Effects of packaging materials and storage on total phenolic content and antioxidant activity of *Centella asiatica* drinks
[Kesan bahan pembungkus dan masa penyimpanan terhadap jumlah kandungan fenol dan aktiviti antioksida di dalam minuman pegaga (*Centella asiatica*)]

W.M. Siah*, H. Faridah*, M.Z. Rahimah*, S. Mohd Tahir* and D. Mohd Zain*

Keywords: *Centella asiatica* drinks, packaging, storage, antioxidant activity, total phenolic content

Abstract
The effects of packaging materials and storage time on total phenolic content and antioxidant activity of *Centella asiatica* drinks were studied. The total phenolic content and antioxidant activity were determined using Folin-Ciocalteau method and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay respectively. All analyses were carried out monthly for a period of one year for drinks packaged in high density polyethylene (HDPE) bottles, polypropylene (PP) bottles, standing pouches and cans, which were stored at ambient temperature (27 ± 5 °C, RH = 75–85%). The total phenolic content and antioxidant activity remained stable for the first month of storage regardless of the types of packaging materials used. Significant decrease (*p* <0.05) was noticed after 2 months storage. Drinks in cans had the greatest decrease, followed by drinks in PP bottles and HDPE bottles, while drinks in standing pouches gave the highest reading throughout the storage period.

Introduction
*Centella asiatica*, a popular Eastern longevity herb and brain food, is enjoying new attention. This herb contains chemicals called triterpenes such as asiaticoside, madecassoside, asiatic acid and madecassic acid that showed various physiological effects as health tonic, anti inflammatory, wound healing, diuretic, laxative and sedative. It is also used to treat skin infections including leprosy and skin ulcers and appears to activate blood cleaning and immunity while stimulating deep replacement. It is also a nerve tonic to improve memory and reduce mental fatigue (Inamdar et al. 1996).

In Malaysia, *C. asiatica* locally known as ‘pegaga’, is commonly eaten raw as ‘ulam’ in the Malay cuisines. With the advent in food processing and packaging technology, this herb can be converted into other convenience and more tasty products which can be consumed at anytime and anywhere. There are many types of *Centella* products available in the market, namely *Centella* juice, *Centella* tea and *Centella* cordial. Most of these products are displayed on shelves for a period of 6 to 12 months before they deteriorated in terms of sensory quality. However, many chemical reactions can occur during this period and subsequently may affect the phytochemicals...
available in these products. Currently most of the research on herbal products is concentrated on phytochemical screening, product development, nutritional, biological and clinical aspects (Inamdar et al. 1996; Abdul Hamid et al. 2002; Zainol et al. 2003; Marimuthu et al. 2005; Susana et al. 2006). No studies have so far been conducted on the effect of storage on the bioactive compounds of these products and how packaging can preserve their desirable properties. Thus, the objective of this study was to investigate the effects of packaging methods on the total phenolic content and antioxidant activity of C. asiatica drinks during storage.

Materials and methods
Preparation of Centella asiatica drinks
Centella asiatica drinks were prepared based on the formulation developed by Faridah et al. (2004). Fresh C. asiatica plants were obtained from a grower at Paya Rumput, Malacca. The plants were trimmed to discard roots and dried leaves and subsequently washed twice in a rotary washer for 10 min each. After washing the herb was fed to an automatic cutter (Emura ECA 20, Japan) and the juice was extracted using the masscolloider (Masuko MKZA 10, Japan). The extracted juice was mixed with sugar, citric acid, xanthan gum and water and the mixing process was done in a homogenizer. The mixture was pasteurized in a jacketed kettle at 80 °C for 5 min.

The pasteurized drinks were then packed in 320 ml cans, high density polyethylene (HDPE) bottles, polypropylene (PP) bottles and oriented polypropylene/aluminium/low density polyethylene (OPP/Al/LLDPE) standing pouches and stored at ambient temperature (27 ± 5 °C, RH = 75–85%) for further analysis. Three samples of each treatment were withdrawn for monthly evaluation up to a period of one year.

Chemicals
DPPH (2,2-diphenyl-1-picrylhydrazyl) and gallic acid were purchased from Sigma Chemical Co. (USA). Folin-Ciocalteau reagent and methanol were purchased from Merck (Germany). All other chemicals were of standard analytical grade.

Preparation of extracts for analysis
Two ml of C. asiatica drinks was extracted with 20 ml of 60% methanol. The mixture was placed in a conical flask (wrapped with an aluminium foil) and kept overnight in the refrigerator at 4 °C. The mixture was then filtered through a Whatman No. 4 filter paper to obtain a clear extract which was used for all analyses.

Determination of total phenolic content
Total phenolic content of C. asiatica drinks extract was determined according to the Folin-Ciocalteau method described by Singh et al. (2002), with some modifications. Methanol solution of the extract (0.5 ml) was mixed with a 10-fold dilution of Folin-Ciocalteau reagents (0.5 ml). The mixture was homogenized with a vortex and incubated for 5 min at room temperature before the addition of a sodium carbonate solution (1 ml, 20% w/v). The mixture was measured at 725 nm using a UV/VIS spectrophotometer (Perkin-Elmer Lambda 25, USA) after standing for 40 min at room temperature.

Gallic acid solutions (0.5 ml) in the concentration of 0.0–0.20 mg/ml were used to prepare a calibration curve. The estimation of phenolic content in the extract was carried out in triplicate. Results were expressed as gallic acid equivalents (GAE), which reflected the phenolic content as the amount of gallic acid in mg per liter of drinks.

Determination of antioxidant activity
The antioxidant activity of the C. asiatica drinks were evaluated by DPPH free radical-scavenging method according to the procedure of Lee et al. (1996), with some modifications. An aliquot of 200 µl sample extract or control sample (60% methanol) was added to 1 ml of 0.2 mM DPPH in
anhydrous methanol. The mixture was homogenized by a vortex for 5 min and left to stand in the dark at room temperature for 30 min. The absorbance of the samples and control were measured at 517 nm with 60% methanol served as a blank using the UV/VIS spectrophotometer (Perkin-Elmer Lambda 25, USA). For each sample, three separate determinations were carried out. The antioxidant activity was expressed as the percentage of scavenging, calculated using the following formula:

\[
\text{Scavenging (\%)} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

where \(A_0\) is the absorbance of the control sample, and \(A_1\) the absorbance of the sample.

**Results and discussion**

**Effects of packaging materials and time of storage on total phenolic content**

Phenolic compounds are ubiquitously present in vegetables, fruits, herbs, tea and juices; thus they are an integral part of the human diet (Klimczak et al. 2007). Recently, they received much attention since many epidemiological studies suggest that consumption of polyphenol-rich foods and beverages was associated with a reduced risk of cardiovascular diseases, stroke and certain forms of cancer (Prior and Cao 2000; Kaur and Kapoor 2001).

The stability of polyphenols in food and beverages is influenced by many external factors such as exposure to light, air, or different storage temperatures (Van der et al. 2005). Table 1 presents the total phenolic content of *C. asiatica* drinks packaged in four types of packaging materials, over the course of 12 months in storage at ambient temperature. As shown in the table, packaging materials and time of storage did not show any significant difference on the total phenolic content of the drinks for the first month of storage even though increasing readings.

### Table 1. Total phenolic content (mg/litre GAE) of *Centella asiatica* drinks in different packaging materials during 12 months storage

