Optimization of extraction of phenolic antioxidants from peanut skins

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Abstract: This work was conducted to optimize the extraction conditions for the best recovery of antioxidant compounds from peanut skins. The extracts from the peanut skins were obtained by different extraction methods. The extraction conditions were: different ethanol proportions as the solvent (0, 30, 50, 70 and 96% v/v in distilled water), different peanut skin particle sizes (0–1, 1–2 and 2–10 mm and non-crushed skins), different proportions of solvent/skins (20, 30, 40, 50 and 60 ml g\(^{-1}\)), different extraction times (by maceration and shaking) and different numbers of extractions. The different extracts obtained under different extraction conditions were compared with special regard to yield, total phenolic compounds and radical scavenging activity. The results showed that the best delivery of phenolic compounds was reached using 70% ethanol, non-crushed peanut skins, ratio of solvent/solid of 20 ml g\(^{-1}\), at 10 min shaking and three extractions. The maximum yield of 0.118 g g\(^{-1}\) was recorded for phenolic compounds when extracted at the optimum conditions.

Keywords: Arachis hypogaea; antioxidant compounds; peanut skins; phenolic compounds

INTRODUCTION

Argentina is a major producer and exporter of peanuts, along with China, India and USA, among others. In 2001/2002 the Argentinean peanut production was about 360 000 tons.1 Blanched peanut is one of the most important products obtained. Blanched peanuts are used to obtain products like salted fried peanuts, peanut butter, roasted peanuts, etc.2

Peanut skins are a by-product from the blanching process of peanut kernels. In Argentina, peanuts skins are used to feed cattle. However, their value could be increased if other more valuable uses were found for that waste.

Recently, some works have been undertaken to study antioxidant compounds obtained from Argentinean peanut skins.3 In those studies, the content of phenolic compounds was found to be between 0.115 and 0.149 g g\(^{-1}\). The ethanolic extracts exhibited high radical-scavenging and antioxidant activity, as demonstrated in sunflower oil.

Proanthocyanidins have been described as the most important phenolic and antioxidant compounds in peanut skins.3 Recently, resveratrol was found in peanuts as well as in peanut skins.3–5 It has been demonstrated that resveratrol possesses cancer chemopreventive activity in mice and acts as an antioxidant and antimutagen.8 It is also associated with a reduction in risk of cardiovascular disease by inhibiting or altering platelet aggregation and coagulation, or modulating lipoprotein metabolism.9–14 It has been proved that wine consumption in moderate quantities is associated with a significantly lower risk of cardiovascular death, which could possibly be explained by higher consumption of wines containing resveratrol.15

The purpose of this work was to determine the best conditions of extraction for antioxidant compounds from peanut skins at laboratory scale using ethanol–water mixtures as the extraction solvent.

MATERIALS AND METHODS

Materials

Peanut skins from Argentinean peanut (Arachis hypogaea cv Plorunner, 2001 crop year) were obtained by blanching and were provided by the Company ‘Lorenzati, Ruestch y Cía,’ Ticino, Córdoba, Argentina in July 2001. The peanut skins were kept in a sealed plastic bag and stored at 4°C until use.

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Optimization of ethanol–water mixtures as the extraction solvent

Phenolic compounds were extracted from peanut skins and defatted peanut skins using different ethanolic solutions in distilled water: 96, 70, 50, 30 and 0% (v/v). To obtain the extracts, the peanut skins (2.5 g) were macerated with the extraction solvent (50 ml) for 24 h at room temperature in a dark room. The extract was filtered and the filtrate was evaporated to dryness under vacuum in a rotary evaporator (Buchi R 110, Flawil, Switzerland) at 35 °C. The defatted peanut skins were prepared using soxhlet apparatus for 16 h with n-hexane (Anedra, San Fernando, Buenos Aires, Argentina).

Yield of dry extracted matter
It was determined on dry peanut skins basis following the formula: \( g \text{g}^{-1} \text{dry extracted matter} = (g \text{ dry extracted matter})/(g \text{ dry peanut skins}) \).

