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Antioxidant properties of Pecan nut [*Carya illinoinensis* (Wangenh.) C. Koch] Shell infusion

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RESUMEN

Propiedades antioxidantes de la infusión de la cáscara de nuez Pecana [*Carya illinoinensis* (Wangenh.) C. Koch]

La composición nutricional de la cáscara de nuez Pecana [*Carya illinoinensis* (Wangenh.) C. Koch] y los contenidos de fenoles totales y de taninos condensados de la infusión de la cáscara de nuez Pecana se determinaron en este trabajo. La actividad antioxidante de la infusión se evaluó a través de los sistemas ABTS, DPPH y β-caroteno/ácido linoleico. La cáscara presentó un contenido elevado de fibras (48% ± 0,06), el contenido de fenoles totales varió de 116 a 167 mg GAE/g y el de taninos condensados se encontró entre 35 y 48 mg CE/g. La actividad antioxidante varió entre 1112 y 1763 µmol TEAC/g en el sistema ABTS. Por el método DPPH, la actividad antioxidante fue de 305 a 488 mg TEAC/g (30 minutos de reacción) y de 482 a 683 mg TEAC/g (24 h de reacción). El porcentaje de inhibición de la oxidación que se obtuvo en el sistema β-caroteno/ácido linoleico varió de 70 a 96%. Los resultados indicaron un elevado contenido de fenoles y una elevada actividad antioxidante para la infusión de la cáscara de nuez Pecana.

PALABRAS CLAVE: Actividad antioxidante – Cáscara de nuez Pecana – Compuestos fenólicos – Composición nutricional – Infusión – oxidación.

SUMMARY

Antioxidant Properties of Pecan Nut [*Carya illinoinensis* (Wangenh.) C. Koch] Shell Infusion

The nutritional composition of Pecan nut [*Carya illinoinensis* (Wangenh.) C. Koch] shells and the total phenolic and condensed tannin contents of Pecan nut shell infusion were determined and the antioxidant activity of the infusion was evaluated through ABTS, DPPH and β-carotene/linoleic acid systems. The shell presented high fiber content (48% ± 0.06), the total phenolic content ranged from 116 to 167 mg GAE/g and the condensed tannin content was between 35 and 48 mg CE/g. The antioxidant activity varied from 1112 and 1763 µmol TEAC/g in the ABTS system. In the DPPH method, the antioxidant activity was from 305 to 488 mg TEAC/g (30 minutes reaction) and from 482 to 683 mg TEAC/g (24 h reaction). The oxidation inhibition percentage obtained in the β-carotene/linoleic acid system varied from 70 to 96%. The results indicated the high phenolic content and antioxidant activity of Pecan nut shell infusion.

KEY-WORDS: Antioxidant activity – Iinfusion – Nutritional composition – Oxidation – Pecan nut shell – Phenolic compounds.

1. INTRODUCTION

Many studies have shown an inverse relationship between the consumption of fruit, vegetables and cereals and the incidence of diseases such as cardiovascular diseases and cancer, among others. These diseases are associated with oxidation in biological systems due to the action of free radicals (FR) in the organism (Scalbert and Williamson, 2000). FR are molecules that possess an isolated electron which makes them unstable and extremely reactive species, responsible for alterations in certain cellular components, possibly initiating oxidation reactions in the fatty acids of the lipoprotein membrane, affecting its structural and functional integrity. Oxidative processes can be retarded by the modification of ambient conditions or by the use of antioxidant substances (Soares 2002, Rice-Evans *et al*., 1997).

Several *in vivo* and *in vitro* assays have shown the antioxidant activity of a wide variety of substances, among them the phenolic compounds. This biological activity has been attributed to the oxidation-reduction properties of phenolic compounds, playing an important role in the absorption or neutralization of free radicals (Basile *et al*., 2005).

