Comparison of total antioxidant activity between fresh and commercial mango juices available in Bangladesh

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Abstract
Fresh mango and commercial mango juices contain various natural antioxidants which have beneficial health effects. In this study, we compared total antioxidant activity of fresh mango (fazlee, amrapali, mohanvog, langra, and gopalvog) juices with that of commercial mango juices (pran frooto, sezan, frutika, and sundrop mango fruit drink), which are available in Bangladesh. Total antioxidant activity was measured by DPPH method. Enzymatic browning in fresh and commercial mango juices was determined by tyrosinase inhibitory assay. The IC50 values of fazlee, amrapali, mohanvog, langra, gopalvog, pran frooto, sezan, frutika, sundrop mango fruit drink juices, vitamin C, and vitamin E were 1.159, 1.367, 0.575, 1.675, 0.725, 0.435, 1.025, 0.925, 0.678, 0.057, and 0.039 mg/ml, respectively. No sample shows tyrosinase inhibitory activity. In conclusion, commercial mango juices can be considered as a good source of antioxidant.

Keywords: Fresh mango; commercially available mango juices; DPPH; Tyrosinase inhibition activity

1. Introduction
Antioxidants are group of molecules that inhibit the oxidation (a chemical reaction in which free radical is produced) of other molecules. Natural antioxidants present in the diet increase the resistance to oxidative damage and they may have a substantial impact on human health [1]. Fresh fruits, vegetables and juices are rich in antioxidant compounds such as polyphenols, ascorbic acid and tocopherols which may protect us against chronic disease [2-3]. Nowadays plant foods especially vegetable and fruits have gained great attention due to their health benefits. In the past decade, a number of studies have found that they are the great source of natural antioxidant [4]. Fruits are normally consumed as fresh, also as salad and desserts. They are sometime blended to consume as fruit juice. Antioxidant from fruits and vegetables protect our biological immunity via inhibition or prevention of oxidative stress induced by reactive oxygen substances generated from normally metabolic activity [5]. Thus antioxidant’s deficiency in human may lead to damage of DNA, lipids and proteins which may induce various diseases such as cancer and increase cell death especially epithelial cell of skin [6]. A difference across chronologic season in the quantity of fruits and vegetables consumed has been documented in several previous epidemiologic investigations [7-11]. We usually consume vegetables whole year but we consume fruits in a single season [7]. In vegetable and fruit consumption, significant seasonal variation has been observed [8-11]. Bangladesh is a well-known for fruits and vegetables. The subtropical climate, abundant sunshine, sufficient humidity and fertile plain land help to grow different kinds of crop throughout the year in this country [12].
Mangoes grow widely throughout Bangladesh and are raised mostly as homestead plantations. The climatic conditions and soil quality of northern regions of Bangladesh are suitable for mango cultivation. There are many mango varieties in Bangladesh including Amrapali, Chausa, Fazlee, Gopalbhog, Himsagar, Khirsapat, Kohitoor, Laksmanbhog, Langra, Mallika, and Mohanbhog [13]. Mango contains various nutrients such as, prebiotic dietary fiber, vitamins, minerals, flavonoid compounds, sugar, protein, fats. Generally, mango is eaten fresh as a dessert. It is used to produce juices, jams, jellies etc. [14]. Presently consumers are aware of quality of their food and drink. To fulfill consumer demand, food industries modify their product to provide better quality and more convenient food and drink [15]. However the level of antioxidant preserved in the commercial mango juices when compared to fresh mango juice is still unclear. Commercial mango juice manufacturer Companies normally advertise and teach their customer that their mango juices are rich in multivitamin (Such as vitamin A, B, C and E). All of those vitamins are normally synthetic and added during the process of the mango juices. Additionally some synthetic antioxidant such as butylatedhydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertbutylhydroxyquinone (TBHQ) are used as preservative those of beverages. These synthetic preservatives as antioxidant may cause genotoxicity and carcinogenicity at high concentration [16]. Most of antioxidants are heat sensitive and deactivated by the heat during evaporation [17]. The aim of this study was to analyze and compare the antioxidant activities of three commercial mango juices with five fresh mango juices which are available in Bangladesh. By studying the total antioxidant activities, concentration and the effect of this health promoting content can be identified and used by the consumer as a factor to consider before they make up their mind to go for fresh mango juices or ready to drink commercial mango juice.

