Changes of antioxidant activity and total phenolic compounds during storage of selected fruits

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Abstract

The antioxidant activity and total phenolic compounds in various fruits (common fruits—two varieties of mangoes (ripe and unripe), guava, papaya, mangosteen and banana; and two indigenous fruits—makiang and maluod) were investigated. Banana and papaya showed the lowest activity, and mangosteen, mango and guava exhibited high levels of antioxidant activity (oxygen radical absorbance capacity (ORAC), and ferric-reducing antioxidant power (FRAP)) as well as total phenolic compounds. The studied indigenous fruits (makiang and maluod) are high in both antioxidant activity (ORAC and FRAP) and total phenolic compounds. Three independent batches of selected fruits—guava (Psidium guajava), makiang (Cleistocalyx nervosum var. paniala) and maluod (Elaeagnus iatifolia, Linn)—were used to determine the changes in antioxidant activity (AO) and total phenolic compounds (TP) during storage at −20 °C for 3 months and at 5 °C for 10 days. The ORAC-AO during storage at −20 °C for 2 wk decreased significantly in homogenised guava (23%) and in whole fruits of maluod (62%), whereas that of makiang was constant. A continuous decrease in TP was found in homogenised guava throughout the 3-months storage period (69% retention) whereas constant levels were found in other fruits. At 5 °C, a decrease in the ORAC-AO in the whole fruits of makiang (14%) and maluod (70%) was found after a 3-days storage, whereas a gradual increase in the activity (120–190%) was found in the whole fruit of guava throughout the storage period. Among the factors which can affect the levels of antioxidant activity and total phenolic compounds in fruits could be the species, size and texture of fruits, the prepared form of the samples and the conditions of storage (e.g. time, temperature). Preliminary studies on the effect of storage in individual types of fruits are suggested before making a sampling plan for systematic analyses of their antioxidant activity.

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1. Introduction

Due to the increased prevalence of chronic degenerative diseases, people are more aware of their food consumption. This is to prevent the occurrence of the diseases that will affect their quality of life and economic status. Many nutritional factors are widely considered to be critical for human health. Among them, free radicals have been of concern as one of the factors contributing to chronic degenerative disease (Bray, 2000).

In the body, free radicals derive from two sources: endogenous sources, e.g. nutrient metabolism and the ageing process and exogenous sources, e.g. air pollution (Elsayed, 2001; Lachance et al., 2001). Free radicals can attack various substrates in the body and contribute to chronic disease development, for example, oxidatively modified LDL has been hypothesised to be a causative agent in the development of cardiovascular disease (Touyz, 2004). Oxidatively modified DNA may also play an important role in human carcinogenesis (Lim et al., 2004). Usually the human body has mechanisms for eliminating the free radicals by some nutrients in the diet that have antioxidant activities. The Food and Nutrition Board has defined a dietary antioxidant as a substance in commonly consumed foods that significantly decreases the adverse effects of chemically reactive species on normal physiological functions in humans (Food and Nutrition Board, 1999).
Board, 2000). Many researchers around the world have been studying antioxidant activity in various foods, especially in grains, vegetables and fruits (Ou et al., 2002a, b; Sun et al., 2002; Karl and John, 2002). Fruits are more interesting because they are rich in antioxidants and can be consumed on various occasions and as fresh, dried, juice and other processed foods. Antioxidant activities in many varieties of common western fruits have been studied, for example, blueberry, grape, banana, cranberry, prune, apple, plum, tomato and raspberry (Imeh and Khokhar, 2002; Sun et al., 2002; Connor et al., 2002). The data of these components in Thai fruits are limited. Thus, the common fruits such as mango, guava, papaya, mangosteen and banana were therefore investigated for total antioxidant activities and total phenolic compounds. Some indigenous fruits from specific provinces in Thailand that are commonly used for producing local beverages and marmalades were also studied. A common fruit that contains high antioxidant activity and two indigenous fruits were selected for further investigation of the changes of antioxidant activity and total phenolic compounds during storage. Storage of samples prior to analysis could be one of the major factors responsible for variation of the reported antioxidant activities in various food samples. In addition, to preserve nutrients in fresh fruits, it is generally recommended to keep them at a low temperature. The main objective of this study was therefore to investigate the changes of antioxidant activity and total phenolic compounds during storage at a low temperature (in a freezer or a refrigerator) to be used as a guideline for analysts to design their studies or for consumers to appropriately handle the fresh fruits.

2. Materials and methods

2.1. Samples and sample preparation

Three batches each of six common fruits (Table 1), about 500–1000 g each, were purchased from three representative markets in metropolitan areas in Bangkok. They were packed in plastic bags and transported to the laboratory at the Institute of Nutrition, Mahidol University, within 4 h after purchasing. Three batches of indigenous fruits: makiang (Cleistocalyx nervosum var paniala), about 3 kg each, and maluod (Elaeagnus iatifolia, Linn.), about 10 kg each, were collected from their local cultivated areas, Lampang and Chiangrai provinces, respectively, and transported to the Institute within 48 h after harvesting. The samples were used for analyses of total antioxidant activities and total phenolic compounds on the same day of arrival.

To study the effect of storage in a freezer at −20 °C, three batches of fresh guava fruits (Psidium guajava) were purchased from a local market. Each individual batch was homogenised using a food blender and divided into five plastic screw-cap bottles. Another three batches of the whole indigenous fruits (makiang and maluod) were individually divided into five packages in sealed plastic bags. All samples were kept in a freezer at −20 °C. They were taken at 0 and 2 wk, 1, 2 and 3 months for analyses of antioxidant activities and total phenolic compounds.

To study the effect of storage in a refrigerator (at 4–6 °C), three batches of fresh guava (whole fruits without homogenising) and whole fruits of makiang and maluod were individually divided into four packages and kept in a refrigerator for 10 days. They were taken at 0, 3, 6 and 10 days for analyses of antioxidant activities and total phenolic compounds.

2.2. Sample extraction

Without adding water, all the studied common fruits and maluod were homogenised by a food blender whereas a mortar was used to homogenise makiang due to the small size of fruits. Two sets of samples, 2 g each and in duplicate were prepared. One set of duplicate samples was extracted with 25 mL of 50% acetone using a shaker at 400 rpm for 1 h. The extract was diluted to 25 mL with 50% acetone and centrifuged at 2500 rpm for 30 min. The supernatant was used for the analysis of antioxidant activities. Another set of duplicate samples was extracted with 25 mL of 40% ethanol using a shaker at 400 rpm for 1 h. The extract was diluted, centrifuged and the supernate was used for the analysis of total phenolic compounds.

2.3. Oxygen radical absorbance capacity (ORAC) method

The method of Ou et al. (2002a, b) was modified as follows: the reaction was carried out in 75 mM phosphate buffer (pH 7.2), and the final reaction mixture was 4.0 mL. Sample extract (0.5 mL) and fluorescein (3.0 mL) solutions...
were mixed and pre-incubated for 10 min at 37°C. Exactly 0.5 mL of 2,2'-azo-bis, 2-amidinopropane (AAPH) dihydrochloride solution was added and immediately the loss of fluorescence (FL) was followed at 1 min intervals for 35 min. The final results were calculated using the differences of areas under the FL decay curves between the blank and a sample and were expressed as micromole trolox equivalents (TE) per gram (μmol TE/g). All the reaction mixtures were prepared in duplicate, and at least three independent assays were performed for each sample.

2.4. Ferric-reducing antioxidant power (FRAP) method

The FRAP assay was performed as previously described by Benzie and Strain (1996). The experiment was conducted at 37°C, and at pH 3.6 with a blank sample in parallel. In the FRAP assay, reductants (“antioxidants”) in the sample reduce Fe (III)/tripyridyltriazine complex to the blue ferrous form, with an increase in absorbance at 593 nm. The final results were expressed as micromole TE per gram (μmol TE/g).

2.5. Folin–ciocalteu method

A modified method of Waterman and Mole (1994) with an incubation time of 120 min for colour development was performed. Results are expressed as mg gallic acid equivalent (GAE)/100 g sample.

2.6. Quality control system

Tomato sauce (as purchased) was used as an in-house quality control sample for antioxidant activity and total phenolic compound determination. The homogeneity and stability of the sample were investigated. The assigned value of each parameter was developed from the analysis of nine independent control samples, in duplicate, on 3 different days. The quality control sample was then analysed in every batch, along with the unknown samples. The values of each studied component in the in-house quality control samples were accepted when they were within mean ± 2 S.D.

2.7. Statistical analysis

Descriptive statistical analysis was performed using Microsoft Excel. The data of moisture contents, antioxidant activity and total phenolic compounds are expressed as mean ± standard deviation (SD) from three independent samples in duplicate. Analysis of variance (ANOVA) was applied to investigate the change of the components during storage at different temperatures (~20 and 5°C), conditions (whole fruit or homogenised) and periods (days to weeks or months). Difference was considered statistically significant at the level of p < 0.05.

3. Results and discussion

In view of the fact that several food components, i.e. carotenoids, vitamin C, vitamin E, phenolic compounds and their interactions contribute to the overall antioxidant activity of foods, it is difficult to measure total antioxidant activity on the basis of individual active components (Pinelo et al., 2004). Therefore, we decided to investigate the antioxidants in the form of total activity in this study. Two representative methods, ORAC and FRAP, based on different principles were selected for measurement of antioxidant activity in test materials. ORAC measures the reaction between the antioxidants and the peroxyl radicals whereas the FRAP measures the total reducing capacity of any compounds in the test materials. Thus, different levels of antioxidant activity can be expected from these two methods.

The determination of total phenolic compounds was included in this study because strong correlations between total phenolic compounds and antioxidant activity in various kinds of fruits were found in previous studies (Gorinstein et al., 2004; Sellappan et al., 2002). Their determination by the Folin–Ciocalteu method was used since many individual phenolic compounds, which provide antioxidant activity in fruits, cannot be identified and measured by HPLC methods (Ferreira et al., 2002). The HPLC method presented only 50–60% of the level analysed by the Folin–Ciocalteu method (Scalbert et al., 2000; Ferreira et al., 2002).

3.1. The antioxidant activity and total phenolic compounds in selected fruits

Mangoes (ripe and unripe varieties), guava, papaya, mangosteen and banana (Table 1) were selected as fruits commonly consumed in Thailand and ASEAN countries. Makiang (C. nervosum var. paniala) and maluod (E. latifolia, Linn) are indigenous fruits from specific provinces in the northern part of Thailand. The taste of makiang and maluod is sweet and sour with a good flavour and, so they can be transformed into many products, e.g. local beverages and marmalades, with the natural colour of dark red. They are included in the Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn. The selected fruits were analysed for antioxidant activity and total phenolic compounds as soon as possible after collection.

As shown in Table 2, individual fruits collected from different places contained similar levels of moisture content. In contrast, a wide variation of their antioxidant activity was observed. Differences in cultivation location (Wang et al., 2002; Hakkinen and Torronen, 2003) and practices (Carbonaro et al., 2002), ripening stage (Raffo et al., 2002), harvested condition and seasons (Wu et al., 2004b) could be the most likely factors. Higher values of antioxidant activity analysed by ORAC than by the FRAP
method were found in most of the studied fruits except those of papaya, banana, and maluod (with a linear regression correlation of $R = 0.711$ between ORAC and FRAP for all the studied fruits). It is likely that not all ORAC-active antioxidants in the studied fruits are reducing agents (detected by FRAP). The rank of ORAC antioxidant activity values in fruits was makiang > maluod > mangosteen > ripe mango (nam-dok-mai) > guava > unripe mango (kiew-sa-weya) > papaya > banana. The rank of FRAP antioxidant activity values in fruits was not similar to the ORAC values: maluod > makiang > guava. The others contained low levels, 4–8$\mu$mol TE/g, of FRAP antioxidant activity. It is not surprising that a different ranking order of antioxidant activity among the studied fruits was shown by ORAC compared to the FRAP method because in theory the measurement of each method is based on different mechanisms of reaction (Benzie and Strain, 1996; Prior and Cao, 1999). Therefore, the antioxidant activity should be investigated by more than one method (Aruoma, 2003). As with papaya, a low level of antioxidant activity—analysed by both ORAC and FRAP methods—was found in banana, which agreed well with the values reported by Wang et al. (1996), 2.6 and 2.2$\mu$mol TE/g, respectively, whereas Wu et al. (2004a) reported a higher value. No data of antioxidants in other studied fruits are available in the literature for comparison.