Among traditionally used foods, nuts have been recommended in the diet due to their positive health effects. The Pecan nut [*Carya illinoinensis* (Wangenh.) K. Koch] belongs to the *Juglandaceae* family and is native of the south of the United States and north of Mexico (Hancock, 1997). In Brazil, the *Carya* genus, known as "nogueira-pecã", grows in the southern and south-eastern regions (Joly, 1993). The Pecan nut presents low saturated fatty acid contents and high levels of monounsaturated and polyunsaturated fatty acids. Evidence also indicates the presence of bioactive molecules, such as sterols and tocopherols, and a high content of total phenolic compounds, with possible natural antioxidant activity (Kornsteiner *et al*., 2006, Kris-Etherton *et al*., 1999). An important group of phenolic compounds present in vegetables are tannins, secondary plant metabolites, which can be found in the condensed or hydrolyzed form. These molecules are of great nutritional and medicinal interest due to their potent antioxidant capacity (Santos-Buelga and Scalbert, 2000).

Pecan nut processing results in a relatively high volume of shell (40 to 50%), an industrial byproduct of reddish color, that can constitute an alternative source of compounds with high antioxidant capacity (Worley, 1994). In the south of Brazil, Pecan nut shell is used for infusion, but there are no studies related to the composition of the shell and to the presence of compounds with possible positive effects. Thus, the objective of the present work was to determine the nutritional composition, the total phenolic compound and condensed tannin contents of Pecan nut shells and to evaluate the *in vitro* antioxidant activity of Pecan nut shell infusion through ABTS [2,2'-azino-bis-(3-ethylbenzotiazoline-6-sulphonic acid)]; DPPH (2,2-diphenyl-1-picrylhydrazyl) and β-carotene/linoleic acid systems. The antioxidant activity of the infusion determined through the β-carotene/linoleic acid system was compared to a commercial antioxidant composed of a mixture of natural tocopherols recommended for use in fats and oils and to mixtures of infusion and tocopherols in equal proportions.

2. MATERIALS AND METHODS

2.1. Material

Pecan nut shells from 3 different batches (2 kg each) of a mixture of the following varieties: Barton (approximately 50%), Shoshone, Shawnee, Choctaw and Cape Fear harvested in 2006, were used.

A commercial antioxidant, indicated for use in animal and vegetable fats and oils (from 100 to 500 ppm), composed of a mixture of natural tocopherols (MT), containing 0.64 g tocopherols/g of product (of which 58.6% was γ-tocopherol, 25.1% δ-tocopherol, 14.7% α-tocopherol and 1.6% β-tocopherol) was used for the comparison of antioxidant activity.

2.2 Nutritional composition

The nutritional composition of the Pecan nut shells was determined according to official Association of Official Analytical Chemists (AOAC, 2005) methodology through the following analyses: moisture (925.09); protein (920.87); total lipids (920.85); total, soluble and insoluble dietary fiber (991.43) and minerals (923.03); carbohydrates were estimated by difference.

2.3. Preparation of Pecan nut shell infusion

After grinding in a food processor, Pecan nut shells were dried in a renewed air oven (model 400/D 200 °C, Nova Ética®), milled in an analytical laboratory mill (model A-11, IKA Works®) and passed through a 60 mesh sieve. Two grams of sample were used to prepare an infusion in 100 mL distilled water (20 g/L) at a temperature of 98/100 °C for 10 minutes followed by immersion in an ice bath. Filtration was then carried out under vacuum, using Whatman 541 (125 mm) filter paper. Volumes were completed to 100 mL and the extracts obtained in triplicates were stored in amber flasks, in a nitrogen atmosphere, and frozen until further analysis (Wilkinson, 2000). Dry weight was determined gravimetrically by placing a 5 mL aliquot in a previously weighed porcelain crucible followed by drying in an oven at 105.0 ± 0.5 °C until reaching a constant weight (AOAC, 2005).