Total phenolic compound

The phenolic compound of the extracts was determined spectrophotometrically using the Folin–Ciocalteu method described above. Peanut skin extract (3 mg) was dissolved in 10 ml of the extracting solvent. This solution (0.1 ml) was transferred into 10 ml volumetric flask. Deionized water (8.4 ml) and 0.5 ml Folin–Ciocalteu reagent (Anedra, San Fernando, Buenos Aires, Argentina) were added and the contents of the flask mixed thoroughly. After 1 min, 1 ml Na\(_2\)CO\(_3\) solution (20 g in 100 ml of water) was added and finally made up to 10 ml with deionized water. After 1 h, absorbance was measured with a spectrophotometer (Spectronic 21, Bausch and Lomb, USA) at 760 nm. The concentration of total phenolic compounds in extracts of peanut skins was determined by comparison with the absorbance of phenol (Merck, Darmstadt, Germany) used at different concentration as standard. All tests were run in triplicate, and analyses of all samples were run in duplicated and averaged. The yield of total phenolic compound was expressed in grams of total phenolic extracted per gram of dry peanut skins.

Radical-scavenging activity

The radical-scavenging activity of the extracts was determined using diphenyl picryl hydrazyl radical (DPPH) (Aldrich, Milwaukee, WI, USA) according to Schmeda-Hirschmann et al. Ethanolic solution of peanut skins extract (5 µl; 300 µg ml\(^{-1}\)) was added to a 1.5 ml ethanolic solution of DPPH radical (20 µg ml\(^{-1}\)) to give a final extract concentration of 1 µg ml\(^{-1}\). The mixture was shaken vigorously and left for 5 min. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Spectronic 21, Bausch and Lomb, USA). All tests were run in triplicate, and analyses of all samples were run in duplicate and averaged. The radical-scavenging activity was calculated using the formula: percentage inhibition = \[ 1 - \left(\frac{\text{absorbance of DPPH}}{\text{absorbance of sample}}\right) \times 100 \].
means were determined by Duncan’s test. Regression analysis was performed to determine the relation between the radical-scavenging activity and the content of total phenolic compounds of the extracts. Second order polynomial regression equation was obtained for each treatment: \( y = Ax^2 + Bx + C \), where \( y \) = percentage inhibition (radical-scavenging activity) and \( x \) = g total phenolic compounds g\(^{-1}\) extract.

**RESULTS AND DISCUSSION**

Optimization of ethanol–water mixtures as the extraction solvent

The yields of dry extracted matter of peanut skins and defatted peanut skins in relation to the ethanol content in the extraction solvent are shown in Fig 1. In a two-way ANOVA (\( \alpha = 0.05 \)), the independent variables were: \( A \)-ethanol content in the solvent, \( B \)-type of peanut skins (peanut skins and defatted peanut skins), and the dependent variable-yield of dry extracted matter. The two independent variables and the interaction between both had a significant effect on the dependent variable. In other words, the effect on the yield of dry extracted matter of ethanol content in the solvent was different in non-defatted peanut skins than in defatted peanut skins. When ethanol content increased from 0 to 96% (v/v), the yield of dry extracted matter on peanut skins increased from 0.085 ± 0.001 to 0.179 ± 0.006 g g\(^{-1}\). However, the dry extracted matter from defatted peanut skins had a maximum yield when 50 and 70% (v/v) ethanol was used (0.181 ± 0.006 and 0.182 ± 0.000 g g\(^{-1}\), respectively). This effect could be explained because lipids are partially soluble in ethanol. As a consequence, when 96% ethanol was used to extract peanut skins, it extracted phenolic compounds and some lipid components. Lipids were absent in defatted peanut skins, therefore 96% ethanol extracted only phenolic compounds in less yield than 50 or 70% ethanol in this material. In general, no significant differences were found in the yields of extraction between peanut skins and defatted peanut skins using 50 and 70% (v/v) ethanol in the solvent.

Duh et al\(^{18} \) compared the yield of extraction of peanut hulls using methanol and ethanol. Methanol extracted 104.9 ± 2.19 mg soluble constituents from 2.5 g dried peanut hulls (0.042 g g\(^{-1}\)) and ethanol extracted 49.7 ± 2.05 mg (0.020 g g\(^{-1}\)). In our case, the ethanol (96% v/v) extracted 0.179 ± 0.006 g g\(^{-1}\) soluble constituents from the peanut skins and 0.099 ± 0.010 g g\(^{-1}\) from the defatted peanut skins. These results indicate that peanut skins contain about nine times more ethanol soluble constituents than peanut hulls.

The yields of total phenolic compounds extracted from peanut skins and defatted peanut skins in relation to the ethanol content in the solvent are presented in Fig 2. When the dependent variable in the two-way ANOVA was the yield of total phenolics compounds extracted, the two independent variables \( A \) and \( B \) had a significant effect on the dependent variable but they did not present any effect of interaction. Thus, the effect on this dependent variable of the ethanol content in the solvent showed no difference between non-defatted peanut skins and defatted peanut skins.

In general, no significant differences were found between peanut skins and defatted peanut skins using the same ethanol–water mixture in the extraction solvent (Duncan test). In both skins, the maximum values of extraction of 0.085–0.099 g g\(^{-1}\) in peanut skins and 0.103–0.114 g g\(^{-1}\) in defatted peanuts skins were obtained with 30, 50 and 70% (v/v) ethanol in the extraction solvent.

Yen et al\(^{19} \) found levels between 33.4 and 71.3 mg total phenolic compounds g\(^{-1}\) peanut hulls (0.033–0.071 g g\(^{-1}\) of total phenolic compounds). The extraction was developed in two stages, with a relation of 50 ml methanol from 5 g of peanut hulls in each stage. The ethanol–water mixture used in this work extracted between 0.055 and 0.099 g g\(^{-1}\) total phenolic compounds from the peanut skins. That means almost twice the total phenolic compounds obtained by Yen et al\(^{19} \) using methanol with peanut hulls.

Radical-scavenging activity of the extracts of peanut skins and defatted peanut skins is shown in Fig 3. When the dependent variable in the two-way ANOVA

![Figure 1. Yield of dry extracted matter of peanut skins and defatted peanut skins (g dry matter extracted g\(^{-1}\) dry peanut skins) obtained by the ethanol–water mixture as extraction solvent. The same letter indicates that yields of dry extracted matter are not significantly different at \( \alpha = 0.05 \).](image1)

![Figure 2. Yield of total phenolic compounds extracted from peanut skins and defatted peanut skins (g total phenolic compounds g\(^{-1}\) dry peanut skins) in relation to the ethanol content in the extraction solvent. The same letter indicates that values of yield of total phenolic compound are not significantly different at \( \alpha = 0.05 \).](image2)
was the percentage of inhibition, the independent variable \( A \) (ethanol content in the solvent) had a significant effect on the dependent variable, but the independent variable \( B \) (type of peanut skins—peanut skins and defatted peanut skins) and the interaction between both did not have a significant effect on the dependent variable. In other words, the effect on this dependent variable of the ethanol content in the solvent showed no difference between non-defatted peanut skins and defatted peanut skins.

This result was similar to the one obtained for the yields of phenolic compounds (Fig 2) with a maximum in the percentage of inhibition in both peanut skins using 30, 50 and 70% (v/v) ethanol in the solvent mixture. The maximum values were 29.7–36.2% for peanut skins and 31.8–40.7% for defatted peanut skins.

Yen et al\(^{20}\) studied the scavenging effect of methanolic extracts of peanut hulls on DPPH. Their extracts showed about 90% inhibition when the concentration was 1.5 mg·ml\(^{-1}\). In our work, the concentration of the peanut skins extracts showed half the percentage of inhibition when the concentration of the extracts was 1 μg·ml\(^{-1}\), 1500 times less than in peanut hulls extracts. As a consequence, the peanut skins extracts are much more active than peanut hulls extract.

