Antioxidant and Phenolic Content of Nuts, Oil Seeds, Milk and Milk Products Commonly Consumed in India

Dande Sreeramulu*, Manchala Raghunath

Endocrinology and Metabolism Division, National Institute of Nutrition, Jamai-Osmania, India.
Email: *dandesr@yahoo.com

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ABSTRACT

Food provides not only essential nutrients required for life, but also bioactive compounds useful to maintain good health and disease prevention. Abundant epidemiological evidences suggest that consumption of food rich in antioxidants (non-nutritional components) can prevent degenerative diseases. A total of 26 commonly consumed nuts, oil seeds, edible oils, milk and milk products were chosen for the study. Considering the fact that antioxidant content (AOC) and phenolic contents (PC) of these foods was not established systematically in Indian context. Therefore, we have assessed and correlated the AOC and PC, an important antioxidant constituents of plant foods. AOC was assessed by DPPH (2, 2'-Diphenyl-1-picryl hydrazyl) scavenging activity and FRAP (Ferric reducing antioxidant power) methods and phenolic content (PC), using Folin-Ciocalteu reagent. Among the nuts and oil seeds arecanut had the highest phenolic and antioxidant content 10841, 4220341 mg/100g respectively. In milk, edible oils and sugars the values ranged from 336 - 11674 mg/100g. Jaggery had the highest PC and AOC among the foods studied. Although AOC and PC showed wide variation among the foods, AOC was correlated significantly with PC. Indeed the “r” value between PC and AOC (DPPH and FRAP) was 0.99 (p < 0.01) among the nuts and oil seeds, while in milk, milk products and sugars, the “r” values ranged from 0.93 and 0.99 (p < 0.01) respectively. The overall results indicate that the phenolic compounds may be significant contributors to the AOC of the foods studied.

Keywords: Antioxidant Content (AOC), 2,2'-Diphenyl-1-picryl hydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), Polyphenols, Phenolic Content (PC), 2,4,6-Tripyridyl-s-triazine (TPTZ)

1. Introduction

Antioxidants are essential for human health. Dietary antioxidants play an important role in controlling oxidative stress [1]. During normal metabolism, the oxidants and antioxidants are maintained in equilibrium [2]. Excess free radical production underlies the pathogenesis of diseases like atherosclerosis, carcinogenesis, diabetes, cataract and accelerated ageing [3]. Robust epidemiological evidence suggests the crucial role of diets in prevention of chronic degenerative diseases [4]. Supplementation of natural antioxidants through a balanced diet containing enough antioxidants could be most effective in protecting against various oxidative stressors [5]. In recent times, natural antioxidants have raised considerable interest among nutritionists, food manufacturers and consumers because of their presumed safety and potential therapeutic value. Indeed, recent research trends indicate a shift towards identifying non-nutritional antioxidants in functional foods [6].

More than 5000 phytochemicals have been identified in plant foods and many more remain to be discovered [7]. Phenolic compounds have been proposed to be the potent and important contributors in reducing oxidative stress due to their antioxidant activity, which are of great importance predominantly in Indian diets [8]. Therefore, food industry is concentrating on foods containing various bioactive compounds for health promotion and disease prevention [9].

There are few studies from India (including our studies) on antioxidant activity and phenolic content of plant foods commonly consumed in India [10,11]. The foods chosen in this study were not attempted systematically for their AOC and their correlation with phenolic content. Therefore, to the best of our knowledge, we have
determined for the first time the AOC and its correlation
with PC in 26 commonly consumed foods in India.

2. Materials and Methods

Chemicals and reagents: 2,2’-Diphenyl-1-picryl hydrazyl (DPPH), Gallic acid, 2,4,6-tripyridyl-s-triazine (TPTZ)
and Ferric Chloride were obtained from Sigma Chemical
Inc, USA. All other reagents and chemicals used were of
analytical grade procured from local sources. Milli Q
water was used in the study.

2.1. Sample Collection and Extraction

Three samples of each food were purchased from each
of the three local markets of Hyderabad and Secunderabad
cities (India). Food samples were pooled from each area
and represented as a single sample from that particular
market. The samples were analyzed separately and data
presented as mean value. Each sample was extracted in
duplicate according to Sing et al. and Zielinski et al.,
[12,13] with slight modifications. Methanol extraction
was adopted as per the procedure described by Matthaus
[14].