2.4. Determination of total phenolic compounds

The total phenolic compound content was estimated using the Folin-Ciocalteau colorimetric method with some modifications (Beal, 2006, Budini *et al.,* 1980). Aliquots (100 μL) of appropriate dilutions of the infusion extracts were oxidized with 500 μL Folin-Ciocalteau reagent. The reaction was neutralized with saturated sodium carbonate (75 g/L; 1.5 mL) and the volumes were completed to 10 mL by adding deionized water. After incubation for 2 hours at ambient temperature, the absorbance of the resulting blue solution was measured at 764 nm. The analyses were carried out in triplicate. The quantification was made using a gallic acid standard curve and the results were expressed as mg GAE/g (gallic acid equivalent) of dry weight.

2.5. Determination of condensed tannin content

To determine the condensed tannin content, the procedure described by Price *et al.* (1978) and adapted by Villarreal-Lozoya *et al.* (2007) was used. Aliquots of 1 mL of appropriate dilutions of the extracts were collected and placed in 2 separate test tubes (1 for the sample and the other for the blank). Then, 5 mL of vanillin reagent (0.5 g reagent and 200 mL 4% HCl/methanol) were added to the samples and 4% HCl/methanol to the blank. The test tubes were kept in a dark place for 20 minutes and the absorbances were measured in a spectrophotometer at 500 nm. The analyses were carried out in triplicate and the results were expressed as mg CE/g (mg catechin equivalent/g of defatted sample) of dry weight.

2.6. Determination of antioxidant capacity

ABTS assay

The ABTS [2,2'-azino-bis-(3- ethylbenzotiazoline-6-sulphonic acid)] assay was carried out according to Re *et al.* (1999), with some modifications. After preparing the ABTS radical (7 mM − 0.03836 g ABTS dissolved in 10 mL deionized water), a potassium persulfate solution (2.45 mM - 10 mL ABTS and 10 mL persulfate mixed, homogenized and kept in an amber flask for at least 16 hoursand protected from light) was prepared. For the sample, an aliquot of 200 μL of the radical formed was

pipetted and diluted in 10 mL 96° ethanol of analysis grade. Absorbance readings at 734 nm, in 10 mm cuvettes, were made to certify that optical density was around 0.700 ± 0.05. An aliquot of 980 μL of the diluted radical was pipetted and transferred to a 10 mm (1 mL) cuvette, absorbance was measured (A_{734} = A_0) and 20 μL of the sample were added immediately, homogenizing and agitating for a few seconds. Trolox was used as standard (0.13209 g/500 mL H_2O). The calculation of the radical inhibition percentage was made using the following formula: per cent radical inhibition = (1 − A_f/Ao) × 100, where, "Ao" is initial absorbance and "A_f" is the final absorbance. The calculations were made for each concentration of the samples analyzed, so a dose-response curve could be obtained with the results. The results were expressed as μmol TEAC/g (Trolox equivalent antioxidant activity) of dry weight. Analyses were carried out in triplicate.

DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was carried out according to Brand-William *et al.* (1995) followed by modifications, as described by Kim *et al.* (2002) and Villarreal-Lozoya *et al.* (2007). After preparing the radical (0.1 mM – 0.03943 g DPPH dissolved in 10 mL 80% methanol), an aliquot of 2.9 mL was pipetted and placed in test tubes with 0.1 mL infusion extract. The samples were kept in a dark place, and absorbance was measured in a spectrophotometer at 515 nm, after 30 minutes and after 24 hours. Trolox was used as standard (150 mg/L). The calculation of the radical inhibition percentage was made using the following formula: per cent radical inhibition = (1 – A_f/Ao) x 100, where, "Ao" is the initial absorbance and "A_f" is final absorbance. The calculations were made for each concentration of the samples analyzed, so a dose-response curve could be obtained with the results. The analyses were carried out in triplicate and the results expressed as mg TEAC/g (Trolox equivalent antioxidant activity) of dry weight.

