Apple: Extraction Optimization of Antioxidant Compounds and Determination of Total Phenolic Amount and Antioxidant Activity on Product and Co-Product

M. Vizzotto¹, M. da Rosa Fetter², M. Couto Pereira³ and D. Dutra Corbelini²
¹ Embrapa Clima Temperado, Rodovia BR 392, Km 78, CEP 96001-970, Pelotas, RS, Brazil
² Universidade Católica de Pelotas, Rua Félix da Cunha, 412, CEP 96010-000, Pelotas, RS, Brazil
³ Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, Avenida Bento Gonçalves, 9500, CEP 91501-970, Porto Alegre, RS, Brazil

Keywords: health, functional food, solvent extractors, bioactive compounds, phytochemicals

Abstract

Apple (Malus domestica) is one of the most commercialized fruit in Brazil, having great importance to the Brazilian fresh fruit market. The main destination of the apple Brazilian production is the fresh market; however, new alternatives are being sought since the fresh consumption has not increased in the last few decades. The alternatives to develop new products pass through the food, pharmaceutical and cosmetic industries. Beverages such as apple juice concentrate, extraction of pectin, apple flour to make cakes, breads and shakes and cosmetics are good examples of high added value products made of apple fruits. Apples have a series of bioactive compounds, also called phytochemicals, which can prevent chronic non-communicable diseases. Genetic factors, as well as environmental factors may affect the content of these bioactive compounds. To increase the knowledge of apples produced in Brazil, Embrapa Temperate Agriculture, Pelotas, has been conducting research activities on chemical composition and antioxidant activity of the main cultivars produced in Brazil and some co-products. Some products and co-products were analyzed such as the cultivar ‘Fuji’, apple flour, concentrate apple juice and fruits from the pollination apple (Malus everest) plant. Also, a methodology to extract the antioxidant compounds from apple was developed. Regarding to the results, fruits from the pollination apple plant show the highest antioxidant activity among all the studied products and co-products. This is important information to the farmers and to the industries since this product is not usually commercialized. Cosmetic industry can use this material to extract antioxidant compounds to be used as natural extract in cosmetic formulations. Regarding the extraction of antioxidant compounds methodology, several solvents and mixtures of solvents were tested and a mix of acetone and ethanol was the most efficient to extract antioxidant compounds from apples.

INTRODUCTION

The production of apples in Brazil has developed on a larger scale, just over 30 years ago, with a very high technological standard and good quality results. The production technology was largely introduced by traditional Japanese and European producers, which have invested in production systems well suited to the climate and soil of Brazil. Beyond productivity, we have obtained very good quality of the final product, which allow Brazilian apples to compete with the best products on the world market.

Apple is one of the most commercialized fruit in Brazil. The main destination of the Brazilian apple production is the fresh market; however, new alternatives are being sought since the fresh consumption has not increased in the last few decades. The alternatives to develop new products pass through the food, pharmaceutical and cosmetic industries. Beverages such as apple juice concentrate, extraction of pectin, apple flour to make cakes, breads and shakes and cosmetics are good examples of high added value products made of apple fruits.
Apples have a number of bioactive compounds, including quercetin, catechin, phloridzin and chlorogenic acid, all of which are strong antioxidants, which can prevent chronic non-communicable diseases if consumed as part of the usual diet. Epidemiological studies show evidences that the consumption of fruits is linked to the prevention of chronic non-transmissible diseases (Hertog et al., 1995), mainly because of the presence of bioactive phytochemical compounds such as phenolic acids, flavonoids and carotenoids (Feldman, 2001; Shahidi and Naczk, 2004). Apple extracts has demonstrated activity in preventing type II diabetes (Song et al., 2005), the oxidation of LDL cholesterol (Vidal et al., 2005; Davis et al., 2006), and several types of cancer as leukemia, ovarian, breast, lung, liver, mouth (Ramos, 2007), colon (Veeriah et al., 2007). The way these compounds act include reduction of cell proliferation (Kern et al., 2005), protection of cellular DNA and inhibition of invasion of tumors (McCanns et al., 2007).

The phytochemical composition of apples varies with cultivars, maturation and ripening of the fruit, storage and processing (Boyer and Liu, 2004). The concentration of these compounds can vary also with the extraction process utilized. To increase the knowledge of apples produced in Brazil, Embrapa Temperate Agriculture, Pelotas, has been conducting research activities on chemical composition and antioxidant activity of the main cultivars produced in Brazil and some co-products. Some products and co-products were analyzed such as the cultivar ‘Fuji’, apple flour, concentrate apple juice and fruits from the pollination apple (Malus everest) plant. Also, a methodology to extract the antioxidant compounds from apple was developed.

