Total antioxidant potential of juices, beverages and hot drinks consumed in Egypt screened by DPPH in vitro assay

By Mohamed Fawzy Ramadan-Hassanien

Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt (e-mail: mframadan@zu.edu.eg)

1. INTRODUCTION

Fruit juice, beverages and hot drinks have received particular attention, because they contain high amounts of known antioxidants such as polyphenols, vitamin C, vitamin E, Maillard reaction products, β-carotene, and lycopene. The consumption of fruit juices, beverages and hot drinks has been inversely associated with morbidity and mortality from degenerative diseases (Gillman et al., 1995; Rimm et al., 1996; Cohen et al., 2000; La Vecchia et al., 2001; Terry et al., 2001; Rodríguez-Bernaldo de Quirós and Costa, 2006). It is not known which dietary constituents are responsible for this association, but antioxidants appear to play a major role in the protective effect of plant foods (Gey, 1990; Gey et al., 1991; Willett, 1991; Liyana-Pathirana et al., 2006; Rodríguez-Bernaldo de Quirós and Costa, 2006). Epidemiologic studies that analyze the health implications of dietary components rely on the intake estimates in sample populations found in databases that list the component’s content in commonly consumed foods. Therefore, the availability of appropriate and complete food composition data is crucial. Due to the chemical diversity of antioxidant compounds present in foods, complete databases on food antioxidant contents are not yet available. In addition, levels of single antioxidants in food do not necessarily reflect their total antioxidant potential (TAP); this also depends on the synergic and redox interaction among the different molecules present in the food (Ramadan et al., 2003; Ramadan and Moersel, 2007). Finally, geographical differences in food composition data should be considered when applying compositional databases to regional surveys.

The relevance of TAP as a new tool for investigating the relationship between dietary antioxidants and oxidative stress-induced pathologies seems confirmed by the data from a recent population-based case control study, which showed an inverse correlation between the diet TAP and the risk of both cardiac and distal gastric cancer (Serafini et al., 2002). These relationships emerged in spite of the use of a very incomplete database of total antioxidant potential, highlighting the potentiality of the TAP as a descriptor of the diet. Several methods were developed recently for measuring the total antioxidant capacity of food and beverages (Wang et al., 1997; Benzie and Strain, 1999; Brenzie and Szeto, 1999; Pellegrini et al., 2000; Ramadan et al., 2003; Ramadan and
According to the Industrial Modernization center (IMC) 2007 report (www.aegypten.ahk.de), Egypt can become the regional leader in the export of fruit juices. The report estimates Egypt's potential for market expansion of fruit juice to be as much as 730 million US$ by 2020. Overall exports of fruit juice have grown from 713,000 US$ in 1998, to 15 million US$ in 2006, with the US market now absorbing more than 65% of Egyptian fruit exports. On the other hand, Egypt is the largest market for tea and coffee in the Middle East, not only because of its 70 million citizens, but also due to the penetration of black tea and coffee as the main drink both indoors and outdoors (www.euromonitor.com/Hot_Drinks_in_Egypt). The objective of this work was to examine if the TAP assay in its recently published improved version (Ramadan and Moersel, 2006, 2007) is suitable for characterizing the antioxidant properties of commonly marketed Egyptian fruit juices, hot drinks and beverages to obtain robust data useful for determining the potential intake of antioxidants in Egyptian population studies as well as contributing to a better understanding of the role of different antioxidant juice ingredients.

2. EXPERIMENTAL

2.1. Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH, approximately 90%) was from Sigma (St. Louis, Mo, USA). Methanol of HPLC grade was used throughout the experiments.

2.2. Fruit juices and beverages

Three samples for each item were purchased from different markets in Zagazig (Egypt). Samples were then prepared and analyzed for TAP, as reported below. Like other nutrients, the estimation of the overall dietary intake of TAP does not require an estimate of the variance for any single food item if the value of the given food as consumed by the responder is sufficiently close to the average value. The same approach was used previously to generate food TAP data (Proteggente et al., 2002). The following fruit juices were purchased in local supermarkets: orange, cherry, apple, pineapple, guava, mango, and fruit cocktail (mixed fruits). Beverages (Anise, Hibiscus, Tilia, Cinnamon, Chamomile, Caraway and Peppermint) and teas (black, green and with lemon) were prepared by infusion (2 g) in 200 mL boiling water for 5 min. Soluble coffee, Turkish coffee and Cappuccino were prepared by solubilizing the samples (2 g) in 200 mL of boiling water for 5 min. To avoid the presence of insoluble components, soluble coffee, Turkish coffee and Cappuccino (cold to room temperature) were adequately and equally diluted in high purity water (1:10 v/v). Diluted samples were centrifuged for 5 min at 1000 x g, and the supernatant was collected and analyzed without further preparation.