β-*carotene/linoleic acid assay*

The *in vitro* antioxidant activity of the β-carotene and linoleic acid model system was determined following the methodology described by Marco (1968) and modified by Miller (1971). Beta-carotene was dissolved in chloroform (20 mg/mL). An aliquot of 20 μL of this solution was put in an erlenmeyer flask with 40 μL linoleic acid, 1 mL chloroform and 500 mg Tween 40. Chloroform was completely evaporated with nitrogen vapor. Then, 150 mL distilled deionized water saturated with oxygen (30 min.) were added to the β-carotene emulsion and the mixture was shaken. Two hundred, 300 and 500 ppm of the infusion extracts were added along with equal concentrations of a commercial antioxidant composed of a mixture of natural tocopherols (MT), recommended for use in fats and oils in general, and used as standard antioxidant. Also, the synergistic effect was evaluated through the mixture of infusion extracts (E) and tocopherols (MT) in concentrations of 100, 150 and 250 ppm each (E + MT, 1:1). Absorbance readings at 470 nm were carried out at 15 minute intervals over a period of 2 hours, keeping the cuvettes in a water bath at 50°C. Antioxidant activity was calculated as the inhibition percentage, relative to the control, using the following equation: AA = [(As − Ac)/(Ao − Ac)] × 100, where As and Ac are, respectively, the absorbances of the sample and of the control at the end of the analysis and Ao is the absorbance of the sample (470 nm) at the beginning of the assay. Analyses were carried out in triplicate.

2.7. Statistical analysis

The statistical analysis of the data was carried out using the SAS "for Windows" program. Results were submitted to analysis of variance (ANOVA) and the Tukey test ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Nutritional composition

The results of the nutritional composition of Pecan nut shells are presented in Table 1.

According to the results, fibers represent the main fraction of Pecan nut shells (48.6%), followed by carbohydrates (29.6%), moisture (16.8%) and low contents of proteins (2.2%) and total lipids (1.1%).

3.2. Phytochemical Analysis and Antioxidant Activity (ABTS and DPPH)

The contents of Dry Extract (DE), Total Phenolic Compounds (TP) and Condensed Tannins (CT) and the Antioxidant Capacity (AC) of Pecan nut shell infusion can be observed in Table 2.

The results obtained for the dry extracts of the different batches show a higher yield in the extraction procedure of batch 3 (29.6 ± 2.3 g/100 g) which also presented a significantly ($p < 0.05$) higher content of phenolic compounds (167 ± 9.8 mg GAE/g). Batches 1 and 2 presented similar extraction yields (21 g GAE/100 g) and the difference in their phenolic compound content was not significant (117 ± 6.7 and 131 ± 11.5 mg GAE/g, respectively).

Villarreal-Lozoya *et al.* (2007) reported a total phenolic compound content of 448 mg CAE/g (chlorogenic acid equivalents) or 80 mg GAE/g (gallic acid equivalents) in Pecan nut shell extracts in an acetone-water (70:30, v/v) mixture, assuming a conversion factor of 0.6 from CAE to GAE (Kyoung Chun and Kim, 2004). These values are lower than those found in the present study, which were also higher than those found for raw materials

Table 1
Nutritional composition of Pecan nut shells

Component (g/100 g)[1]	Mean ± SD (n = 3)
Protein[2]	2.2 ± 0.04
Moisture	16.8 ± 0.1
Total lipids	1.1 ± 0.1
Minerals	1.4 ± 0.03
Total fiber	48.6 ± 0.06
Soluble fiber	3.1 ± 0.09
Insoluble fiber	45.4 ± 0.4
Carbohydrates[3]	29.6
Caloric value[4] (kcal/100 g)	331

[1]Means ± SD (n = 3); [2]N x 6.25; [3]Calculated by difference: 100 − (% total lipids + % proteins + % moisture + % minerals + % total fiber). [4]Calculated by the sum of the percentages of proteins and carbohydrates multiplied by the factor 4 (kcal/g) plus the content of total lipids multiplied by 9 (kcal/g).

considered rich in phenolic compounds, such as cloves, which, according to Wu *et al.* (2004), presented 113.19 mg GAE/g and green infusion with 117.30 mg GAE/g, according to Samman *et al.* (2001).

The condensed tannin content determined did not differ significantly ($p < 0.05$) among the batches (35 ± 12.8; 47 ± 8.30 and 48 ± 14.8 mg CE/g for batches 1, 2 and 3, respectively). These values are in accordance with those found by Villarreal-Lozoya *et al.* (2007) who reported a mean of 42 ± 1.5 mg CE/g in different cultivars analyzed.