The rank of phenolic compounds in the studied fruits was makiang > guava > ripe mango (nam-dok-mai) > maluod > mangosteen > unripe mango (kiew-sa-weya) > papaya > banana. A moderate level of correlation (with a linear regression correlation of $R = 0.821$) between the values of total phenolic compounds and ORAC antioxidant activity in these fruits was found. The correlation increased ($R = 0.967$) when the values of mangosteen and maluod were excluded. A lower level of correlation (with a linear regression correlation of $R = 0.559$) was found between the values of total phenolic compounds and FRAP antioxidant activity. A similar finding was obtained when the values of maluod were excluded; the linear regression correlation increased to 0.973. The results indicated that in some fruits for e.g. maluod, the antioxidant activity is not mainly contributed by phenolic compounds.

At present, there is no information on the recommended intake level for total antioxidant activity and phenolic compounds. Prior et al. (1998) estimated the normal intake of antioxidants by measuring the antioxidant activity in foods consumed by the American people using the ORAC method. A range of 1200–1700$\mu$mol TE/day was reported. A normal intake of approximately 1000 mg GAE/day of total phenolic compounds was estimated by Scalbert and Williamson (2000). According to the Thai FBDG, Ministry of Public Health (1999), the recommended amount of fruits consumed per day should be about 250 g; therefore, the studied common fruits could provide an average of 3230$\mu$mol TE/day of ORAC antioxidant activity and 200 mg GAE of total phenolic compounds. The studied indigenous fruits—makiang and maluod—which are normally consumed as beverages (25% and 40% concentration, respectively), contribute 820 and 1120$\mu$mol TE of ORAC antioxidant activity and 74 and 98 mg GAE of total phenolic compounds per 200 mL serving, respectively (unpublished data). This study confirms that fruits are excellent sources of antioxidants.

### 3.2. Changes of antioxidant activity and total phenolic compounds during storage at $-20^\circ$C

Storage of food samples at $-20^\circ$C when analyses cannot be performed immediately is a common practice. This also applies for the examination of antioxidant activity and total phenolic compounds; therefore, the effect of sample storage in a freezer on the levels of both components was investigated. Guava was selected as a representative of common fruits with high antioxidant activity (Table 2) that are available all year round whereas mango and mangosteen are seasonal. Since guava is a large size fruit, 10–12 cm
in diameter, compared to the small size of makiang (1 cm × 1.5 cm) and maluod (2 cm × 3 cm), the guava was homogenised before freezer storage whereas the latter indigenous fruits were kept whole.

As shown in Table 3, the moisture content of each fruit was stable throughout the storage period of 3 months. Among different kinds of studied fruits, different rates and degrees of loss in the ORAC and FRAP antioxidant activity occurred during storage. The ORAC antioxidant activity in the homogenised guava and the intact maluod (small, soft flesh fruit) decreased significantly during storage at −20 °C for 2 weeks and continued to decrease during 3 months of storage, whereas that of makiang (small, firm flesh fruit) was stable. In contrast, FRAP antioxidant activity did not decrease in homogenised guava but those of the whole fruits of makiang and maluod decreased during freezer storage.

