parameter do not differ significantly according to ANOVA (Tukey's test) at $P < 0.05$. CNB or CNT: Bread or Tortilla with 100% wheat flour, 5%PNB: Bread with 95% wheat flour and 5% non-extruded pecan shell powder, 5%PEB: Bread with 95% wheat flour and 5% extruded pecan shell powder, 10% PNT: Tortilla with 90% wheat flour and 10% non-extruded pecan shell powder, 10%PET: Tortilla with 90% wheat flour and 10% extruded pecan shell powder. DPPH:2,2-diphenyl-1-picrylhydrazi; ABTS^{+}: 2, 2′-Azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid); GAE: Gallic acid equivalent; TE: Trolox equivalent

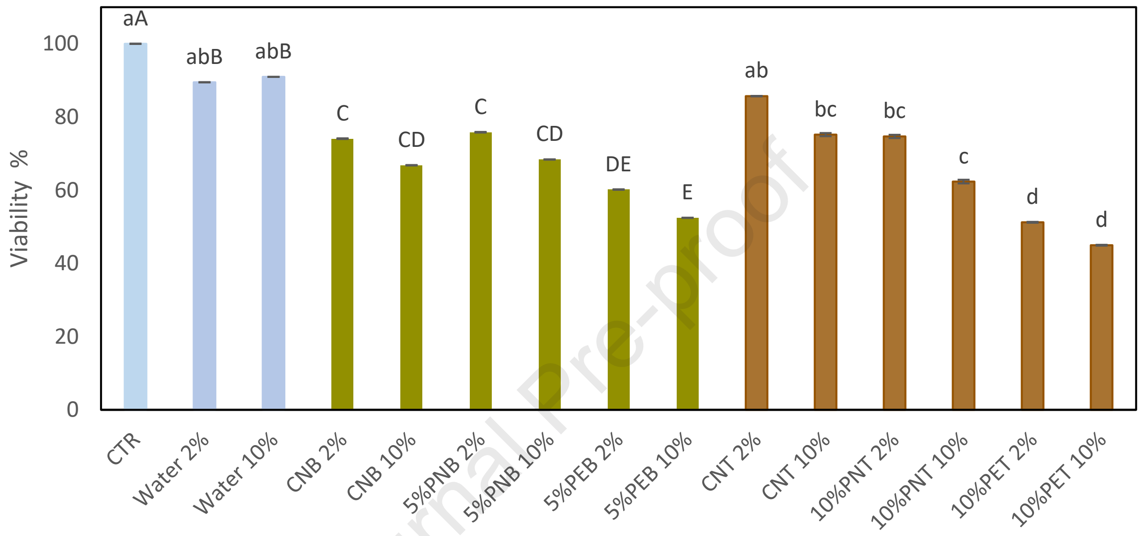

Fig 1. Growth inhibitory potency of breads and tortillas enriched with non-extruded and extruded pecan shell powder in comparison to control wheat bread (CNB) and tortillas (CNT) in cancer hepatic cell line (HepG2). Cell viability was assayed at 48 h using two different concentrations (2 and 10%) of each extract. Negative controls consisted in non-treated cells (CTR), cells treated with 2 and 10% of water, and with 2 and 10% of extract of wheat bread and tortilla (CNB and CNT). CNB or CNT, bread or tortillas produced with 100% wheat flour, respectively; 5%PNB, bread produced with 95:5 wheat flour: non-extruded pecan shell powder; 5%PEB, bread produced with 95:5 wheat flour: extruded pecan shell powder); 10%PNT, tortilla produced with 90:10 wheat flour: non-extruded pecan shell powder; 10%PET, tortilla produced with 90:10 wheat flour: extruded pecan shell powder. Different letters: a-d indicate significant differences between tortilla treatments and the negative controls (CTR, water, CNB and CNT) ($P < 0.05$). Different letters: A-E indicate significant differences between bread treatments and the negative controls (CTR, water, CNB and CNT) ($p < 0.05$). Data are means ± standard deviations of 3 replicates.