With respect to the antioxidant activity determined through the ABTS system, the extracts obtained from batch number 3 presented significantly ($p < 0.05$) higher values than those found for batches 1 and 2 (1763 ± 8.6; 1112 ± 13.8 and 1338 ± 19.5 μmol TEAC/g, respectively).

Similar results were observed for the antioxidant activity determined using the DPPH radical during a 30 minute reaction, with significantly ($p < 0.05$) higher values for batch 3 (488 ± 42 mg TEAC/g) when compared to the values determined for batches 1 and 2 (305 ± 43.4 and 361.32 ± 53.94 mg TEAC/g, respectively). Using the same procedure, but evaluating after a 24 hour reaction with the DPPH radical, batch 3 also presented greater activity (683.75 ± 34.61 mg TEAC/g) followed by batch 2 (550 ± 63.72 mg TEAC/g). Batch 1 presented significantly ($p < 0.05$) lower activity when compared to batches 2 and 3 (482 ± 61.5 mg TEAC/g).

Villarreal-Lozoya *et al.* (2007) reported a lower mean antioxidant activity (487 ± 42 mg TEAC/g) for Pecan nut shell extracts in an acetone-water (70:30, v/v) mixture, when compared to the results obtained for batches 2 and 3 in the present study. The same researchers demonstrated a high antioxidant activity of Pecan nut shells, presenting total phenolic compound content, antioxidant capacity (DPPH) and condensed tannins approximately 6, 4.5 and 18 times higher, respectively, than that of the Pecan nut.

In Figure 1, the correlation between the total phenolic compound content (TP) and the antioxidant activity determined through the ABTS and DPPH systems can be observed.

According to the results, it is possible to observe a significant ($p < 0.05$) correlation between the total phenolic compound content in Pecan nut shells and the antioxidant activity of the extracts, measured in both ABTS ($r = 0.9973$) and DPPH ($r = 0.9980$) systems. The sample that presented the highest phenolic compound content (batch 3), also presented a significantly ($p < 0.05$) higher antioxidant activity in both of the systems studied.

3.3. Antioxidant Activity of the β-carotene and Linoleic Acid Model System

The oxidation inhibition percentage of the mixture of natural tocopherols used as standard (MT), of Pecan nut shell infusion and of the mixtures of infusion and natural tocopherols in equal proportions (100, 150 and 250 ppm each) measured *in vitro* through the β-carotene and linoleic acid model system can be observed in Table 3.

The oxidation inhibition percentage was significantly ($p < 0.05$) different for the different concentrations of each batch, except for the concentrations of 200 and 300 ppm of batch 2, which did not present a significant statistical difference.

Table 2
Dry extract, total phenolic compounds, condensed tannins and antioxidant capacity of Pecan nut shell infusion

	DE (g/100 g)*	TP (mg GAE/g)*	CT (mg CE/g)*	AC $_{ABTS}$ (μmol TEAC/g)*	AC $_{DPPH}$ 30 min (mg TEAC/g)*	AC $_{DPPH}$ 24 h (mg TEAC/g)*
Batch	01 21^{b} ± 1.3	117^{b} ± 6.7	35^{a} ± 12.8	1112^{b} ± 13.8	305^{b} ± 43.4	482^{b} ± 61.5
Batch	02 21^{b} ± 3.3	131^{b} ± 11.5	47^{a} ± 8.3	1338^{b} ± 19.5	361^{b} ± 54	550^{ab} ±63.7
Batch 03	29^{a} ± 2.3	167^{a} ± 9.8	48^{a} ± 14.8	1763^{a} ± 8.6	488^{a} ± 42	684^{a} ± 34.6
Mean	23 ± 5	138 ± 26	43 ± 7	1404 ± 330	385 ± 94	572 ± 102