2.3. Radical scavenging activity toward DPPH radical

Radical scavenging activity and the presence of hydrogen donors in juice and beverage samples were examined by reduction of DPPH in methanol according to Ramadan et al. (2003). A methanolic solution of DPPH radicals was freshly prepared at a concentration of 10-4 M. The radical, in the absence of antioxidant compounds, was stable for more than 3 h of normal kinetic assay. For evaluation, 100 µL of each sample was mixed with 500 µL Methanolic solution of DPPH radicals (10-4 M) and the mixture was vortexed for 10 s at ambient temperature. Against a blank of methanol without DPPH, the decrease in absorption at 515 nm was measured in 1-cm quartz cells after 30, 60 and 120 min of mixing using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). Antiradical action toward DPPH radical was estimated from the difference in absorbance with or without the sample (control) and the percent of inhibition was calculated from the following equation.

\[
\text{% Inhibition} = \frac{[\text{absorbance of control} - \text{absorbance of test sample}]}{\text{absorbance of control}} \times 100
\]

The variation in the DPPH values for replicates was always between 3 to 10% relative standard deviation (RSD). When the RSD was higher than 10%, the analyses were repeated to confirm the value. Three samples of each item were analyzed and the main values as well as the SD were given.
Brenes et al., 2005, Davalos et al., 2005), which flavonoids and other phenolics in the human diet compounds. Thus, grape juice is a rich source of high concentration and a great variety of phenolic able to quench 63.7%. Vitis vinifera fruits show a incubation, 82.6 percent of DPPH radicals were highest TAP followed by mango juice. After 120 min were obtained (data not shown). Table 1 shows that spectrometry and similar rankings in the activities evaluated using galvinoxyl radicals also dissolved in potential of the selected fruit juices, was further experiments were carried out using different doses as well as different DPPH reagent volumes. In general, a linear relationship was noted. When higher doses of juices and beverages were used stronger antiradical action was obtained, while when larger volumes of DPPH reagent were used lower antiradical performance was observed.

3.1 Total antioxidant potential of fruit juices

Using the same per volume basis the antiradical performance of fruit juices toward DPPH radicals was measured and compared. The order of effectiveness of fruit juices in inhibiting free radicals was as follow: red grape juice > mango juice > guava juice > cocktail juice > pineapple juice > orange juice > cherry juice. The antioxidative potential of the selected fruit juices, was further evaluated using galvinoxyl radicals also dissolved in methanol by means of electron spin resonance spectrometry and similar rankings in the activities were obtained (data not shown). Table 1 shows that during the test period, red grape juice had the highest TAP followed by mango juice. After 120 min incubation, 82.6 percent of DPPH radicals were quenched by red grape juice while mango juice was able to quench 63.7%. Vitis vinifera fruits show a high concentration and a great variety of phenolic compounds. Thus, grape juice is a rich source of flavonoids and other phenolics in the human diet (Rice-Evans et al., 1996). It is also well known that red grapes and berries have high antioxidant capacity and this capacity is likely due to their high contents of phenolics and flavonoids such as anthocyanins (Macheix et al., 1990; Kahkonen et al., 1999; Kalt et al., 1999; Kakkinen et al., 1999; Halvorson et al., 2002; Pellegrini et al., 2000, Brines et al., 2005, Davalos et al., 2005), which have been demonstrated to produce strong antioxidant activities in different model systems (Wang et al., 1997; Satue-Gracia et al., 1997). Positive health benefits of the consumption of grape juice, such as improvement in the endothelial function, increase in the serum antioxidant capacity, protection of LDL against oxidation, decrease in native plasma protein oxidation, and reduction in platelet aggregation, have also been reported (Davalos et al., 2005). On the other hand, mango, guava and pineapple fruit juices exhibited intermediate antioxidant capacity. This result is in agreement with the higher concentration of phenolic compounds, carotenoids and vitamin C present in these juices (Yen and Lin, 1999; Shivashankara et al. 2004; Rodríguez-Bernaldo de Quiros and Costa, 2006). Among the fruit juices, cherry, followed by apple, had the lowest antioxidant capacity. In Egypt, the increase in consumption of ready-to-drink juices has been associated with a reduction in the consumption of fresh fruits. Mango, guava and orange juices, both fresh and packed, are the most consumed fruit juices by the Egyptian population. Differences in the antioxidant activities among fruit juices could be attributed to their differences in phenolic contents and compositions and to other non-phenolic antioxidants present in the samples.