On the basis of the results observed in Figs 1–3, the partial conclusion is that the best solvents to extract a high yield of total phenolic and antioxidant compounds were 50 and 70% (v/v) ethanol in water. However, when considering the evaporation time and cost, the preferred solvent mixture was 70% (v/v) ethanol in water because the evaporation time is lower. As a consequence, the possibility of oxidation of the extracted phenolic compounds decreased. Because of this, the mixture ethanol–water (70% v/v) was chosen for the optimization of particle size, solvent volume, contact time and extraction stages to extract antioxidant components from peanut skins.

Correlation and regression analyses where \( y = \) percentage inhibition (radical-scavenging activity) and \( x = g \) total phenolic compounds g\(^{-1}\) extract. The lineal regression was:

\[
y = 92.29x^2 - 38.69x + 21.90 (R^2 = 0.638).
\]

The Pearson coefficient = 0.78

These results showed a good relation between both variables, and they implied that it is possible to predict the radical-scavenging activity in the extract by knowing the content of total phenolic compounds using the above equation.

**Optimization of peanut skin particle size**

The yield of total phenolic compounds extracted by maceration and shaking from the different particle sizes of peanut skins is shown in Table 1. From a two-way ANOVA (\( \alpha = 0.05 \)), independent variable \( A \)-particle size, independent variable \( B \)-maceration and shaking, and the dependent variable-yield of total phenolics extracted, it could be concluded that the two independent variables, and the interaction of both, had a significant effect on the dependent variable. The effect of the particle size was observed only in the extraction by maceration; the peanut skins between 0 and 1 mm exhibited the least values of total phenolic compound extracted (0.069 ± 0.001 g·g\(^{-1}\)). This value could be due to the compactness of the solid, resulting in a smaller contact surface and a higher value of retained solvent (6.85 ml) in comparison to the largest particle sizes (retained solvent between 5.00 and 5.11 ml). That effect was compensated when the extraction was conducted by shaking. In this case, the particle size did not produce an effect in the independent variable. As a consequence, the crushing of the peanut skins did not produce an increase in the total phenolic compound extracted by a single extraction stage using maceration or shaking. The maximum values obtained in yield of total phenolic compounds extracted were between 0.111 (non-crushed peanut skins, maceration) and 0.121 g·g\(^{-1}\) (2–10 mm particle size, maceration) with no significant differences between these results (Duncan test).

**Optimization of relation solvent–solid for the extraction**

The yields of dry matter and total phenolic compounds extracted with different relations solvent/peanut skins

<p>| Table 1. Yields of total phenolic compounds extracted from different particle sizes of peanut skins by maceration and shaking |
|-----------------------------|-----------------------------|-----------------------------|</p>
<table>
<thead>
<tr>
<th>Particle size (mm)</th>
<th>Maceration</th>
<th>Shaking</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>0.068 ± 0.001ab</td>
<td>0.112 ± 0.004b</td>
</tr>
<tr>
<td>1–2</td>
<td>0.112 ± 0.003b</td>
<td>0.114 ± 0.006b</td>
</tr>
<tr>
<td>2–10</td>
<td>0.121 ± 0.000b</td>
<td>0.114 ± 0.002b</td>
</tr>
<tr>
<td>Non-crushed skins</td>
<td>0.119 ± 0.002b</td>
<td>0.111 ± 0.002b</td>
</tr>
</tbody>
</table>

\( ^a \) g total phenolic compounds g\(^{-1}\) dry peanut skins.

\( ^b \) Means ± standard error followed by the same letter are not significantly different at \( \alpha = 0.05 \).
Extraction of phenolic antioxidants (v/w) are presented in Table 2. The yields did not show significant differences (one-way ANOVA, Duncan test, \( \alpha = 0.05 \)). The increase of the solvent volume did not produce an increase in the total phenolic compounds extracted. The chosen volume solvent used to extract non-crushed peanut skins was the minimum required to cover the skins, resulting in a relation 20 ml solvent g\(^{-1}\) peanut skins, obtaining 0.176 ± 0.007 g of dry matter extracted and 0.113 ± 0.008 g total phenolic compounds g\(^{-1}\) dry peanut skins.