*DE = Dry Extract (gravimetric assay), TP = Total Phenolics - mg GAE/g (gallic acid equivalent) of dry weight (Folin-Ciocalteau assay), CT = Condensed Tannins - mg CE/g (mg catechin equivalent/g of defatted sample) of dry weight (vanillin assay), AC = Antioxidant Capacity - μmol TEAC/g (Trolox equivalent antioxidant activity) of dry weight (ABTS), Antioxidant Capacity (DPPH – 30 minutes), Antioxidant Capacity - mg TEAC/g (Trolox equivalent antioxidant activity) of dry weight (DPPH – 24 hours); [a, b] means with the same superscript letter in the same column are not significantly different (Tukey test, $p < 0.05$); overall mean values ± SD (n = 3).

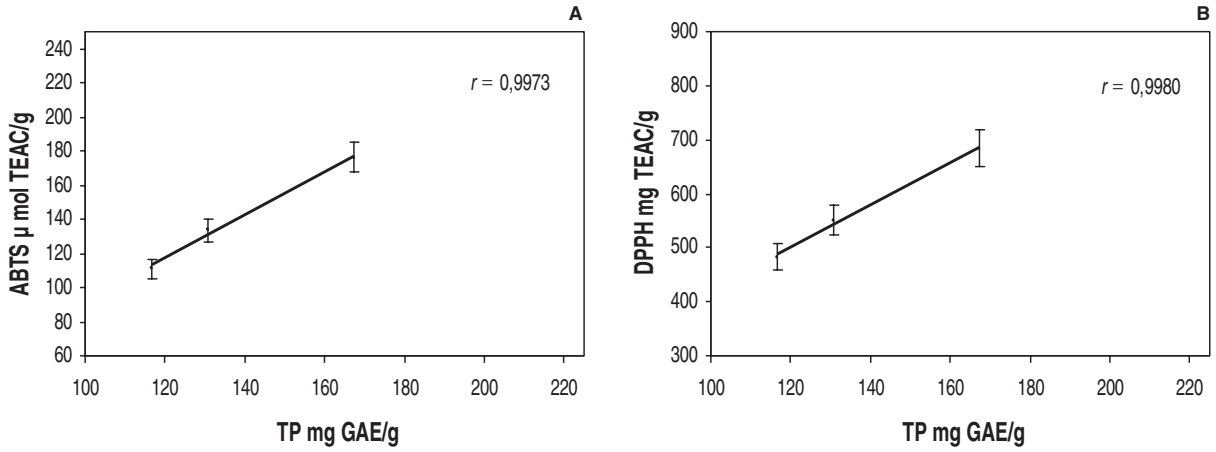

Figure 1
(A) Correlation between antioxidant capacity measured in the ABTS assay and total phenolic compound content (TP).
(B) Correlation between antioxidant capacity measured in the DPPH assay (24 hours) and total phenolic compound content (TP).

Comparing the results obtained for the different batches, batch 3 presented significantly ($p < 0.05$) higher values than those obtained for batch 1 for all the infusion and infusion plus tocopherol mixture concentrations. Between batches 2 and 3, a significant difference was observed only for the 300 ppm concentration. Between batches 1 and 2, there were significant differences for the concentrations of 200 and 500 ppm.

The 500 ppm concentration in the 3 conditions studied (tocopherols, infusion and mixture of tocopherols and infusion) presented a significantly ($p < 0.05$) higher inhibition percentage and Pecan nut infusion presented a significantly ($p < 0.05$) higher oxidation inhibition percentage in all the concentrations studied when compared to the tocopherol standard and the mixtures of tocopherols and Pecan nut infusion.

The infusion extracts presented the highest oxidation inhibition percentages when compared to the tocopherols and the infusion plus tocopherol mixtures, indicating that there was no synergistic effect between the components. These results do not agree with the "polar paradox theory" according to which hydrophilic antioxidants are more effective in non-polar media, while lipophilic coumpouds are better antioxidants in polar media (Frankel *et al*., 1994). On the other hand, some authors have reported that some compounds do not follow the polar paradox rule or that the partitioning of antioxidants between the oil and water phases depends on different factors. This suggests that antioxidant activity is affected by complex phenomena and that polarity is not the only parameter to be taken into account (Cuvelier *et al*., 2000; Gordon *et al*., 2001). According to Prat and Hudson (1990), the structural characteristics of an antioxidant, such as the localization of hydroxyl groups, seem to be more important than extract polarity. Besides, the complex composition of an extract can lead to some interactions between its components such as a synergistic, additive or antagonic effect.

Table 3
Oxidation inhibition percentage of tocopherols (MT), Pecan nut shell infusion extracts (E) and mixtures of extracts and tocopherols in the same proportions (E+MT 1:1), measured *in vitro* through the β-carotene and linoleic acid model system

Sample	Oxidation inhibition percentage in different concentrations		
	200 parts per million	300 parts per million	500 parts per million
MT (standard)	$21.7^{C} \pm 0.2$	$40.6^{B} \pm 0.2$	$57.6^{A} \pm 0.6$
Extract - Batch 01	$70.2^{Cb} \pm 2.2$	$79^{Bc} \pm 0.6$	$89.5^{Ab} \pm 0.7$
Extract - Batch 02	$81.1^{Ba} \pm 0.9$	$85.3^{Bb} \pm 0.1$	$95.3^{Aa} \pm 0.1$
Extract - Batch 03	$84.3^{Ca} \pm 1.5$	$89^{Ba} \pm 0.9$	$96.4^{Aa} \pm 0.8$
E + MT - Batch 01	$55.5^{Cb} \pm 0.2$	$70.9^{Bb} \pm 1.2$	$79^{Ab} \pm 0.1$
E + MT - Batch 02	$70.8^{Ba} \pm 1.9$	$75.4^{Bab} \pm 1.9$	$85.4^{Aa} \pm 1$
E + MT - Batch 03	$67.9^{Ca} \pm 0.9$	$79.2^{Ba} \pm 1.2$	$87.9^{Aa} \pm 1.7$

E+MT 1:1 (100, 150 and 250 ppm each); ^{A,B,C} values with the same superscript uppercase letter in the same line are not significantly different (Tukey $p < 0.05$); $^{a, b, c}$ values with the same superscript lowercase letter in the same column are not significantly different (Tukey $p < 0.05$) among the batches, for the same kind of sample (Extract or E + MT); results expressed as percentage (per cent); overall mean values ± SD ($n = 3$).

4. CONCLUSIONS

The results obtained indicated high total phenolic compound and condensed tannin contents in Pecan nut shell infusion, as well as a high antioxidant activity determined through different methodologies. More research is needed to isolate, identify and quantify phenolic compounds from Pecan nut shells so as to investigate the individual contribution of each compound on the total antioxidant activity. Studies related to *in vivo* activity and toxicological experiments are necessary for the safe use of Pecan nut infusion.

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REFERENCES

AOAC 2005. Association of Official Analytical Chemists. Official methods of analysis of the AOAC. 18th. Ed. AOAC, Arlington, VA.

Basile A, Ferrara L, Del Pozzo M, Mele G, Sorbo S, Bassi P, Montesano D. 2005. Antibacterial and antioxidant activities of ethanol extract from *Paullinia cupana* Mart, *Journal Ethnopharmacol* **102**, 32-36.

Beal BH. 2006. *Atividade antioxidante e identificação dos ácidos fenólicos do gengibre (Zingiber officinale Roscoe)*. 87 p. Dissertação (Mestrado em Ciência dos Alimentos) – Centro de Ciências Agrárias, Universidade Federal de Santa Catarina, Florianópolis.

Brand-Williams W, Cuvelier AE, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity, *Lebensm.-Wiss. u.- Technol* **28**, 25 - 30.

Budini R, Tonelli D, Girotti S. 1980. Analysis of total phenols using the Prussian blue method. *Journal of Agricultural and Food Chemistry* **28**, 1236-1238.

Cuvelier ME, Bondet V, Berset C. 2000. Behavior of phenolic antioxidants in a partitioned medium: structure-activity relationship. *Journal of the American Oil Chemists' Society* **77**, 819-823.