<table>
<thead>
<tr>
<th>Storage time (month)</th>
<th>HDPE bottle</th>
<th>PP bottle</th>
<th>Standing pouch</th>
<th>Can</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>314.17 ± 9.50aA</td>
<td>304.51 ± 7.29aA</td>
<td>293.03 ± 27.13bA</td>
<td>286.78 ± 10.46bA</td>
</tr>
<tr>
<td>1</td>
<td>316.35 ± 39.86aA</td>
<td>316.81 ± 58.43aA</td>
<td>326.89 ± 16.99aA</td>
<td>337.60 ± 25.85aA</td>
</tr>
<tr>
<td>2</td>
<td>157.35 ± 10.74bA</td>
<td>156.18 ± 14.25bA</td>
<td>163.12 ± 7.17cA</td>
<td>148.02 ± 15.58cA</td>
</tr>
<tr>
<td>3</td>
<td>71.80 ± 0.43cB</td>
<td>63.88 ± 3.24cC</td>
<td>79.52 ± 0.38dA</td>
<td>53.99 ± 2.46 dD</td>
</tr>
<tr>
<td>4</td>
<td>49.33 ± 0.75deA</td>
<td>47.80 ± 1.74cdA</td>
<td>47.95 ± 2.55eA</td>
<td>30.37 ± 0.37eB</td>
</tr>
<tr>
<td>5</td>
<td>58.79 ± 1.16cA</td>
<td>48.60 ± 1.26cdB</td>
<td>57.83 ± 1.90eA</td>
<td>37.67 ± 3.04deC</td>
</tr>
<tr>
<td>6</td>
<td>25.30 ± 0.52AB</td>
<td>39.94 ± 3.94cA</td>
<td>23.48 ± 5.69gB</td>
<td>32.10 ± 14.01eAB</td>
</tr>
<tr>
<td>7</td>
<td>20.97 ± 4.95C</td>
<td>48.60 ± 1.26cA</td>
<td>18.55 ± 0.92gC</td>
<td>40.73 ± 1.94deD</td>
</tr>
<tr>
<td>8</td>
<td>39.82 ± 1.61defB</td>
<td>38.69 ± 2.34cdB</td>
<td>46.19 ± 2.67eA</td>
<td>42.08 ± 0.55deB</td>
</tr>
<tr>
<td>9</td>
<td>58.79 ± 1.16cA</td>
<td>48.60 ± 1.26cdB</td>
<td>57.83 ± 1.90eA</td>
<td>37.61 ± 3.12deC</td>
</tr>
<tr>
<td>10</td>
<td>33.20 ± 0.17eB</td>
<td>34.04 ± 1.07cdB</td>
<td>53.06 ± 3.00eA</td>
<td>32.78 ± 0.20eB</td>
</tr>
<tr>
<td>11</td>
<td>21.77 ± 0.54B</td>
<td>23.15 ± 2.15dB</td>
<td>32.23 ± 0.66fgA</td>
<td>23.73 ± 0.47eB</td>
</tr>
<tr>
<td>12</td>
<td>37.94 ± 2.46defB</td>
<td>38.12 ± 6.51cdB</td>
<td>62.91 ± 9.01eA</td>
<td>40.61 ± 3.04deB</td>
</tr>
</tbody>
</table>

Means within a column with the same lower case letters are not significantly different at 5% level (\(p >0.05\))

Means within a row with the same upper case letters are not significantly different at 5% level (\(p >0.05\))
Packaging and storage of *Centella asiatica* drinks

were noticed. These results indicated that one month ambient storage did not lower the concentration of total phenolic content, which was in agreement with the observation of Kevers et al. (2007) and Jasenka et al. (2009) who showed that the phenolic content of many fruits and vegetables remained stable during storage. Van der et al. (2005) also found that one month storage of apple juice in a refrigerator or even at room temperature did not lower the concentration of polyphenolic antioxidants.

Total phenolic content of the packaged drinks decreased significantly \( (p < 0.05) \) starting from the second month onwards. At the second month of storage, about 50% of the phenolic content was lost from all the drinks in the different packaging materials, with the canned *Centella* drinks showing the lowest reading at 148.02 mg/litre GAE, and the highest reading was observed in drinks in standing pouches. Although the can and standing pouch packed *Centella* drinks gave the lowest and highest reading respectively, there was no significant difference between the packaging materials applied until this period.

At the third month, total phenolic content again decreased significantly as compared to the previous month. At this time, total phenolic content of drinks in HDPE bottles, PP bottles, standing pouches and cans had decreased by 77, 79, 72 and 81% respectively, compared with the freshly prepared drinks. The drinks in cans showed significant lower reading among all the packaging materials used, followed by PP bottles, HDPE bottles while standing pouches showed the highest.

