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

By Ana Cristina Pinheiro do Prado, Analu Monalise Aragão, Roseane Fett and Jane Mara Block*

Federal University of Santa Catarina, Center of Agrarian Sciences, Department of Food Science and Technology, UFSC – Florianópolis – SC, Brazil.

(*)Corresponding author: jmblock@cca.ufsc.br

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).

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 content of phenolic compounds was 116 to 167 mg GAE/g and the condensed tannin content was between 35 and 48 mg CE/g. The antioxidant activity varied from 305 to 488 mg TEAC/g (30 minutes reaction) and from 482 to 683 mg TEAC/g (24 h reaction). The percentage of inhibition of the oxidation that was observed in the β-carotene/linoleic system varied from 70 to 96%. The results indicated a high phenolic content and antioxidant activity of Pecan nut shell infusion.
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-picyrylhydrazyl) 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
Analyses were carried out in triplicate. The antioxidant activity (Trolox equivalent antioxidant activity) of dry weight.

### DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was carried out according to Brand-Williams 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/Ao) × 100, where, “Ao” is initial absorbance and “A” 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.

### 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.
considered rich in phenolic compounds, such as cloves, which, according to Wu et al. (2004), presented 113.19 mg GAE/g and green infusion and 133.8 mg TEAC/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). 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.

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.

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 1. Nutritional composition of Pecan nut shells

<table>
<thead>
<tr>
<th>Component (g/100 g)</th>
<th>Mean ± SD (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>2.2 ± 0.04</td>
</tr>
<tr>
<td>Moisture</td>
<td>16.8 ± 0.1</td>
</tr>
<tr>
<td>Total lipids</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Minerals</td>
<td>1.4 ± 0.03</td>
</tr>
<tr>
<td>Total fiber</td>
<td>48.6 ± 0.06</td>
</tr>
<tr>
<td>Soluble fiber</td>
<td>3.1 ± 0.09</td>
</tr>
<tr>
<td>Insoluble fiber</td>
<td>45.4 ± 0.4</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>29.6</td>
</tr>
<tr>
<td>Caloric value</td>
<td>331</td>
</tr>
</tbody>
</table>

3Means ± SD (n = 3); 4 % total lipids; 5 % proteins; 2.2 % moisture; 1.4 % minerals; 42 % total fiber; 100 – (% total lipids + % proteins + % moisture + % minerals + % total fiber); 3.3 mg CT/g (mg catechin equivalent/g of defatted sample) of dry weight (vanillin assay); 2.2 mg TEAC/g (mg catechin equivalent/g of defatted sample) of dry weight (Folin-Ciocalteau assay); 1.3 mg GAE/g (gallic acid equivalent) of dry weight (Folin-Ciocalteau assay).
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 compounds 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

<table>
<thead>
<tr>
<th>Sample</th>
<th>200 parts per million</th>
<th>300 parts per million</th>
<th>500 parts per million</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT (standard)</td>
<td>21.7±0.2</td>
<td>40.6±0.2</td>
<td>57.6±0.6</td>
</tr>
<tr>
<td>Extract - Batch 01</td>
<td>70.2±2.2</td>
<td>79.9±0.6</td>
<td>89.5±0.7</td>
</tr>
<tr>
<td>Extract - Batch 02</td>
<td>81.1±0.9</td>
<td>85.3±0.1</td>
<td>95.3±0.1</td>
</tr>
<tr>
<td>Extract - Batch 03</td>
<td>84.3±1.5</td>
<td>89.9±0.9</td>
<td>96.4±0.8</td>
</tr>
<tr>
<td>E + MT - Batch 01</td>
<td>55.5±0.2</td>
<td>70.9±1.2</td>
<td>79.9±0.1</td>
</tr>
<tr>
<td>E + MT - Batch 02</td>
<td>70.8±1.9</td>
<td>75.4±1.9</td>
<td>85.4±1.7</td>
</tr>
<tr>
<td>E + MT - Batch 03</td>
<td>67.5±0.9</td>
<td>79.2±1.2</td>
<td>87.9±1.7</td>
</tr>
</tbody>
</table>

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.

ACKNOWLEDGMENTS
The authors would like to thank Divinut Ind. de Nozes Ltda., from Cachoeira do Sul – RS, Brazil, for supplying the raw material used in this study, and the Coordination for the Improvement of Higher Education Personnel (CAPES), for the scholarship.

REFERENCES