3.2. Total antioxidant potential of beverages, teas and coffees

The tap of hot drinks and caffeine-rich beverages and the ranking order are presented in Table 2. Among the beverages analyzed, tea drinks were the most effective, with lemon-flavored tea followed by green tea then black tea having the greatest antioxidant capacity. After 2 h incubation, 93.5 percent of DPPH radicals were quenched by lemon-flavored tea while green tea was able to quench only 89.9%. In teas, the antioxidant potential of green tea is considerably higher than that of black tea, according to the literature (Daglia et al., 2000; Richelle et al., 2001; Wei et al., 2006). This different behavior of teas is due to the changes occurring during the process of fermentation; the flavanols in green tea leaves (mainly catechins and their gallic esters) undergo an oxidative polymerization by polyphenol oxidase, which turns the leaves black. During oxidation, much of the catechin content in green tea is converted to oxyproducts, such as thearubingens and theaflavins, with a loss of antioxidant capacity (Richelle et al., 2001). In Egypt, per capita consumption of black tea in 2005 was around 1.26 kg (www.euromonitor.com/Hot_Drinks_in_Egypt).

Soluble coffee followed by Turkish coffee was also found to have high TAP. Turkish coffee, preferably flavored with cardamom, accounts for around 95% of fresh coffee value sales in Egypt (www.euromonitor.com/Hot_Drinks_in_Egypt). During coffee making, the roasting process leads to profound changes in the chemical composition and
pyrolysis of organic compounds (Daglia et al., 2000; López-Galilea et al., 2006). In roasted coffee most polyphenolic compounds are destroyed, but biological activities of the coffee bean, resulting in the generation of compounds derived from Maillard reaction, carbohydrates caramelization and

<table>
<thead>
<tr>
<th>Fruit Juice</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red grape juice</td>
<td>30.5 ± 0.05</td>
<td>21.7 ± 0.03</td>
<td>17.4 ± 0.03</td>
</tr>
<tr>
<td>Mango juice</td>
<td>41.3 ± 0.06</td>
<td>38.3 ± 0.02</td>
<td>36.3 ± 0.04</td>
</tr>
<tr>
<td>Guava juice</td>
<td>53.3 ± 0.03</td>
<td>47.1 ± 0.02</td>
<td>43 ± 0.02</td>
</tr>
<tr>
<td>Cocktail juice</td>
<td>67.5 ± 0.05</td>
<td>66.3 ± 0.05</td>
<td>66.1 ± 0.03</td>
</tr>
<tr>
<td>Pineapple juice</td>
<td>78.1 ± 0.06</td>
<td>74.3 ± 0.05</td>
<td>71.9 ± 0.04</td>
</tr>
<tr>
<td>Orange juice</td>
<td>81.5 ± 0.06</td>
<td>80.6 ± 0.04</td>
<td>79.4 ± 0.04</td>
</tr>
<tr>
<td>Moncherry juice</td>
<td>88.3 ± 0.03</td>
<td>85.9 ± 0.04</td>
<td>84.7 ± 0.03</td>
</tr>
<tr>
<td>Apple juice</td>
<td>91.6 ± 0.07</td>
<td>89.7 ± 0.06</td>
<td>89.5 ± 0.04</td>
</tr>
</tbody>
</table>