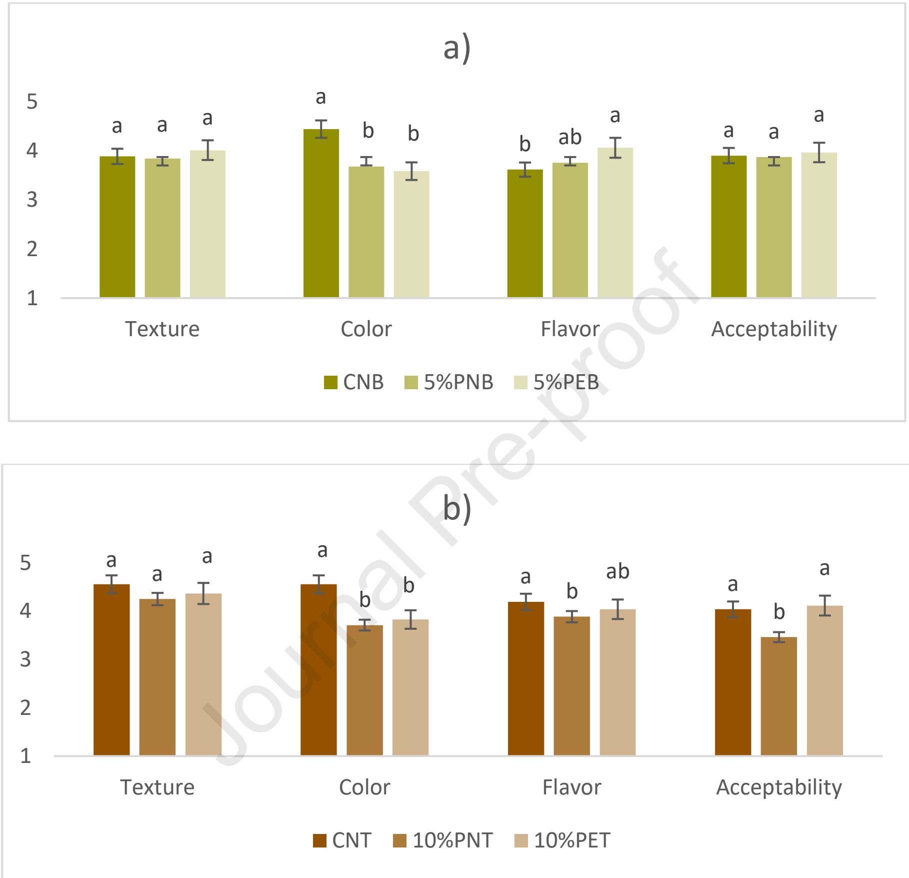

Figure 2. Score of sensory analysis of breads (a) and tortillas (b) enriched with non-extruded and extruded pecan shell powder. Letters on the top of each bar indicate significantly different values between samples, according to ANOVA (Tukey's test) at $P < 0.05$. *Note*. Panelists scored using a 5-point hedonic scale: 1, nothing; 2, a little bit; 3, regular; 4: moderate; 5: a lot. Data are means ± standard deviations of 70 replicates. CNB or CNT, breads or tortillas produced with 100% wheat flour, respectively; 5%PNB, bread produced with 95:5 wheat flour: non-extruded pecan shell powder; 5%PEB, bread produced with 95:5 wheat flour: extruded pecan shell powder); 10%PNT, tortilla produced with 90:10 wheat flour: non-extruded pecan shell powder; 10%PET, tortilla produced with 90:10 wheat flour: extruded pecan shell powder.

- Extruded pecan shell powder increased total dietary fiber in bread and tortillas
- Extruded pecan shell increased *in vitro* antioxidant activity in bread and tortillas
- Breads and tortillas containing extruded pecan shell powder had good acceptability
- Extracts enriched with extruded shell powder showed viability reduction of cancer cells

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: