Research Paper

In vitro Antioxidant Capacities of Star Fruit (Averrhoa carambola), an Underutilised Tropical Fruit

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Abstract

Star fruit is an underutilised tropical fruit that could serve as an alternative food source to the world. The current study was, therefore, undertaken to investigate the functional potentials of green and ripe star fruits by determining its antioxidant contents and capacities. The antioxidant capacities of star fruit were increased significantly with ripening, except for the total ascorbic acid content. The ripe star fruit peel contained higher total polyphenol (98.19 g TAE/100 g FW), total flavanol (33.31 g CAE/100 g FW) and ascorbic acid contents (1.56 g AAE/100 g FW) than green star fruit peel. Ripe star fruit peel also demonstrated stronger FRAP (1.41 M FEA/100 g FW) and DPPH (75% inhibition) values than green star fruit peel. The antioxidant capacities of peel were greater than pulp. On the basis of results obtained, the ripe star fruit is a potential source of natural antioxidants owing to its significant antioxidant activities.

Keywords: Star Fruit, FRAP, DPPH, Polyphenol, Ascorbic Acid

1. Introduction

During normal activities, various processes inside the human body produce reactive oxygen species (ROS) which often cause cell death and are involved in other degenerative processes associated with ageing (Babu & Rao, 2011 and Gao et al, 2011). As a natural defense system, our body is protected against these free radicals by antioxidant molecules and antioxidant enzymes (Gao et al, 2011). Fruits and vegetables are good plant sources that provide dietary antioxidant to human health (Shui & Leong, 2006 and Yogesh & Ali, 2012). This dietary antioxidant is complemented to antioxidants that naturally produced in human body. It can enhance the potency of antioxidant in scavenging oxidative stress in human body and thereby reduce the risk of having some chronic diseases (Chakraborty et al, 2009 and Kedar & Singh, 2011). Furthermore, the advantages of natural antioxidants in foods are high consumer acceptance due to these health issues (Loganayaki et al, 2011 and Yogesh & Ali, 2012). Star fruit (Averrhoa carambola) is one of the underutilised tropical fruits that belong to Oxalidaceae family. It is popular in Southeast Asia countries and has been cultivated in several countries for its edible fruits. Star fruit is sweet in taste and usually consumed fresh or made into juice. Several studies revealed that star fruit containing as high antioxidant level as guava, papaya and banana (Lim et al, 2007). With substantial promotions and research evidences, this underutilised fruit could play an important role as an alternative food source to the world and in improving human health. However, only a few publications were reported on the functional potentials of star fruit. Therefore, the current study was undertaken to investigate the total antioxidant contents and capacities of star fruit.

2. Materials and Methods

2.1. Sample Preparation
5 grams of star fruit’s peel and pulp were extracted with 50 ml of 80 % (v/v) methanol and mixed for 2 hours at room temperature using an orbital shaker (Yih Der, Taiwan) set at 150 rpm. The extracts were filtered with No.1 Whatman paper and concentrated using a rotary evaporator (Buchi, USA) at 45 °C for 1 hour. The concentrates were then redissolved in 5 ml of 80 % (v/v) methanol and stored at -80 °C freezer till used.

2.2. Extraction of Phytochemicals

5 grams of star fruit’s peel and pulp were extracted with 50 ml of 80 % (v/v) methanol and mixed for 2 hours at room temperature using an orbital shaker (Yih Der, Taiwan) set at 150 rpm. The extracts were filtered with No.1 Whatman paper and concentrated using a rotary evaporator (Buchi, USA) at 45 °C for 1 hour. The concentrates were then redissolved in 5 ml of 80 % (v/v) methanol and stored at -80 °C freezer till used.

2.3. Determination of Total Polyphenol Content

Total polyphenol content was determined using the Folin-Ciocalteau method adopted from Ramamoorthy & Bono (2007) with minor modifications. Star fruit extract of 0.125 ml was mixed with 0.2 N Folin-Ciocalteau reagent (Merck, Germany) and incubated for 5 minutes at room temperature. Then, 0.5 ml of 7.5 % (w/v) of Na2CO3 (Merck, Germany) was added and topped up with 5 ml of distilled water. The mixture was incubated at room temperature for 2 hours. Absorbance was then measured at 760 nm against a reagent blank. The polyphenol content of sample was determined based on the tannic acid (QRëC, Thailand) calibration curve (0 to 1.0 g/l). Result was expressed as g Tannic acid equivalent (TAE)/100 g of sample fresh weight (FW).