2. Material and methods

2.1. Preparation of fresh and commercial mango juices

Fresh mangoes (Fazlee, Amrapali, Mohanvog, Langra, and Gopalvog) and commercial mango juices were purchased from supermarket in the city of Dhaka. Mango juice samples were collected from four different juice company (pran frooto mango juice, sezan, frutika, sundrop mango fruit drink). All of fresh mangoes were ripped. Before experiment, mangoes were washed and then skins were removed. 5 g of each sample was blended in 100 ml of deionized water. Next, the supernatant was filtered with Whatman filter paper after centrifugation the extracts at 10,000 rpm for 30 min. Based on the level of nutritional fact and on the availability of fruit, the commercial fruit juices had been chosen. Concentration in the juices was used for this study. Three commercial mango juices were selected and pelleted at 10,000 rpm for 30 min and filtered through Whatman filter paper. All of the filtered samples were stored overnight in -80 °C freezer. Then samples were placed in a single layer a solid anodized aluminum trays and freeze and dried for 22 h. The freeze dried extracts were stored in -60 °C freezer prior to further use in the antioxidant scavenging assay.

2.2. In vitro DPPH free radical scavenging assay

The total antioxidant activity was determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method according to the procedure reported elsewhere [18-24, 42]. Fresh and commercial mango juices were dissolved in methanol at different concentrations (5, 3, 1.5, 0.575, 0.285, 0.085 mg/ml). The solutions were done vortex and allowed to stand at room temperature in dark. The examinations were tested in triplicate and vitamin C (sigma, USA) and vitamin E (sigma, steinheim Germany) were used as standard controls. IC50 of DPPH free radicals was compared between vitamin C, vitamin E, fresh mango juices and commercial mango juices.

All reactions were subjected to thermal auto oxidation at 37 °C in incubator. The absorbance of the sample was measured at 15 min time interval until the color of vitamin C & E and others sample had disappeared. The time of inhibition of the absorbance is related to the concentration of antioxidant compound. All samples were assayed in triplicate. The antioxidant activity was expressed as percentage of absorbance decrease, corresponding to the percentage of DPPH that was scavenged. The percentage of DPPH was calculated.

\[
\text{Percentage of DPPH radical scavenge} = \frac{[1-\{(A_t-A_i)/(A_{t0}-A_i)\}]}{x100}
\]

\( A_i = \) Measured absorbance value of sample at zero time

\( A_{t0} = \) Measured absorbance value of sample after incubation such as 120 min at 37 °C

\( A_i = \) Measured absorbance value of control at zero time

\( A_{t} = \) Measured absorbance value of sample after incubation such as 120 min at 37 °C.
2.3. Tyrosinase inhibitory assay

The tyrosinase inhibitory activity assay was performed as previously described by Lee et al. [25]. In brief, 100 µl of L-tyrosine was mixed with 600 µl of 0.1 M phosphate buffer (pH 6.8) and incubated at room temperature for 30 min. Next, 50 µl of test samples and 33 µl of the aqueous solution of tyrosinase (1380 U/ml) were added to the mixture. The assay mixture was incubated at room temperature for 7 min, and the absorbance at 475 nm was measured using a spectrophotometer. Kojic acid and L-cysteine were used as positive control in this assay. Tyrosinase inhibitory activity was expressed as the percentage inhibitory of tyrosinase.

2.4. Statistical analysis

The experiment was calculated using a completely randomized designed with three replications. A preliminary test was run prior to the main experiment reported here. Data were analyzed as a two factor linear model via IBM SPSS software (version 20.0), where treatment and storage were the factors.

3. Results

3.1. Total antioxidant activity of fresh and commercial mango juices

The DPPH scavenging activity of fresh and commercial mango juice has been shown to be strongly correlated with their phenolic and antioxidant content (Figure 1). All fresh mangos give IC\textsubscript{50} in this assay. In this process commercial mango juices gave just below antioxidant activity with the highest IC\textsubscript{50} value (Table 1). However, all of the fresh and commercial mango juices gave much higher IC\textsubscript{50} value when compared to vitamin C (0.057 mg/ml) and vitamin E (0.039 mg/ml).

<table>
<thead>
<tr>
<th>Type of item</th>
<th>IC\textsubscript{50} (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fazlee</td>
<td>1.159±0.004</td>
</tr>
<tr>
<td>Amrapali</td>
<td>1.367±0.006</td>
</tr>
<tr>
<td>Mohanvog</td>
<td>0.575±0.006</td>
</tr>
<tr>
<td>Langra</td>
<td>1.675±0.002</td>
</tr>
<tr>
<td>Gopalvog</td>
<td>0.725±0.005</td>
</tr>
<tr>
<td>Pranfruto</td>
<td>0.435±0.006</td>
</tr>
<tr>
<td>Sezan</td>
<td>1.025±0.006</td>
</tr>
<tr>
<td>Frutika</td>
<td>0.925±0.002</td>
</tr>
<tr>
<td>Sundrop</td>
<td>0.678±0.004</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.057±0.001</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.039±0.001</td>
</tr>
</tbody>
</table>
Figure 1 DPPH scavenging activity of fresh and commercial mango juices.