This observation could be explained by the characteristics of the packaging materials used. The reaction between the coating of the cans and the compounds inside the drinks maybe responsible for greater decrease of total phenolic content in canned drinks. As mentioned earlier, the stability of polyphenols in food and beverages are influenced by many external factors such as light and oxygen. This effect can be observed in drinks packed in PP bottles, HDPE bottles and standing pouches. Among these three packaging materials, standing pouches are light and oxygen-proof, thus, the total phenolic content of drinks in this material remained the highest compared to those packed in HDPE which is only light-proof and PP bottles which do not have these characteristics.

The lowest total phenolic values for all drinks were recorded after 6–7 months of storage. Further storage at ambient temperature up to 12 months resulted in a slight increase in total phenolic content in all the drinks. Similarly, in the study of polyphenol content and antioxidant activity of orange juices during storage, Klimczak et al. (2007) first observed a decrease in the total phenol content after 4 months storage, followed by an increase at 6 months storage. It is possible that during storage, some compounds are formed that react with the Folin-Ciocalteau reagent and enhanced the phenolic content.

**Effects of packaging materials and time of storage on the antioxidant activity**

It is known that packaging materials influenced the quality of liquid foods during storage, due to the absorption of flavour compounds or permeation through packaging materials. In addition, degradation of flavour, colour, and nutrients also occurs by oxygen transmission through packages (Ayhan et al. 2001).

The levels of DPPH radical scavenging capacity in the *Centella* drinks packaged in four types of packaging materials with time are shown in Table 2. During 12 months storage at ambient temperature, fluctuations in the antioxidant activity of the *Centella* drinks were observed in all four types of packaging materials. The initial radical scavenging capacity was over 80% for all the evaluated drinks. The reading of over 80% remained after one month storage for all the drinks in different packaging materials except drinks in cans. After one
month storage, the antioxidant activity of drinks in cans decreased significantly ($p < 0.05$) to 73.26%, probably due to the reaction between coating of cans with phytochemical compounds in the drinks.

After two months storage, antioxidant activity for all the drinks decreased significantly compared to the initial and first month, however, there was no specific trend of decrease for all the packaging materials used. The antioxidant activity of drinks showed fluctuation readings towards the end of the storage period with the drinks in standing pouches remaining the highest among all the packaging materials used, and drinks in the PP bottles exhibiting the lowest reading. The decrease in the antioxidant activity maybe linked to a lower content of total phenolic content in stored drinks as compared to fresh, meanwhile the increase in the antioxidant activity is usually ascribed to Maillard’s reaction products (Anese et al. 1999). The antioxidant activity of drinks in HDPE bottles, PP bottles, standing pouches and cans decreased by 87.9, 87.5, 78.5 and 85.8% respectively.

Transformation of existing structure and interaction of phenolic antioxidant with other food components may also explain the reducing value of antioxidant activity in juices (Nicoli et al. 1999). Centella asiatica drinks used in this study contained multi components in its formulation including citric acid, sugar and xantham gum coupled with the pasteurization step during processing which can affect more complex reactions and mechanisms of antioxidant activity. Other factors such as the synergism with other food components or chelating agents particularly citric acid, could also attribute to the level of antioxidant activity in the samples.

**Conclusion**

Centella asiatica drinks exhibited substantial decrease in total phenolic content and antioxidant activity after one month storage. At the end of the storage study, drinks packed in standing pouches exhibited greatest stability, followed by HDPE bottles, cans and lastly, PP bottles. These findings suggest that C. asiatica drinks in all the packaging materials studied should be treated as short shelf life products. They should be consumed

### Table 2. Total antioxidant activity (%) of Centella asiatica drinks in different packaging materials during 12 months storage