Approximately 50 g of the edible portion of the food
was subjected to grinding in a domestic blender. In case
of nuts and oil seeds, 5 grams of the ground sample was
taken. Sample extracts were prepared in 20 ml of 70%
Methanol containing 0.1% HCl by shaking vigorously
for four hours at room temperature. In case of liquid
samples like milk and edible oils, 5 ml of the sample was
taken directly and extracted as above. The sample sus-
pension was centrifuged at 10,000 g for 15 min at 10°C,
the supernatant was collected and filtered through
Whatman #1 filter paper and the resultant filtrate was
stored at –20°C. Analysis was completed within a month
of extraction [15].

2.2. DPPH Radical Scavenging Activity

DPPH radical scavenging activity was determined ac-
cording to Yu et al. [16]. This method is based on the
ability of the antioxidant to scavenge the DPPH cation
radical. Briefly, 100 µl of sample extract or standard was
added to 2.9 ml of DPPH reagent (0.1 mM in methanol)
and vortexed vigorously. The reaction tubes were incu-
bated in dark for 30 min, at room temperature and the
discouloration of DPPH was measured against a reagent
blank at 517 nm. Percentage inhibition of the discoul-
oration of DPPH by the sample extract was expressed as
Trolox equivalents [17].

2.3. FRAP Assay

Ferric reducing antioxidant power (FRAP) was deter-
mined according to Benzie and Strain [18]. This method
is based on the ability of the sample to reduce Fe^{3+} to
Fe^{2+} ions. In the presence of TPTZ, the Fe^{2+}-TPTZ com-
plex exhibits blue colour which has absorption maxima
at 593 nm. Briefly, 3.0 ml of working FRAP reagent was
added to a suitable volume of the sample extract was
taken to suit into standard range. After incubation for 6
min at room temperature the absorbance was measured at
593 nm against FeSO4 as standard.

2.4. Folin-Ciocalteu Assay

Soluble phenolic compounds (PC) were determined in
sample extracts using the Folin-Ciocalteu reagent as per
the method described by Singleton and Rossi [19] with
slight modifications. Briefly, suitable volumes of sample
extract to fit into standard concentrations were taken,
1.0 ml of 10% Folin-Ciocalteu reagent and 0.8 ml of
7.5% Na2CO3 were added, vortexed thoroughly and in-
cubated at room temperature for 90 minutes and the ab-
sorbance was read at 725 nm. The values were expressed
as equivalents of Gallic acid, which is one of the most
commonly, used standards in phenolic estimations. Gallic
acid has indeed been shown to be more stable and a phar-
macologically active antioxidant, quantitatively equivalent
to many other phenolics and gives consistent and repro-
ducible results [20].

2.5. Statistical Analysis

Results are expressed on fresh weight basis and pre-
sented as mean ± SD. Statistical analysis was performed
to know the correlation between AOC and phenolic con-
tent using SPSS 14.0 statistical package.

3. Results and Discussion

Phenols and polyphenols are stronger antioxidants than
the vitamins [21]. Several epidemiological studies
showed a lower risk with increasing intakes of plant
foods [22] and protection against DNA damage [23]. Yet
in India, plant foods have received less attention in terms
of quantifying their AOC [10]. As such, little data exists
on the AOC of plant foods commonly consumed in India,
let alone their relationship with the phenolic content [24,
25]. On the other hand, no single method gives a com-
prehensive estimation of the antioxidant efficacy of the
food sample tested. About twenty different AOC indices
are currently in use, single index by itself is not consid-
ered sufficient to quantify the AOC of foods. Therefore,
use of more than one method is recommended for quan-
tifying the AOC of the foods [26]. FRAP and DPPH are
widely used and well accepted AOC indices [27,28].
Therefore, these two indices are chosen to determine the
AOC of nuts, oil seeds, milk, edible oils and sugars.
Considering that phenolic compounds are important an-
tioxidants of commonly consumed foods, soluble pheno-
lic content (PC) was determined by Folin/Ciocalteu assay.
The results among the 26 commonly consumed foods in India are summarized in Tables 1-4. In general, the coefficient of variation in the AOC and PC among the three samples collected from three different local markets for a given food sample was less than 10% indicating no significant differences among the market samples collected for the study. However, there was a wide variation in the AOC and PC content of the foods studied.

DPPH radical scavenging activity (trolox equivalents) and FRAP (ferrous sulphate equivalent), showed marked variation among the 12 commonly consumed nuts and oil seeds studied (Table 1). DPPH radical scavenging activity presented in Table 1, ranges from 20.0 - 28622.0 mg TE/100g with the highest activity being found in the arecanut followed by the mustard seeds (1155) and the least in the coconut water (20 mg/100g) (Table 1). Similarly, their FRAP activity ranged from 220.0 - 4220341.0 mg /100g. Inline with the DPPH results, FRAP content was highest in the arecanut, followed by the mustard seeds and the least in the coconut water. The PC of nuts and oil seeds ranged from 10.0 to 10841.0 mg/100g, and in line with their AOC (DPPH and FRAP), arecanut has the highest phenolic content (10841.4) and coconut water the least (10.0).