**Optimization of extraction contact time**
The yields of total phenolic compounds extracted from the peanut skins at different contact times by shaking and maceration are presented in Fig 4. From a two-way ANOVA (\( \alpha = 0.05 \), independent variable \( A \)-maceration or shaking, independent variable \( B \)-time, and dependent variable-yield of total phenolics extracted) it could be concluded that the two independent variables had a significant effect on the dependent variable, and the interaction between both had no significant effect on the dependent variable. The maceration process required 60 min for extracting the same quantity of total phenolic compounds in comparison to the shaking method, which required 10 min, six times less than the maceration method. The maximum yield of total phenolic compounds extracted with both methods was between 0.095 and 0.096 g g\(^{-1}\).

**Optimization of number of extraction stages**
The yields of total phenolic compounds extracted with 70% (v/v) ethanol in water from the peanut skins in five stages are shown in Fig 5. The percentages of extraction of the total phenolic compounds extractable from the peanut skins in each stage were 64.6, 87.3, 94.8, 98.21 and 100% for a single, two, three, four and five stages, respectively. The total phenolic compounds obtained with three stages was 0.118 ± 0.000 g g\(^{-1}\), and with five stages was 0.125 ± 0.002 g g\(^{-1}\). As a consequence, using more than three stages is not recommended because the time and solvent required to achieve 100% extraction would not be justified by such a low difference in the yield of extraction.

![Figure 4](image-url)

**Figure 4.** Yield of total phenolic compounds extracted from the peanut skins (g total phenolic compounds g\(^{-1}\) dry peanut skins) at different contact time by shaking and maceration. The same letter indicates that the values of percentage inhibition are not significantly different at \( \alpha = 0.05 \).

![Figure 5](image-url)

**Figure 5.** Yield of total phenolic compounds extracted from the peanut skins (g total phenolic compounds extracted g\(^{-1}\) dry peanut skins) by extraction stages.

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**Table 2.** Yields of dry matter and total phenolic compounds extracted from the peanut skins with different ratios of solvent:peanut skins (v/w)

<table>
<thead>
<tr>
<th>Ratio of solvent (ml): peanut skins (g)</th>
<th>Yield of dry matter extracted(a)</th>
<th>Yield of total phenolic compounds extracted(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20(c)</td>
<td>17.59 ± 0.73a</td>
<td>11.26 ± 0.78a</td>
</tr>
<tr>
<td>30</td>
<td>19.46 ± 0.49a</td>
<td>13.11 ± 1.05a</td>
</tr>
<tr>
<td>40</td>
<td>20.04 ± 0.40a</td>
<td>11.09 ± 0.40a</td>
</tr>
<tr>
<td>50</td>
<td>18.80 ± 0.89a</td>
<td>12.72 ± 0.20a</td>
</tr>
<tr>
<td>60</td>
<td>19.94 ± 0.57a</td>
<td>11.55 ± 0.31a</td>
</tr>
</tbody>
</table>

\(a\) g extracted matter g\(^{-1}\) dry peanut skins.

\(b\) g total phenolic compounds g\(^{-1}\) dry peanut skins.

\(c\) Means ± standard error followed by the same letter within each column are not significantly different at \( \alpha = 0.05 \).
In most of the works related to the extraction of phenolic compounds from peanut hulls,\textsuperscript{19,21,22} the extracts were obtained in two stages, by shaking at room temperature overnight or after 24 h contact. In our study, the maximum time required to obtain the ethanolic extracts was 10 min by shaking, and 1 h by maceration at room temperature.

CONCLUSION
As a conclusion from the results obtained in the extraction of the antioxidant compounds from peanut skins with ethanolic solvent at laboratory level, the best method was using 70\% (v/v) ethanol in water, non-crushed peanut skins, by shaking for 10 min and in three stages. The phenolic compounds obtained for this procedure could be used as additives in food products as natural antioxidants to extend their shelf-life. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG), are used in many foods to prevent rancidity but there is growing concern for the potential health hazards of synthetic antioxidants. One example is the work of Ito et al\textsuperscript{23} that reported BHA to be carcinogenic in animal experiments. The present research renews interest in the increased use of naturally occurring antioxidants.

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