Antioxidant potential of banana: Study using simulated gastrointestinal model and conventional extraction

Anjali Bhatt & Vinayak Patel*

Laboratory of Foods and Nutrition, Post Graduate Department of Home Science, Sardar Patel University, Vallabh Vidyanyagar, Anand, Gujarat-388 120, India.

Received 28 December 2013; revised 30 May 2014

Most reports on fruit antioxidant capacities are based on extraction of antioxidants using polar solvents. In banana, little is known about the fate of bioactive compounds during the digestion process, particularly in the food matrix under the gastric and intestinal conditions. In the present study, an in vitro gastrointestinal digestion method was used to simulate physiological conditions of the stomach and small intestine to evaluate the actual antioxidant capacity of banana. The simulated gastrointestinal extracts showed significantly higher antioxidant properties. The total phenol content of the physiological enzymatic extract was higher by almost 150% than the methanolic extract. Similarly, the flavonoid and flavonol contents were higher in the physiological enzymatic extract by 330.6 and 141.7%, respectively as compared to methanolic extract. These differences were also noticed in the antioxidant capacity measurement parameters. From the results, it can be concluded that the conventional extracts underrate the antioxidant value of banana and that they may have much higher health significance, as an antioxidant in particular.

Keywords: Carotenoids, Flavonoids, Flavonols, Food matrix, Free radical scavenging, Fruits, Musa paradisiaca, Nutrition, Phenols, Plantain

Fruits are health enhancing foods as they have the largest varieties of antioxidants. Antioxidants when consumed and absorbed by the body provide protection against the reactive oxygen species (ROS). ROS is responsible for the deleterious effects caused by the oxidation reactions to important biomolecules like DNA. This may in turn lead to early aging and other lifestyle diseases. Though banana peel has abundant of antioxidants, it is generally considered inedible for humans and is seldom consumed. Cressey et al. proved that daily consumption of banana improves blood glucose and lipid profile in hypercholesterolemic subjects and increases serum adiponectin in type 2 diabetic patients. However, the antioxidant potential of banana stands neglected. It is because the true bioaccessibility of banana antioxidants is unknown as the available reports based on the conventional procedures grossly under rated its actual potential. The extraction of antioxidants from food samples has been mostly done with the help of organic solvents with or without using water. Some studies also involve use of combinations of two or more organic solvents. These procedures are simple and time saving. However, the antioxidants extracted do not reflect the actual uptake of antioxidants by the body. Phenolic compounds mainly exist as glycosides linked to various sugar moieties or as other complexes linked to organic acids, amines, lipids, carbohydrates, and other phenols. The enzymatic treatments hydrolyze starch and protein, which may favour the release of polyphenols. The biological properties of antioxidants depends on the release of phenolic compounds from the food matrix during the digestion process (bioaccessibility) and may differ quantitatively and qualitatively from those produced by the chemical extraction employed in most studies. The amount of nutrients and phytochemicals absorbed during digestion is governed by the physical properties of the food matrix which affects the efficiency of physical, enzymatic and chemical digest. Many studies have reported antioxidant potential of different food stuffs after gastrointestinal digestion.

The conventional extraction procedure does not take into account the digestion and absorption that takes place in vivo. Thus, the true antioxidant capacity of
banana may vary from those reported by these studies. Consequently, to overcome these pitfalls, present study was designed to measure the true antioxidant capacity of banana. It attempts to demonstrate the bioaccessible antioxidant capacity of banana as well as the difference between the two extraction procedures.

Materials and Methods

Chemicals—Pepsin (P-7000), Pancreatin (P-1750), Lipase (L-3126), Bile Extract Porcine (B-8631), α-Amylase (A-3176), Amyloglucosidase (A-7095), ABTS (A-1888), DPPH (D-9132), Catechin (C-1251), Vanillin (V-2375), Rutin (R-5143), Gallic acid (G-7384) and TPTZ (T-1253) were purchased from Sigma Aldrich-Germany and Trolox–56510 was purchased from Fluka.

Sample preparation—Moderate ripe variety of banana (Musa paradisiaca) was purchased from the local market, peeled and the edible part was mashed to homogenize.

Extraction and enzymatic digestion

Extraction by conventional method—Banana sample was extracted twice in 80% aqueous methanol (pH set 2.0 with HCl) by shaking at 35 °C for 45 min. Both the supernatants were pooled to make up a known volume.

Extraction by in vitro gastrointestinal digestion—Banana sample was used for in vitro gastrointestinal digestion. The digestive enzymatic extraction was carried out by using the in vitro procedure previously described. Samples were successively incubated with digestive enzymes to simulate digestion in the small intestine. A control of sample was also incubated similarly with buffers without addition of enzymes.

Both chemical and digestive extracts (control and enzymatic) were used to determine the antioxidant capacity.

Determination of total phenol, flavonoid and flavonol—Folin–Ciocalteu method was used to determine the total phenol content of the chemical and physiological extracts. Different aliquots of known concentration of gallic acid were taken as standard. Flavonoid content was estimated by the method of Zhishen et al. Different aliquots of Rutin were treated as standard. Flavonol content was measured from concentrated samples by the method of Yermakov et al. Different aliquots of known concentration of Catechin were treated as standard.

Determination of Antioxidant Capacity—The antioxidant capacity was measured by four different methods, namely Ferric Reducing Antioxidant Power (FRAP), DPPH Radical Scavenging Activity (DPPHRSA), ABTS Radical Scavenging Activity (ABTSRSA) and Reducing Power Assay (RPA). For each method, a calibrated Trolox curve was standardized and results were expressed in terms of Trolox Equivalent Antioxidant Capacity i.e. TEAC (mg of Trolox equivalents/100 g). The antioxidant activity of the extracts to scavenge the stable DPPH radical was determined by the method of Brand-Williams et al. The radical scavenging capacity of different wheat extracts was determined using the modified ABTS radical decolorization assay as described by Re et al. FRAP and RPA were determined by the methods, Benzie & Strain and Oyaizu, respectively.

Statistical analysis—Experiment was done in duplicate batches with two separate purchases in the same season. Four observations of two different experiments were analyzed statistically. Differences between variables were tested for significance by using a one-way ANOVA, DUNCAN using the level significance of P ≤0.05 by SPSS.

Results and Discussion

In literature, the antioxidant capacity of cereals (viz. wheat flour, bread, raw and boiled rice, wheat bran, and oat bran) has been possibly underestimated because the extraction solvents normally used do not allow a complete release of antioxidant compounds and additionally, non-extractable polyphenols with a high antioxidant capacity are ignored. To overcome these drawbacks of extraction and to know the definite difference between the two extraction procedures, here, we studied the antioxidant potential of banana adopting both the procedures. To the best of our knowledge, so far there are no published data for antioxidant of bananas after simulated digestion.

Results of this study revealed considerable differences in the antioxidant potential of banana when the conventional and physiological extracts were compared. Table 1 shows the mean values of the antioxidants in terms of the contents of total phenol (TPC), flavonoid and flavonol of different banana extracts. The TPC of the physiological enzymatic extract (PE) was higher by almost 150% than the methanolic extract (ME). However, when the enzymes were absent in the physiological control
(PC) i.e., physiological digestion without enzymes, the TPC was less by almost 80% compared to the methanolic extract (ME). Similarly, the flavonoid and flavonol contents were higher in the PE by 330.6 and 141.7%, respectively than that of ME. Their PC extracts were both lower by almost 77% as compared to control. The increased values of flavonoid in the physiological enzymatic extract group could be due to the increase in the flavonol content after the enzymatic digestion which in turn could have increased the TPC. Absorption of flavonoids in the small intestine happens only when their sugar (glycosides) components are removed. This process is controlled by the action of enzymes manufactured in the small intestine (for example, mammalian β-glucosidase), resulting in the release of the flavonoid skeleton (the aglycone) from its sugar.18 The newly released free, low molecular weight phenolic compounds show enhanced antioxidant potential. Antioxidant capacity of banana was also affected by these differences in the extraction procedures. The results of antioxidant capacity of banana extracts are given in Table 2. When extracted physiologically without enzymes (PC), FRAP of banana was found less by 42.6% and RPA, 50% as compared to ME. This reduction could be attributed to the fall in the phenolic content after PC. On the other hand, the PE was higher in FRAP and RPA by 196% and 69%, respectively as compared to ME. The radical scavenging activities were also low by 46% and 90% in ABTSRSA and DPPHRSA, respectively compared to ME when banana was extracted in the buffers without enzymes (PC). However, on subjection to enzymatic treatment (PE), they had 66% and 167% higher radical scavenging potentials compared to ME. The increase in phenolic compounds after the enzymatic treatment could have possibly boosted the antioxidant capacity of banana. Analysis of the antioxidant capacity has revealed that they are highly dependent on the phenolic compounds. A significantly strong and positive relationship (<i>P</i> ≤0.05) was recognized between TPC and antioxidant potential of banana. The present observations have also been compared with other similar studies2,19-22 as well (Table 2).

These results are in agreement with our previous study on garlic23. Similar results using simulated digestion for extraction of carotenoids and phenolic compounds were previously shown by Epriliati et al.24 in tomato, papaya, and mango as well as Perez-Jimenez and Saura-Calixto17 in cereals. Other studies

<table>
<thead>
<tr>
<th>Table 1—Total phenol content, flavonoid content and flavonol content of different banana extracts</th>
<th>ME</th>
<th>PC</th>
<th>PE</th>
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<tr>
<td><strong>Parameter</strong></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
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<tr>
<td>TPC (mg GAE/100g)</td>
<td>404.68&lt;sup&gt;b&lt;/sup&gt;±23.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.76&lt;sup&gt;a&lt;/sup&gt;±5.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1263.82&lt;sup&gt;c&lt;/sup&gt;±86.75&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flavonoid (mg RE/100g)</td>
<td>138.97&lt;sup&gt;b&lt;/sup&gt;±10.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.66&lt;sup&gt;a&lt;/sup&gt;±2.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>598.45&lt;sup&gt;c&lt;/sup&gt;±16.22&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flavonol (mg CE/100g)</td>
<td>39.48&lt;sup&gt;b&lt;/sup&gt;±4.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.31&lt;sup&gt;a&lt;/sup&gt;±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.42&lt;sup&gt;c&lt;/sup&gt;±5.03&lt;sup&gt;c&lt;/sup&gt;</td>
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Values are mean ± standard deviation of four observations. Within rows, values with the different following superscript letter differ significantly from each other (<i>P</i> ≤0.05).

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<th>Table 2—Antioxidant Potential of different banana extracts compared to existing values in literature</th>
<th>ME</th>
<th>PC</th>
<th>PE</th>
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<tr>
<td><strong>Parameter</strong></td>
<td>Different extract procedures (Mean ± SD)</td>
<td>Available value in literature</td>
<td></td>
</tr>
<tr>
<td>FRAP (mg TE/100g)</td>
<td>962.63±50.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>552.65±28.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2853.77±155.20&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>RPA (mg TE/100g)</td>
<td>931.04±20.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>465.23±12.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1575.90±34.60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ABTSRSA (mg TE/100g)</td>
<td>931.04±20.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>465.23±12.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1575.90±34.60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DPPHRSA (mg TE/100g)</td>
<td>184.53±114.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>955.21±20.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3061.77±128.63&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>TPC (mg GAE/100g)</td>
<td>404.68±23.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.76±5.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1263.82±86.75&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

Values are mean ± standard deviation of four observations. Within rows, values with the different following superscript letter differ significantly from each other (p≤0.05). [Extract procedure for literature reports: #, ME; ^, 50% ethanol; *, 60% acidic methanol; $, Ethyl acetate. Numbers in superscript refers to particular reference in literature]
have reported similar results after measurement of antioxidant potential of different food stuffs after gastrointestinal digestion\textsuperscript{6-9}. According to Ryan and Prescott\textsuperscript{25} when phenolic compounds are exposed to \textit{in vitro} digestion, they are transformed into different structural forms and possess different chemical properties and functions. Gawlik-Dziki \textit{et al.}\textsuperscript{8} evaluated the bioactivity of wheat and buckwheat after digestion with simulated intestinal fluid and reported an increase in the phenolic acids content as well as free radicals scavenging properties with the digestion time. Serrano \textit{et al.}\textsuperscript{6} have evaluated and shown a wide difference in the antioxidant capacity of plant foods in the Spanish diet measured by both chemical and physiological approach.

**Conclusion**

The present study has shown the wide differences in the chemical and physiological extracts of banana, thus proving that mere extraction by organic solvents may not be sufficient for the determination of antioxidant capacity. The reason behind the differences is that significant part of the antioxidants contained in plant foods are not analyzed in most antioxidant capacity assays, where the antioxidant extraction is incomplete. Also, the quantity and quality of antioxidant compounds extracted by organic solvents may not imply to their physiological bioavailability. Such conventional extraction procedures may prove misleading for assessment of the antioxidant potential of foods. A physiological perspective on antioxidant capacity bioavailability yields more useful information about possible health effects of antioxidants of foods.

**Acknowledgement**

The first author AB is thankful to University Grants Commission, New Delhi for award of “Research Fellowship in Sciences to Meritorious Student” under Basic Scientific Research (BSR) scheme.

**References**