AOC and PC of milk, milk products, edible oils, sugar, and sugarcane juice findings have been highlighted in Table 3. The DPPH activity ranges from 3.4 - 208.0 mg /100g with Jaggery having the highest DPPH content (208) followed by ground nut oil (22.1) and lowest activity found in whole milk (3.4). On the other hand, the FRAP activity ranges from 36 - 11674 mg/100g, with the highest activity in jaggery (11674.0) followed by sugar-cane juice (872.0) and lowest was in sunflower oil (36.0 mg/100g). The PC content of milk, milk products, edible oils and sugars (Table 3) showed a wide range of values 0.72 - 336 mg/100g. Here again, jaggery had the highest PC (336.4) followed by honey (140.4) and the least phenolic content was observed in vanaspathi (0.70).

Indeed, the AOC values reported here for the foods studied (Tables 1 and 3) are the first of their kind from India. However very scanty data is available from India on the phenolic content of some of the foods studied but not AOC [29]. Available information from other parts of the world on the PC content of sugar (cane) reported ranged from 11 - 41 mg/100g [30] and our findings (12.28 mg/100g) are comparable for sugar. Where as sugarcane juice is reported to have a phenolic content of 16 mg/100g [31], we observed 27.1 ± 6.00 mg/100g. The reported data on the phenolic contents of different brands of honey ranges from 234 - 394 mg/100g [32], whereas our values (140.4 mg/100g) are found to be lower (Table 3). This discordance could be due to factors like agro-economic, genomic and post—harvesting conditions, which may affect the chemical composition of foods studied [33,34]. However, there is no published data on the AOC of the foods studied from India to compare our findings. Among the 26 foods studied, a few of them were not rich sources of AOC/PC, inspite of that they were included to have reference values from India.

Even though the foods studied belong to different food groups, significant correlation was observed between AOC (as estimated by both DPPH and FRAP methods) and PC (Tables 2 and 4). The correlation coefficient “r” values between PC and AOC was 0.99 in nuts and oil seeds (Table 2), whereas the “r” value was 0.93 in other foods given in Table 4. The positive correlation between PC and AOC (as assessed by DPPH and FRAP) among all the foods studied, indicate the importance of PC to antioxidant content.

4. Conclusions

The data reported here is the first of its kind from India

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Common name of the nuts &amp; oil seeds</th>
<th>Botanical name</th>
<th>Phenolic content (Gallic acid Eq mg/100g)</th>
<th>Antioxidant Content (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DPPH (Trolox Eq)</td>
<td>FRAP (FeSO4 Eq)</td>
</tr>
<tr>
<td>1</td>
<td>Areca nut</td>
<td>Areca catechu</td>
<td>10841 ± 2258</td>
<td>28622 ± 5251</td>
</tr>
<tr>
<td>2</td>
<td>Coconut (dry)</td>
<td>Cocos nucifera</td>
<td>40 ± 1.9</td>
<td>99 ± 0.0</td>
</tr>
<tr>
<td>3</td>
<td>Coconut tender</td>
<td>Cocos nucifera</td>
<td>39 ± 3.7</td>
<td>74 ± 5.0</td>
</tr>
<tr>
<td>4</td>
<td>Coconut milk</td>
<td>Cocos nucifera</td>
<td>31 ± 4.9</td>
<td>129 ± 13.0</td>
</tr>
<tr>
<td>5</td>
<td>Coconut water</td>
<td>Cocos nucifera</td>
<td>10 ± 1.3</td>
<td>20 ± 1.0</td>
</tr>
<tr>
<td>6</td>
<td>Gingelly seeds</td>
<td>Sesamum indicum</td>
<td>148 ± 35.3</td>
<td>154 ± 15.4</td>
</tr>
<tr>
<td>7</td>
<td>Linseed seeds</td>
<td>Linum usitatissimum</td>
<td>119 ± 11.0</td>
<td>135 ± 32.1</td>
</tr>
<tr>
<td>8</td>
<td>Mustard seeds</td>
<td>Brossicaniagra</td>
<td>725 ± 38.4</td>
<td>1155 ± 82.5</td>
</tr>
<tr>
<td>9</td>
<td>Niger seeds</td>
<td>Guizota abyssinica</td>
<td>143 ± 3.0</td>
<td>154 ± 3.0</td>
</tr>
<tr>
<td>10</td>
<td>Safflower seeds</td>
<td>Carthamus tinctorius</td>
<td>599 ± 51.5</td>
<td>228 ± 33.1</td>
</tr>
<tr>
<td>11</td>
<td>Sunflower seeds</td>
<td>Helianthus annus</td>
<td>207 ± 7.3</td>
<td>850 ± 126.9</td>
</tr>
<tr>
<td>12</td>
<td>Water melon seeds</td>
<td>Citrullus vulgaris</td>
<td>74 ± 11.0</td>
<td>54 ± 4.0</td>
</tr>
</tbody>
</table>