2.4. Determination of Ascorbic Acid Content

Ascorbic acid content was determined using modified method of Hussain et al (2010). Star fruit extract of 0.5 ml was added with 4.5 ml of 0.05 M oxalic acid (R&M Chemicals, USA), 0.5 ml of 3 % (w/v) metaphosphoric acid (Merck, Germany), 1 ml of 5 % (v/v) H2SO4 (System ChemAR®, Poland), 2 ml of 5 % (w/v) ammonium molybdate (Fisher Scientific, UK) and topped up to 25 ml with distilled water. Absorbance was then measured at 760 nm against a reagent blank. The ascorbic acid content of sample was determined based on the ascorbic acid (Fluka, USA) calibration curve (0.01 to 0.09 mg/ml). Results were expressed as mg ascorbic acid equivalent (AAE)/100 g sample FW.

2.5. Determination of Flavanol Content

Total flavanol content was determined using the p-dimethylaminocinnamaldehyde (DMACA) method adopted from Arnous et al (2001) with minor modifications. Star fruit extract of 0.2 ml was mixed with 1 ml of 0.1 % (v/v) DMACA (Sigma-Aldrich, USA) and incubated for 10 minutes at room temperature. Absorbance was then measured at 640 nm against a reagent blank. The total flavanol content of sample was determined based on the Catechin (Sigma-Aldrich, USA) calibration curve (0 to 16 mg/l). Result was expressed as g Catechin equivalent (CAE)/100 g of sample FW.

2.6. Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay was carried out according to the method of Ahmed & Beigh (2009) with minor modifications. The FRAP reagent was prepared by mixing 25 ml of 0.3 M acetate buffer (pH 3.6), 2.5 ml of 10 mM 4,6-tripyridyl-s-triazine (TPTZ; Fluka) solution and 2.5 ml of 20 mM FeCl3. After that, 100 μl of star fruit extract was mixed with 3 ml of FRAP reagent and incubated in water bath at 37 °C for 10 minutes. Absorbance was then measured at 593 nm against a reagent blank. The ferric reducing antioxidant power of sample was determined based on the FeSO4 (R&M Chemicals, USA) calibration curve (0.0 to 1.0 mM). Results were expressed as mM FeSO4 equivalent (FEA)/100 g sample FW.

2.7. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical-Scavenging Assay

The ability of antioxidants to scavenge the DPPH free radical was carried out according to the modified method of Lachman et al (2006). The DPPH solution (Sigma, USA) was prepared freshly by mixing 2.5 mg of DPPH with 100 ml of absolute methanol. After that, 5 μl of star fruit extract was mixed with 3 ml of DPPH reagent and incubated at room temperature for 30 minutes. Absorbance was then measured at 515 nm wavelength. The radical scavenging activity of sample was calculated based on decrease of absorbance in percentage, designated as percent inhibition. The calculation was as follows:

\[
\% \text{ Inhibition} = 100 - \left( \frac{A_{\text{Sample}} - A_{\text{Blank}}}{A_{\text{Blank}}} \right) \times 100
\]

The \( A_{\text{Sample}} \) is the absorbance of tested sample extract. \( A_{\text{Blank}} \) is the absorbance of control reaction (containing all reagents except the test sample).

2.8. Statistical Analysis

All tests were carried out in triplicates. The data were presented as the mean ± standard deviation of the mean. Analysis of variance was calculated using the General

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The antioxidant capacities of star fruit was increased significantly (p<0.05) with ripening (Table 1). The ripe star fruit (RS) exhibited higher antioxidant activities and contents than green star fruit (GS), except for the total ascorbic acid content. No significant difference (p>0.05) was observed in the total ascorbic acid content, suggested that vitamin C does not contribute significantly to the increase of antioxidant activities during ripening.