The total IC\textsubscript{50} values were presented in the bar chart (Figure 2).

Figure 2 Comparison of antioxidant activity of fresh and commercial mango juices at IC\textsubscript{50} level.

Total antioxidant activity of fresh and commercial mango juices were calculated at 15 minutes time interval within 120 minutes by taking absorbance at 470 nm (Figure 3). In this process total antioxidant activity was determined during time dependant manner.
3.2. Tyrosinase inhibitory activity of fresh and commercial mango juices

All fresh and commercial mango juices were unable to inhibit auto oxidation of tyrosinase activity, where positive control L-cystein was able to inhibit 50% of tyrosinase activity at 0.597 mg/ml.

4. Discussion

Due to developing economy in a country, consumer has shown increasing interest in food materials that are rich in natural ingredients which are good for human health [26-27]. Antioxidants are able to inhibit the oxidation of easily oxidisable biomolecules such as lipids, proteins and DNA. Total antioxidants are divided into two groups as enzymatic and non-enzymatic antioxidants. Superoxide dismutase and glutathione reductase are example of enzymatic antioxidant. On the other hand, non-enzymatic antioxidants are water soluble (Vitamin C and polyphenolic compounds) and lipid soluble (vitamin E and pre vitamin A) biomolecules. Fruit, vegetable and plant materials contain high level of different antioxidants such as polyphenols, tocopherols, carotinoids and ascorbic acid [28-30]. These antioxidants contain health promoting properties including inhibition on the neoplasms, coronary disease and other degenerative disease [15]. Fruit is the most widely consumed plant food due to their taste and the presence of antioxidant ingredients [31]. When we talk about mango, this is not only delicious but also rich in prebiotic dietary fiber, vitamins, minerals and polyphenolic flavonoid antioxidant compounds. There are some reports on the physicochemical characteristics of different mango varieties [32-38]. But the physicochemical and nutritional characteristics of most of the varieties of mango grown in Bangladesh are not addressed based on variety. Considering these, we have designed the present study by evaluating the nutritional status of five different mango varieties such as Amrapali, Fazlee, Gopalbhog, Langra, and Mohanvog grown in Bangladesh which is recommended commercial juice instead of fresh mango. Otherwise, mango is an important commercial fruit worldwide that contains dietary antioxidants such as ascorbic acid, carotinoids and phenolic compounds [31]. On the other side, fruit juice concentrate is the result of an industrial process where mango juice is subjected to evaporation to remove majority of the water to provide better condition for storage, transport and preservation of liquid food materials with lowest handling cost. Besides, this process which normally involves heating was believed to be able to increase the phenolic content of mango through the extraction step. Therefore mango juice concentrate or commercial mango juice that reconstitute from the concentrate was believed as they can be served as good source of functional food to replace carbonated soft-drink [17,39]. Decrease of antioxidant activity in commercial mango juice may be due to storage of the raw fruit or the fruit concentrate before the production and packaging of the ready to drink mango juice [40]. It has found that increase of oxygen, PH, and temperature during storage reduces the antioxidant activity of the fruit concentration.

Previously researcher has found that short term in vivo consumption of orange juice was not able to affect the oxidation stress that related to cardiovascular risk [41-42]. This may be due to the insufficient daily phenolic compounds was too dilute as reported in this study.
However all samples were unable to inhibit auto-oxidation of tyrosinase. Where control L-cystein gives positive result. This means that taking either fresh or commercial mango juice did not help in protection against skin darkening.

This research clearly expresses the potential value of commercial mango juice as the substitute for fresh mango. Various mangoes contain different level of antioxidant capacity as we observed in this study. Also sweetened and aroma of different mango are different. According to the result, commercial mango juices still contain high level of antioxidant capacity as compared to fresh mango. Therefore, commercial mango juice can be considered as a good source of natural antioxidants besides fresh mango. However, fresh mango still contains much higher antioxidant capacity. Thus, commercial mango juices are normally more expensive that those of fresh mangoes. Commercial mango juice should be considered as the secondary choice of dietary fruit, when seasonal mango is not available.

5. Conclusion

Langra and Amrapali mangoes showed the highest antioxidant activity. The level of antioxidant capacity was comparatively same in commercial mango juices. So, commercial mango juices can be considered as a good source of antioxidant and replaced as a dietary source during off season of fresh mangoes.

Compliance with Ethical Standards

Acknowledgments

Experiments were performed at the Department of Lab Science Diagnostics, Dhaka-1205, Bangladesh; Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh; Department of Clinical and Molecular Sciences, Polytechnic University of Marche, Ancona 60126, Italy.

Disclosure of conflict of interest

The authors have no conflict of interest to declare.

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