The level of total phenolic compounds in the homogenised samples of guava was gradually decreased throughout the 3 months storage period while those of the whole makiang and maluod did not decrease. Homogenisation of guava fruits before storage could induce the reaction of the endogenous polyphenol oxidase during frozen storage at −20 °C. This could result in the observed gradual reduction of the phenolic compound level in guava during storage in the freezer. Not only the amount of phenolic compounds but also other antioxidants were affected by homogenisation of the sample, as can be seen by the same reduction pattern in the ORAC antioxidant activity. Therefore, in the study of phenolic compounds and antioxidant activity, if possible, the sample should be analysed as soon as possible to prevent oxidation and enzymatic degradation. It was noticed that there were some fluctuations in the results of both antioxidant activity and total phenolic compounds in makiang and maluod but not in guava (Table 3). This is because several composite samples of the whole fruits of makiang and maluod were used for study at each individual storage period compared to the homogenised samples of guava. Variations of those components among the tested fruits in each composite sample of the whole tested fruits are likely involved. Although the level of total phenolic compounds in the whole fruits of makiang and maluod seemed to be stable when they were kept at −20 °C, a higher level of fluctuation was observed in maluod than makiang that could be due to the sample size effect.

Maluod is twice as large as makiang; therefore, fewer fruits were sampled in each set, which might affect the homogeneity of their components.

As noted earlier, correlations between total phenolic compounds and antioxidant activity in various kinds of fruits have been found in previous studies (Gorinstein et al., 2004; Sellappan et al., 2002). We found a correlation between ORAC and total phenolic compounds in guava (decreasing with storage) and makiang (unchanged with storage) but not with maluod (Table 3). This is likely due to the various types and amounts of antioxidants, including various types of phenolic compounds present in the fruits, which are responsible for the measured antioxidant activity.
and which have different rates of degradation during storage (Sakakibara et al., 2003).

3.3. Changes of antioxidant activity and total phenolic compounds during storage at 5 °C

Storage at 5 °C was selected as being representative of the common storage temperature for fruits used by consumers. Thus, the effect of the storage period on the levels of antioxidant activity and total phenolic compounds in the whole fruits of guava, makiang and maluod was followed for 10 days. Normally consumers would not keep these fruits so long even under refrigeration.

As shown in Table 4, the moisture content of the fruits did not change during storage at 5 °C. The ORAC and FRAP antioxidant activity in guava tended to increase with a storage of 10 days whereas those of makiang and maluod decreased slowly in the former and quickly in the latter. During this short storage period, the content of total phenolic compounds in the intact studied fruits was stable. A higher degree of variation in the values of the components within and between the studied periods was found, in guava more than maluod, and the least in makiang, corresponding to the relative sizes of the fruits. This is consistent with the effect noted during frozen storage. This effect makes it difficult to evaluate the relationship between ORAC and FRAP and total phenolic compounds.

Based on the findings in this study, in order to maximise antioxidant concentration, makiang should not be kept in a refrigerator for more than 3 days and maluod should be consumed or analysed as fresh as possible.

4. Quality control system

The quality control system is essential for this type of study to show the consistency of sequential measurement of the analyst. Tomato sauce was selected as an in-house quality control sample for antioxidant activity and total phenolic compound determination because it is well recognised to contain antioxidants, is commercially available with good homogeneity, and is not expensive. Duplicate values for FRAP antioxidant activity and total phenolic compounds resulting from analysis of 10 single samples of tomato sauce were determined. The within-sample variation, which was evaluated by Cochran’s maximum variance test, showed that the variation within all samples could be ignored (the maximum variance or range divided by the sum of variance or range was less than the critical values for Cochran’s maximum variance test at the 5% level). The results indicated good precision of the analysts who performed homogeneity testing of the antioxidant activity and total phenolic compounds in the test material. According to the F-values (MSB/MSW), which were less than the critical F-values, the tomato sauce was considered sufficiently homogenous in terms of the analysed components. The assigned value of each parameter was developed from the duplicate analysis of nine random single samples on 3 different days; they were 13.71 ± 1.89, 4.70 ± 0.38 (μmol TE/g) and 80.68 ± 4.69 mg GAE/100 g for ORAC, FRAP antioxidant activity and total phenolic compounds, respectively. The levels of FRAP antioxidant activity and total phenolic compounds analysed in five random single samples of tomato sauce at 1-year storage were found to be within the values of mean ± 2 SD of the assigned values. The quality control