**Table 1. Antioxidant Capacities of Green and Ripe Star Fruits**

<table>
<thead>
<tr>
<th>Star Fruit</th>
<th>Total Polyphenol Content (g TAE/100 g FW)</th>
<th>Total Flavanol Content (g CAE/100 g FW)</th>
<th>Total Ascorbic Acid Content (g AAE/100 g FW)</th>
<th>FRAP (% Inhibition)</th>
<th>DPPH (% Inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS peel</td>
<td>36.95 ± 7.17(^b)</td>
<td>13.12 ± 0.82(^b)</td>
<td>1.24 ± 0.18(^ab)</td>
<td>0.48 ± 0.09(^b)</td>
<td>56.41 ± 4.01(^b)</td>
</tr>
<tr>
<td>RS peel</td>
<td>98.19 ± 13.83(^a)</td>
<td>33.31 ± 4.35(^a)</td>
<td>1.56 ± 0.24(^a)</td>
<td>1.41 ± 1.03(^a)</td>
<td>75.00 ± 6.93(^a)</td>
</tr>
<tr>
<td>GS pulp</td>
<td>16.18 ± 1.40(^c)</td>
<td>7.06 ± 0.82(^b)</td>
<td>0.86 ± 0.09(^b)</td>
<td>0.16 ± 0.02(^c)</td>
<td>22.82 ± 2.21(^d)</td>
</tr>
<tr>
<td>RS pulp</td>
<td>39.89 ± 5.29(^b)</td>
<td>16.01 ± 2.07(^ab)</td>
<td>1.15 ± 0.38(^ab)</td>
<td>0.52 ± 0.04(^b)</td>
<td>38.85 ± 6.63(^c)</td>
</tr>
</tbody>
</table>

\(^a\), \(^b\), \(^c\) Different letters in each column indicate significant difference at p<0.05

The total polyphenol and total flavanol contents of RS peel were 2.7-fold and 2.5-fold, respectively, higher than GS peel. Whereas in the FRAP and DPPH assays, RS peel exhibited 2.9-fold and 1.3-fold, respectively, higher than GS peel. These results indicated that polyphenols such as flavanol were contributed significantly in ferric reducing capacity and radical scavenging capacity of star fruit peel during ripening. Luximon-Ramma et al (2003) reported that the high antioxidant activities in star fruit were associated with flavanol and flavones. While Mahattanawee et al (2006) reported that star fruit was rich in hydrolysable tannins.

The total polyphenol content of RS pulp were 2.5-fold higher than GS pulp. Nevertheless, the difference of the total flavanol and ascorbic acid contents between GS and RS pulps were not significant (p>0.05). In the FRAP and DPPH assays, RS pulp were 3.3-fold and 1.7-fold, respectively, higher than GS pulp. The increase in the total polyphenol content together with the increase in FRAP and DPPH values in RS pulp suggested that the antioxidant capacities during ripening might be contributed by polyphenols other than flavanol and vitamin C. Earlier study revealed that star fruit contains proanthocyanidins and (-)-epicatechin (Shui & Leong, 2004). Proanthocyanidins are strong antioxidants that protect against free radical damage, lipoperoxidation, cancer and vascular disorders and inhibit platelet aggregation while epicatechin stabilises free radicals through hydrogen donation (Teissedre et al, 1996 and Plumb et al, 1998). The current study also demonstrated that the star fruit peel contained a higher antioxidant capacity than pulp. Similar findings were also reported on fruits, such as pomegranate, plum, nectarines, apple and mango (Li et al, 2006 and Kim et al, 2010). Kim et al (2010) reported that peel extract gave a remarkable effect in protecting cell from oxidative damage. This could be due to the additional phenolic compounds present in peel other than juice and pulp residue (Li et al, 2006).

### 4. Conclusion

The results attained in this study suggested that star fruit’s peel and pulp have significant antioxidant capacities, in which peel exhibited the most promising antioxidant potential. The antioxidant capacities of star fruit was increased significantly during ripening as the ripe star fruit exhibited higher antioxidant activities and contents than green star fruit.

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**References**


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