**MATERIALS AND METHODS**

**Sample Preparation**

The apple samples were harvested and stored at -18°C until further analysis. For the analysis 5 g of sample taken from the equatorial part of the fruit were used. Samples were submitted to some tests (described below) to achieve the maximum phenolic compounds extraction. Samples were always prepared in three replications and homogenized in an ultraturrax homogenizer set to small samples, and centrifuged at 15,000 rpm and temperature of 4°C. After sample preparation, total phenolic compounds and antioxidant activity were assayed.

**Different Solvents**

The following solvents were tested (all solvents were tested at pure form): ultrapure water, methanol, ethanol, acetone, hexane.

**Solvent Mixtures**

The mixtures 70% ethanol:30% ultrapure water; 50% ethanol:50% ultrapure water; 70% acetone:30% ultrapure water; 50% acetone:50% ultrapure water; 50% ethanol:50% acetone; 70% ethanol:30% acetone; 30% ethanol:70% acetone were used.

**Total Phenolic Compounds**

The method used was adapted from Swain and Hillis (1959).Shortly, 250 µl of sample, 4 ml of ultrapure water and 250 µl of Folin-Ciocalteu (0,25N) reagent were added to each falcon tube. The sample was mixed vigorously and allowed to react for 3 min. 500 µl of sodium carbonate (1N) were added and samples were incubated at room temperature for 2 hours. Absorbance was measured at a spectrophotometer previously blanked at 725 nm.

**Antioxidant Activity**

The method used was adapted from Brand-Williams et al. (1995). In summary, 200 µl of sample were added to 3800 µl of diluted DPPH in 15 ml Falcon tubes. Tubes were agitated and let to react for 24 hours. The spectrophotometer was blanked with methanol and the absorbance was read at 525 nm.
RESULTS AND DISCUSSION

Fruits from the pollination apple plant (*Malus everest*) showed the highest antioxidant activity among all the studied products and co-products (Table 1). Comparing *Malus everest* and *Malus domestica*, the total phenolic content was eight and antioxidant activity was 38-folds superior in the *Malus everest*. This is important information to the farmers and to the industries since this product is not usually commercialized. Cosmetic industry can use this material to extract antioxidant compounds to be used as natural extract for cosmetic formulations.

Several solvents were tested for extraction of phenolic compounds in apple. The content of total phenolics and antioxidant activity varied depending on the solvent used (Table 2). The samples extracted with hexane (solvent of lower polarity) had a very low yield of phenolic compounds, and had thus a low antioxidant activity. These results are in agreement with other authors who suggested that high-polarity solvents such as water and solvents with very low polarity or nonpolar, such as hexane or dichloromethane, are not good extractants (Liu et al., 2000; Chirinos et al., 2007). Ethanol was the most efficient solvent, followed by acetone. Methanol is widely used and efficient solvent for extraction of phenolic compounds (Tsao and Deng, 2004; Shi et al., 2005). Methanol and acetone are also suitable for extraction of anthocyanins from various raw materials (Skrede et al., 2000). Pure water as a solvent extractor was not very efficient. It is considered the universal solvent, in combination with other organic solvents helps to create a moderately polar environment, which favors the extraction of polyphenols; however, the use of pure water results in extracts with high impurity (organic acids, sugars, soluble proteins) which may interfere with the quantification of these compounds (Chirinos et al., 2007).

In a second step, to improve the extraction efficiency, mixtures of solvents that had been previously tested isolates were tested (Table 3). In this case the solvent mixture ethanol:acetone (30:70) was more efficient in extracting phenolic compounds from apple fruits. However, the highest antioxidant activity was obtained with a mixture of ethanol:acetone (70:30).

Analyzing all the data generated from apple extraction, we observed no positive correlation between total phenolic content and total antioxidant activity (Fig. 1).

CONCLUSIONS

*Malus everest* have characteristics that can be of interest to the cosmetic and food industries to produce antioxidant extracts.

Acetone and ethanol mixture is the most efficient solvent to extract antioxidant bioactive compounds from apples.

In apple extracts total phenolic content does not correlate to total antioxidant activity.

ACKNOWLEDGEMENTS

We are thankful to EMBRAPA and FAPERGS for the financial support.

Literature Cited

Hertog, M.G.L., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F.