Frankel EN, Huang, SW, Kanner J, German JB. 1994. Interfacial phenomena in the evaluation of antioxidants: bulk oils vs. emulsions. *Journal of Agricultural and Food Chemistry* **42**, 1054-1059.

Gordon MH, Paiva-martins F, Almeida M. Antioxidant activity of hydroxytyrosol acetate compared with that other olive oil polyphenols. 2001. *Journal of Agricultural and Food Chemistry* **49**, 2480-2485.

Hancock, B.G. *1997. Texas Pecan Handbook. Development of Pecan Industry,* Texas Agricultural Extension Service: College Station, TX.

Joly, A.B. 1993. *Botânica: Introdução à taxonomia vegetal,* Nacional, São Paulo.

Kim DO, Lee KW, Lee HJ, Lee CY. 2002. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolics phytochemicals. *Journal Agricultural Food Chemistry* **50**, 3713 - 3717.

Kornsteiner M, Wagner KH, Elmadfa I. 2006. Tocopherols and total phenolics in 10 different nut types. *Food Chemistry* **98**, 381 - 387.

Kris-Etherton PM, Yu-Poth S, Sabaté J, Ratcliffe HE, Zhao G, Etherton TD. 1999. Nuts and their bioactive constituents: effects on serum lipids and other factors that affect disease risk. *American Journal of Clinical Nutrition* **70**, 504 - 511.

Kyoung Chun OK, Kim D. 2004. Consideration on equivalent chemicals in total phenolic assay of chlorogenic acid-rich plums. *Food Research International* **37**, 337 - 342.

Marco GJ. 1968. A rapid method for evaluation of antioxidants. *Journal of the American Oil Chemist's Society* **45**, 494-598.

Miller HE. 1971. A simplified method for the evaluation of antioxidants. *Journal of the American Oil Chemist's Society* **48**, 91.

Prat, DE. 1990. Natural antioxidants not exploited commercially, In: Prat, D.E (Ed). Food antioxidants, Elsevier Apllied Science, London, p. 171-192.

Price ML, Van Scoyoc S, Butler LG. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum. *Journal of Agricultural and Food Chemistry* **26**, 1214 - 1218.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cátion decolorization assay. *Free Radical Biology & Medicine* **26**, 1231 -1237.

Rice-Evans CA, Miller NJ, Pagança G. 1997. Antioxidant properties of phenolic compounds. *Trends in plant science*, **2**, 152 - 159.

Samman, S., Sandström, B., Toft, M.B., Bukhave, K., Jensen, M., & Sorensen, S.S. 2001. Green tea or rosemary extract added to foods reduces nonheme-iron absorption, *American Journal of Clinical Nutrition* **73**, 607 - 612.

Santos-Buelga CE, Scalbert A. 2000. Proantocyanidins and tannin-like compounds-nature, occurrence dietary intake and effects on nutrition and health. *Journal of the Science of Food and Agriculture* **80**, 1094 - 1117.

Scalbert A, Williamson G. 2000. Dietary intake and bioavail-abbility of polyphenols. *Journal of Nutrition*, **130** (Suppl. 8S), 2073S - 2085S.

Soares SE. 2002. Ácidos Fenólicos como antioxidantes. *Revista de Nutrição* **15**, 71 - 81.

Villarreal-Lozoya JE, Lombardini L, Cisneros-Zevallos. 2007. Phytochemical constituents and antioxidant capacity of different pecan [*Carya illinoinensis* (Wangenh.) K. Koch] cultivars. *Food Chemistry* 102, 1241-1249.

Wilkinson J. 2000. Efffect of ginger tea on the fetal development of Sprague-Dawley rats. *Reproductive Toxicology* **14**, 507 - 512.

Worley, R. E. 1994. Pecan physiology and composition, in C. R. Santerre (Ed.). *Pecan technology*, Chapman & Hall, New York, 39 – 45.

Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry* 52, 4026 - 4037.

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