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

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RESUMEN

Potencial antioxidante total de zumos, bebidas y bebidas calientes consumidas en Egipto determinadas mediante ensayo *in vitro* con DPPH.

Las plantas comestibles contienen diferentes clases y tipos de antioxidantes y el conocimiento de su potencial antioxidante total (TAP), que es la capacidad acumulativa de los componentes de los alimentos para captar radicales libres, debería ser útil en estudios epidemiológicos. De acuerdo a esto, una variedad de zumos de fruta, bebidas calientes y bebidas consumidas habitualmente en Egipto fueron analizadas usando un ensayo *in vitro* con DPPH. El orden de efectividad de los zumos de frutas en inhibir los radicales libres fue el siguiente: zumo de uva tinta > zumo de mango > zumo de guayaba > zumo de macedonia de frutas > zumo de piña >zumo de naranja > zumo de cereza > zumo de manzana. Entre las bebidas y bebidas calientes, el té seguido por el café son los que tuvieron mayores TAPs. Estos datos confirman que el zumo de uva, el té y el café son buenas fuentes de antioxidantes.

PALABRAS CLAVE: Bebidas – Bebidas calientes – Café – Ensayos con DPPH – Potencial antioxidante – Té – Zumos.

SUMMARY

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

Plant foods contain different classes and types of antioxidants and knowledge of their total antioxidant potential (TAP), which is the cumulative capacity of food components to scavenge free radicals, would be useful for epidemiological purposes. To accomplish this, a variety of fruit juices, hot drinks and beverages commonly consumed in Egypt were analyzed using *in vitro* DPPH assay. The order of effectiveness of fruit juices in inhibiting free radicals was as follows: red grapes juice > mango juice > guava juice > cocktail juice > pineapple juice > orange juice > cherry juice > apple juice. Among beverages and hot drinks, teas followed by coffees had the greatest TAP. These data confirm grape juice, teas and coffees as good dietary sources of antioxidants.

KEY-WORDS: Antioxidant potential – Juices – Beverages – Hot drinks – Tea – Coffee – DPPH assay.

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

Moersel, 2006, 2007); these assays differ in their chemistry (generation of different radical and/or target molecules) and in the way end points are measured.

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, Titia, 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.

% Inhibition = [(absorbance of control – absorbance of test sample)/ absorbance of control] x 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.

3. Results and discussion

The consumption of foodstuffs rich in antioxidants provides protection against cancer and cardio- and cerebro-vascular diseases. This protection can be explained by the capacity of these active compounds to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins and nucleic acids (Aruoma, 1998, Ramadan *et al.*, 2003). The juices, hot drinks and beverages included in this study were chosen in such a manner that samples with high concentrations of different antioxidant groups are included; for example, berry juice known to be rich in anthocyanins, citrus juices containing flavanones and teas containing flavanols. Thus, the influences of these particular antioxidant groups on the overall antioxidant capacity can be considered. In this survey, the total antioxidant capacity of a variety of fruit juices, hot drinks and beverages found in the Egyptian market were evaluated using DPPH assay. At present, it is hard to find data in literature concerning the TAP of foods and drinks consumed in Egypt. Moreover, a comparison with other data in the literature will be problematic due to the large variability within the food item and the lack of standardization of the assay. For this discussion, the ranking order of the TAP values will be used. The described arrangement for the simple experiment uses the addition of stable radicals 1,1-diphenyl-2-picrylhydrazyl (DPPH) to fruit juices and beverages which will be decomposed by components having antioxidant properties. By means of a spectrophotometric process, the disappearance of the DPPH radicals was recorded. Certain compounds react very rapidly with DPPH radicals, reducing the number of radical molecules corresponding to the number of available hydroxyl group. However, for the majority of the compounds tested, the mechanism is more complex. Free radical scavengers donate an electron to the free radical and this electron donation is associated with the disappearance of the DPPH radical absorbance. The lower the absorbance, the stronger the free radical scavenging activity is. Concerning the dose effect of the different juices and beverages, 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, Brenes *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, caroteinoids and vitamin C present in these juices (Yen and Lin, 1999; Shivashankara *et al*. 2004; Rodríguez-Bernaldo de Quirós 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

Table 1
Scavenging effect (percentage of remaining DPPH radical) of fruit juices during DPPH test as measured by changes in absorbance at 515 nm

	Incubation time		
	30 min	**60 min**	**120 min**
Red grape juice	30.5 ± 0.05	21.7 ± 0.03	17.4 ± 0.03
Mango juice	41.3 ± 0.06	38.3 ± 0.02	36.3 ± 0.04
Guava juice	53.3 ± 0.03	47.1 ± 0.02	43 ± 0.02
Cocktail juice	67.5 ± 0.05	66.3 ± 0.05	66.1 ± 0.03
Pineapple juice	78.1 ± 0.06	74.3 ± 0.05	71.9 ± 0.04
Orange juice	81.5 ± 0.06	80.6 ± 0.04	79.4 ± 0.04
Moncherry juice	88.3 ± 0.03	85.9 ± 0.04	84.7 ± 0.03
Apple juice	91.6 ± 0.07	89.7 ± 0.06	89.5 ± 0.04

Each value is the average of three determinations ± SD

Table 2
Scavenging effect (percentage of remaining DPPH radical) of beverages and hot drinks during DPPH test as measured by changes in absorbance at 515 nm

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

Each value is the average of three determinations ± SD

biological activities of the coffee bean, resulting in the generation of compounds derived from Maillard reaction, carbohydrates caramelization and pyrolysis of organic compounds (Daglia *et al.*, 2000; López-Galilea *et al.*, 2006). In roasted coffee most polyphenolic compounds are destroyed, but

Maillard reaction compounds with antioxidant properties are generated, resulting in an increased antioxidant activity in the ,-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 bioactives (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.

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