Table 1. Total phenolic compound and antioxidant activity of apple cultivars and co-products. Embrapa Clima Temperado, Pelotas, 2010.

<table>
<thead>
<tr>
<th></th>
<th>Phenolic compound$^1$</th>
<th>Antioxidant activity$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Malus domestica</em> ‘Fuji’</td>
<td>186±6 b</td>
<td>499±42 c</td>
</tr>
<tr>
<td><em>Malus everest</em></td>
<td>1444±408 a</td>
<td>19221±933 a</td>
</tr>
<tr>
<td>Apple flour</td>
<td>875±30 a</td>
<td>1506±204 bc</td>
</tr>
<tr>
<td>Concentrate apple juice</td>
<td>1426±149 a</td>
<td>2239±218 b</td>
</tr>
</tbody>
</table>

Average of three repetitions ± standard deviation.

$^1$ Phenolics = mg chlorogenic acid equivalent/100 g fresh weight.

$^2$ Antioxidant activity = µg trolox equivalent/g fresh weight.

Table 2. Total phenolic compound and antioxidant activity of apple using different solvent extractor. Embrapa Clima Temperado, Pelotas, 2010.

<table>
<thead>
<tr>
<th></th>
<th>Phenolic compound$^1$</th>
<th>Antioxidant activity$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>53.8±8.1 d</td>
<td>534.0±54.7 d</td>
</tr>
<tr>
<td>Methanol</td>
<td>130.6±3.7 c</td>
<td>819.8±55.3 c</td>
</tr>
<tr>
<td>Ethanol</td>
<td>241.4±6.0 a</td>
<td>1705.7±103.9 a</td>
</tr>
<tr>
<td>Acetone</td>
<td>208.5±16.2 b</td>
<td>1341.0±93.0 b</td>
</tr>
<tr>
<td>Hexane</td>
<td>17.2±2.2 e</td>
<td>-</td>
</tr>
</tbody>
</table>

Mean ± standard deviation of four replicates.

$^1$ Phenolics = mg chlorogenic acid equivalent/100 g fresh weight.

$^2$ Antioxidant activity = µg trolox equivalent/g fresh weight.

Mean separation in columns by Tukey’s HSD test at $P \leq 0.05$. - Antioxidant activity not detected.

Table 3. Total phenolic compound and antioxidant activity of apple using different mixtures of solvent extractor. Embrapa Clima Temperado, Pelotas, 2010.

<table>
<thead>
<tr>
<th></th>
<th>Phenolic compound$^1$</th>
<th>Antioxidant activity$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>196.5±11.8 cd</td>
<td>3775.1±105.2 bc</td>
</tr>
<tr>
<td>Ethanol:water  (70:30)</td>
<td>171.4±28.8 d</td>
<td>3827.8±38.6 bc</td>
</tr>
<tr>
<td>Ethanol:water  (50:50)</td>
<td>212.4±27.8 bcd</td>
<td>3892.2±993.2 ab</td>
</tr>
<tr>
<td>Acetone</td>
<td>255.9±16.4 b</td>
<td>2902.3±313.7 cd</td>
</tr>
<tr>
<td>Acetone:water  (70:30)</td>
<td>268.0±20.5 ab</td>
<td>2348.3±263.3 d</td>
</tr>
<tr>
<td>Acetone:water  (50:50)</td>
<td>251.0±14.6 bc</td>
<td>4046.0±206.6 ab</td>
</tr>
<tr>
<td>Ethanol:acetone (50:50)</td>
<td>211.6±24.1 bcd</td>
<td>4365.4±228.0 ab</td>
</tr>
<tr>
<td>Ethanol:acetone (70:30)</td>
<td>215.1±36.3 bcd</td>
<td>4794.7±149.5 a</td>
</tr>
<tr>
<td>Ethanol:acetone (30:70)</td>
<td>324.7±27.7 a</td>
<td>4459.3±401.1 ab</td>
</tr>
</tbody>
</table>

Mean ± standard deviation of four replicates.

$^1$ Phenolics = mg chlorogenic acid equivalent/100 g fresh weight.

$^2$ Antioxidant activity = µg trolox equivalent/g fresh weight.

Mean separation in columns by Tukey’s HSD test at $P \leq 0.05$. - Antioxidant activity not detected.
Fig. 1. Correlation between total phenolic content and total antioxidant activity on apple extracts. Embrapa Clima Temperado, Pelotas, 2010.

The equation of the regression line is:

\[ y = -0.5517x + 4019.3 \]

The coefficient of determination, \( R^2 \), is 0.001.