Range 10-10841 20-28622 220-4220341

Values are Mean ± SD, n = 3. Decimal points are not given due to higher values, to keep uniform units higher values are not condensed.
Table 2. AOC Vs TPC correlation of nuts and oil seeds.

<table>
<thead>
<tr>
<th>Correlation</th>
<th>r</th>
<th>r²%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC Vs DPPH</td>
<td>0.998</td>
<td>99.71</td>
</tr>
<tr>
<td>PC Vs FRAP</td>
<td>0.997</td>
<td>99.51</td>
</tr>
<tr>
<td>DPPH Vs FRAP</td>
<td>0.999</td>
<td>99.85</td>
</tr>
</tbody>
</table>

Table 3. Antioxidant and phenolic contents of milk, milk products, edible oils and sugars.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Common name</th>
<th>Phenolic content (Gallic acid Eq mg/100g)</th>
<th>Antioxidant Content (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DPPH (Trolox Eq)</td>
</tr>
<tr>
<td>1</td>
<td>Toned milk (Dairy milk)</td>
<td>3.4 ± 0.4</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>WHOLE MILK (BUFFALO)</td>
<td>3.0 ± 0.2</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>Card (Inoculated from dairy milk)</td>
<td>7.4 ± 0.6</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>Groundnut oil (unrefined)</td>
<td>3.2 ± 0.3</td>
<td>22.1 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>Groundnut oil (refined)</td>
<td>3.1 ± 0.1</td>
<td>13.2 ± 0.0</td>
</tr>
<tr>
<td>6</td>
<td>Sunflower oil</td>
<td>1.5 ± 0.2</td>
<td>12.4 ± 0.7</td>
</tr>
<tr>
<td>7</td>
<td>Vanaspathi (Dalda)</td>
<td>0.7 ± 0.0</td>
<td>13.1 ± 1.8</td>
</tr>
<tr>
<td>8</td>
<td>Palm oil</td>
<td>3.2 ± 0.2</td>
<td>12.1 ± 0.6</td>
</tr>
<tr>
<td>9</td>
<td>Til oil</td>
<td>5.1 ± 0.6</td>
<td>19.9 ± 1.7</td>
</tr>
<tr>
<td>10</td>
<td>Ghee (Vijaya)</td>
<td>10.2 ± 1.3</td>
<td>14.7 ± 0.8</td>
</tr>
<tr>
<td>11</td>
<td>Honey (Agmark Girijan)</td>
<td>114.0 ± 26.6</td>
<td>19.6 ± 0.3</td>
</tr>
<tr>
<td>12</td>
<td>Jaggery</td>
<td>336.4 ± 12.8</td>
<td>208.1 ± 27</td>
</tr>
<tr>
<td>13</td>
<td>Sugarcane juice</td>
<td>27.1 ± 6.0</td>
<td>22.0 ± 6.0</td>
</tr>
<tr>
<td>14</td>
<td>Sugar</td>
<td>12.2 ± 3.1</td>
<td>15.1 ± 1.4</td>
</tr>
</tbody>
</table>

Values are Mean ± SD, n = 3. Decimal points are not given due to higher values, to keep uniform units higher values are not condensed.

Table 4. AOC Vs TPC correlation of milk, milk products, edible oils and sugars.

<table>
<thead>
<tr>
<th>Correlation</th>
<th>r</th>
<th>r²%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC vs. DPPH</td>
<td>0.93</td>
<td>86.52</td>
</tr>
<tr>
<td>PC vs. FRAP</td>
<td>0.93</td>
<td>87.55</td>
</tr>
<tr>
<td>DPPH vs. FRAP</td>
<td>0.99</td>
<td>99.03</td>
</tr>
</tbody>
</table>

and these findings would be useful to nutritionists and consumers to know and formulate the antioxidant—rich therapeutic diets. In addition, the present study would be a valuable information to the existing knowledge on non—nutrient antioxidant contents of commonly—consumed Indian foods.

5. Acknowledgement

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