<table>
<thead>
<tr>
<th>Storage time (month)</th>
<th>HDPE bottle</th>
<th>PP bottle</th>
<th>Standing pouch</th>
<th>Can</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>84.50 ± 0.84aA</td>
<td>84.24 ± 0.93aA</td>
<td>84.12 ± 2.04aA</td>
<td>80.11 ± 1.88aB</td>
</tr>
<tr>
<td>1</td>
<td>84.02 ± 1.20aA</td>
<td>83.34 ± 0.96aA</td>
<td>84.89 ± 1.92aA</td>
<td>73.26 ± 2.01bB</td>
</tr>
<tr>
<td>2</td>
<td>46.68 ± 1.47dE</td>
<td>44.92 ± 1.39dEB</td>
<td>56.79 ± 0.81gA</td>
<td>45.02 ± 1.50fB</td>
</tr>
<tr>
<td>3</td>
<td>45.80 ± 1.27eB</td>
<td>44.33 ± 0.88efB</td>
<td>63.85 ± 1.69dA</td>
<td>45.32 ± 1.24fB</td>
</tr>
<tr>
<td>4</td>
<td>47.66 ± 2.14deB</td>
<td>48.16 ± 1.56cB</td>
<td>57.28 ± 1.40fA</td>
<td>47.70 ± 1.45efB</td>
</tr>
<tr>
<td>5</td>
<td>51.79 ± 1.59bcC</td>
<td>44.60 ± 1.66defD</td>
<td>60.83 ± 1.68eA</td>
<td>55.67 ± 1.54cB</td>
</tr>
<tr>
<td>6</td>
<td>49.30 ± 1.25cdC</td>
<td>47.27 ± 1.48cdC</td>
<td>60.15 ± 1.39efA</td>
<td>54.77 ± 1.57cDB</td>
</tr>
<tr>
<td>7</td>
<td>51.97 ± 2.38bcB</td>
<td>48.60 ± 1.26cB</td>
<td>58.22 ± 1.69efgA</td>
<td>56.06 ± 2.36cA</td>
</tr>
<tr>
<td>8</td>
<td>54.49 ± 1.42cB</td>
<td>47.03 ± 2.39cedeC</td>
<td>69.52 ± 3.25cA</td>
<td>51.08 ± 1.35deCB</td>
</tr>
<tr>
<td>9</td>
<td>54.12 ± 1.46dB</td>
<td>45.93 ± 1.51cdefD</td>
<td>59.50 ± 1.06efgA</td>
<td>48.61 ± 1.18efC</td>
</tr>
<tr>
<td>10</td>
<td>53.20 ± 0.17dB</td>
<td>44.04 ± 1.03fC</td>
<td>73.39 ± 1.32bA</td>
<td>56.12 ± 5.71cB</td>
</tr>
<tr>
<td>11</td>
<td>53.44 ± 1.11dB</td>
<td>53.15 ± 2.15bB</td>
<td>63.90 ± 1.01dA</td>
<td>52.73 ± 0.65cDB</td>
</tr>
<tr>
<td>12</td>
<td>49.28 ± 1.72cDB</td>
<td>34.45 ± 2.11gC</td>
<td>56.91 ± 0.43gA</td>
<td>48.28 ± 1.28efB</td>
</tr>
</tbody>
</table>

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Means within a row with the same upper case letters are not significantly different at 5% level ($p > 0.05$)
Packaging and storage of *Centella asiatica* drinks within the first month after processing to get the most out of the potential health benefits of polyphenol antioxidant.

**References**


Abstrak
Kesan bahan pembungkus dan masa penyimpanan terhadap jumlah kandungan fenol dan aktiviti antioksida dalam minuman pegaga telah dikaji. Jumlah kandungan fenol dan aktiviti antioksida masing-masing ditentukan dengan menggunakan kaedah Folin-Ciocalteau dan 2,2-diphenyl-1-picrylhydrazyl (DPPH). Analisis dijalankan setiap bulan selama setahun bagi minuman pegaga yang diisi dalam botol polietilena berketumpatan tinggi (HDPE), botol polipropilena (PP), ‘standing pouch’ dan tin yang disimpan dalam keadaan suhu ambien (27 ± 5 °C, RH = 75–85%). Jumlah kandungan fenol dan aktiviti antioksida kekal stabil untuk bulan pertama penyimpanan tanpa dipengaruhi oleh jenis bahan pembungkus yang digunakan. Selepas 2 bulan penyimpanan, jumlah kandungan fenol dan aktiviti antioksida telah menurun secara signifikan ($p <0.05$). Minuman di dalam tin menunjukkan kadar penurunan yang paling tinggi, diikuti dengan minuman pegaga di dalam botol PP, botol HDPE, manakala minuman dibungkus dengan ‘standing pouch’ tetap menunjukkan jumlah kandungan fenol dan aktiviti antioksida yang paling tinggi sepanjang tempoh penyimpanan.