<table>
<thead>
<tr>
<th>Name</th>
<th>Day</th>
<th>Moisture (g/100 g)</th>
<th>Antioxidant activity (μmol TE/g)</th>
<th>Total phenolic compounds (mg GAE/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ORAC2</td>
<td>FRAP2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>% Retention</td>
<td>Values5</td>
</tr>
<tr>
<td>Guava</td>
<td>0</td>
<td>90.1 ± 0.7</td>
<td>12 ± 3.3a</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>89.8 ± 0.8</td>
<td>14 ± 4.2a</td>
<td>119 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>89.0 ± 0.7</td>
<td>22 ± 3.4b</td>
<td>191 ± 31.5</td>
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<tr>
<td></td>
<td>10</td>
<td>89.4 ± 0.5</td>
<td>16 ± 1.2ab</td>
<td>144 ± 28.4</td>
</tr>
<tr>
<td>Makiang</td>
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<td>85.3 ± 0.1</td>
<td>37 ± 0.8a</td>
<td>100</td>
</tr>
<tr>
<td></td>
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<td>86.4 ± 0.6</td>
<td>31 ± 2.6a</td>
<td>86 ± 8.8</td>
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<td>87.4 ± 0.1</td>
<td>27 ± 1.4c</td>
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<td>10</td>
<td>89.6 ± 0.9</td>
<td>26 ± 0.7c</td>
<td>75 ± 6.9</td>
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<tr>
<td>Maluod</td>
<td>0</td>
<td>88.6 ± 0.1</td>
<td>26 ± 1.6a</td>
<td>100</td>
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<tr>
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<td>7.9 ± 1.0b</td>
<td>30 ± 4.4</td>
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<tr>
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<td>7.5 ± 1.3b</td>
<td>29 ± 6.4</td>
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<tr>
<td></td>
<td>10</td>
<td>89.2 ± 0.1</td>
<td>11 ± 1.1c</td>
<td>43 ± 6.5</td>
</tr>
</tbody>
</table>

1Mean ± SD obtained from analysis of three independent samples, in duplicate.
2Oxygen radical absorbance capacity (ORAC), expressed as μmol of trolox equivalents per gram of fresh weight.
3Ferric reducing antioxidant power (FRAP), expressed as μmol of trolox equivalents per gram of fresh weight.
4Gallic acid equivalent.
5Means within the same column having different superscript indicate significant difference (p < 0.05).
Table 5
Data from analysis of quality control samples—tomato sauce

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Assigned value</th>
<th>%CV</th>
<th>Quality control data (n = 26)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORAC antioxidant activity (μmol TE/g)</td>
<td>13.71 ± 1.89</td>
<td>13.8</td>
<td>14.45 ± 1.69</td>
<td>11.7</td>
</tr>
<tr>
<td>FRAP antioxidant activity (μmol TE/g)</td>
<td>4.70 ± 0.38</td>
<td>8.0</td>
<td>4.42 ± 0.34</td>
<td>7.8</td>
</tr>
<tr>
<td>Total phenolic compounds (mg GAE/100 g)</td>
<td>80.68 ± 4.69</td>
<td>5.8</td>
<td>83.49 ± 5.45</td>
<td>6.5</td>
</tr>
</tbody>
</table>

1 Mean ± SD obtained from analysis of nine independent control samples, in duplicate, on 3 different days.
2 Oxygen radical absorbance capacity (ORAC), expressed as μmol of trolox equivalents per gram of fresh weight.
3 Ferric-reducing antioxidant power (FRAP), expressed as μmol of trolox equivalents per gram of fresh weight.
4 Gallic acid equivalent.

sample was then analysed along with the unknown samples. As shown in Table 5, the component values of the in-house quality control sample within mean ±2 SD of the assigned values were achieved for each set of analysis. The relative standard deviation of less than 10% was obtained, except that of the ORAC antioxidant activity (% CV = 11.7) which is the most complicated measurement.

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References