Each value is the average of three determinations ± SD

<table>
<thead>
<tr>
<th>Beverage/Hot Drink</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tea with lemon</td>
<td>10.4 ± 0.01</td>
<td>9.4 ± 0.01</td>
<td>6.5 ± 0.01</td>
</tr>
<tr>
<td>Green tea</td>
<td>10.3 ± 0.01</td>
<td>9.6 ± 0.01</td>
<td>9.5 ± 0.01</td>
</tr>
<tr>
<td>Black tea</td>
<td>11.7 ± 0.02</td>
<td>10.6 ± 0.01</td>
<td>10.5 ± 0.02</td>
</tr>
<tr>
<td>Soluble coffee</td>
<td>14.0 ± 0.03</td>
<td>13.8 ± 0.02</td>
<td>13.6 ± 0.02</td>
</tr>
<tr>
<td>Titia</td>
<td>24.6 ± 0.03</td>
<td>23.0 ± 0.03</td>
<td>18.7 ± 0.02</td>
</tr>
<tr>
<td>Turkish coffee</td>
<td>33.2 ± 0.02</td>
<td>29.6 ± 0.02</td>
<td>23.5 ± 0.03</td>
</tr>
<tr>
<td>Peppermint</td>
<td>42.6 ± 0.03</td>
<td>35.7 ± 0.03</td>
<td>29.4 ± 0.02</td>
</tr>
<tr>
<td>Hibiscus</td>
<td>63.9 ± 0.04</td>
<td>60.4 ± 0.03</td>
<td>57.1 ± 0.03</td>
</tr>
<tr>
<td>Cappuccino</td>
<td>66.1 ± 0.05</td>
<td>62.1 ± 0.04</td>
<td>61.5 ± 0.03</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>69.3 ± 0.04</td>
<td>69.1 ± 0.03</td>
<td>65.3 ± 0.03</td>
</tr>
<tr>
<td>Anise</td>
<td>85.5 ± 0.06</td>
<td>83.4 ± 0.04</td>
<td>81.9 ± 0.05</td>
</tr>
<tr>
<td>Caraway</td>
<td>87.3 ± 0.05</td>
<td>86.4 ± 0.03</td>
<td>84.7 ± 0.04</td>
</tr>
<tr>
<td>Chamomile</td>
<td>94.1 ± 0.06</td>
<td>92.1 ± 0.05</td>
<td>88.5 ± 0.05</td>
</tr>
</tbody>
</table>

Each value is the average of three determinations ± SD
Maillard reaction compounds with antioxidant properties are generated, resulting in an increased antioxidant activity in the \( \beta \)-carotene-linoleic acid model system (Daglia et al., 2000; Sánchez-González et al., 2005).

It is well known that the antioxidative properties are correlated not only with the total amount of antioxidants, but also with the presence of selected compounds. It could be said that the TAP of fruit juice and beverages can be interpreted as the combined action of different endogenous antioxidants. The significantly stronger antiradical action of some fruit juices or beverages may be due to (i) the differences in content and composition of bioactive (ii) the diversity in structural characteristics of potential phenolic antioxidants present, (iii) a synergism of bioactives with other components present in each juice or beverages and (iv) the differences in kinetic behaviors of potential antioxidants. All these factors may contribute to the radical quenching efficiency of juices and beverages.

4. CONCLUSION

The validity of the TAP approach for investigating the role of antioxidants in the protective effect of food and drinks is growing. The data presented here confirm that the DPPH assay is a well-founded method and appropriate to survey the antioxidant capacities of juice and beverage samples. On the whole, the results are partially in good accordance with the literature data, partially not. Obviously, this is because of basically different survey parameters. Coupled with an appropriate questionnaire, this will allow the evaluation of the overall intake of antioxidant-equivalents in selected groups of the Egyptian population in relation to the incidence of oxidative-stress-induced diseases.

REFERENCES


Ramadan MF, Kroh LW, Moersel JT. 2003. Radical scavenging activity of black cumin (Nigella sativa L.),
coriander (Coriandrum sativum L.) and niger (Guizotia abyssinica Cass.) crude seed oils and oil fractions. *Journal of Agricultural and Food Chemistry* 51, 6961-6969.

Ramadan MF, Moersel JT. 2006 Screening of the antiradical action of vegetable oils. *Journal of Food Composition and Analysis* 19, 838-842.


Rodríguez-Bernaldo de Quirós A, Costa HS. 2006. Analysis of carotenoids in vegetable and plasma samples: A review. *Journal of Food Composition and Analysis* 19, 97-111.


