Available mineral contents and anti-oxidant contents of sag incorporating 20 percent fresh chickpea leaves at 45 days after sowing on dry matter basis

Seema and Neelam Khetarpaul

Abstract
In this experiment data related that available mineral in sag containing 20 percent fresh leaves at 45 days after sowing of desi and kabuli chickpea varieties. The highest available iron content was observed in sag containing the 20 percent fresh leaves of kabuli chickpea variety HK-2 (20.98%) and in desi variety lowest from C-235 (18.66%), calcium content (30.75%) was observed of HK-2 and lowest was observed in variety HC-1 was (30.14%). The highest zinc content in sag containing 20 percent fresh leaves of kabuli chickpea variety HK-2 (49.44%), while lowest from variety HK-1 (48.11%). The phenolic compounds ranged from 0.87 to 0.95 mg GAE/100 g and DPPH free radical scavenging activity were (31.9 to 32.85%) present in the sags incorporating 20 percent fresh chickpea leaves at 45 days after sowing of desi and kabuli chickpea varieties i.e. HC-1, C-235, HK-1 and HK-2. The flavonoid contents in sag ranged from 7.2 to 8.3 mg/g.

Keywords: Sag, minerals, phenolic compounds

Introduction
Chickpea belongs to subfamily Papilionaceae of family leguminosae and is said to be one of the oldest pulses known to be cultivated from ancient time both in Asia and the Europe. It spread to different countries including India and it is now grown as pulse crop throughout tropical and sub-tropical Asia, Northern Africa, Southern Europe, Central and Southern America (Nene and Reddy, 1987) [3]. Chickpea leaves can be used fresh as well as processed and then utilized in value addition of traditional Indian recipes. The chickpea is consumed in different forms, fresh green leaves are used as vegetable (sag). The basic idea is to find novel methods by which consumption of greens can be increased. For chickpea leaves data on leaf mineral concentrations are limited, however, available reports on iron, zinc and copper suggest that this food could be a good source of these minerals. More information is needed on the concentrations of all the human essential minerals in chickpea leaves, and whether certain types and/or cultivars of chickpea might be more nutritious than others (Ibrikei et al., 2003) [2]. Chickpea leaves can be used in various traditional Indian food products especially, raita, sag, poori and paratha etc. The recipes have to be standardized according to the acceptability in the Indian conditions. It is a general perception that the leaves of the desi chickpea can only be used for various products like chutney, sag etc. among the rural population. In Haryana, the area under pulses decreased due to availability of irrigation facilities. In the present scenario, the importance of leaves along with grain yield has been increased due to their nutritional value. Limited work has been done on this aspect. Desi and kabuli chickpea varieties are explored due to their prominent characteristics which cover whole Haryana. Many varieties of chickpea have been developed for irrigated, rainfed, early and late sowing conditions and disease resistance.

Material and methods
Sag from fresh leaves
Total thirty six different types of sags were prepared from different supplementation levels of chickpea leaves of desi and kabuli varieties picked up at 30, 45 and 60 days after sowing and were subjected to sensory evaluation to select the best recipe of sag further nutritional evaluation.
Sag was prepared as per method given below:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh chickpea leaves (g) collected at 30, 45 and 60 days after sowing</td>
<td>I 10 II 15 III 20</td>
</tr>
<tr>
<td>Spinach (g) (cleaned, washed and chopped)</td>
<td>I 55 II 50 III 45</td>
</tr>
<tr>
<td>Onion (g)</td>
<td>I 15 II 15 III 15</td>
</tr>
<tr>
<td>Tomato (g)</td>
<td>I 15 II 15 III 15</td>
</tr>
<tr>
<td>Green chilli (g)</td>
<td>I 5 II 5 III 5</td>
</tr>
<tr>
<td>Salt (g)</td>
<td>I 2 II 2 III 2</td>
</tr>
</tbody>
</table>

Available minerals (Fe, Ca and Zn)

Available iron

Available in the sample was extracted according to the procedure of Rao and Prabhavathi (1978) [4].

Available calcium and zinc

Available calcium and zinc were extracted by the method of Kim and Zemel (1986) [6].

Reagents

1. 0.1% Pepsin in 0.1 N HCl
2. HCl
3. NaHCO₃
4. 0.5% pancreatin in 5% bile

Procedure

One gram of finely ground sample was taken in a conical flask and 3 ml distilled water was added to rehydrate it. To this 20 ml of pepsin solution (0.1% pepsin in 0.1N HCl) was added. The pH was adjusted to 1.5 with dilute HCl. The contents were incubated at 37°C in a shaker-cum-water bath for one hour. After one h, the pH contents were raised to 6.8 with sodium bicarbonate solution. Then 2.5 ml of a suspension containing 0.5 percent pancreatin in 5 percent bile was added and the contents were again incubated at 37°C for one hour. Then the contents were taken out and total volume was made to 50 ml with distilled water. The contents were then immediately centrifuged at 5000 x g for 45 min at 5°C. Supernatant was collected and re-centrifuged at 2500 x g for 45 min at 5°C. The supernatant was collected; oven dried, digested in the diacid mixture and proceeded for the estimation of calcium and zinc by the atomic absorption spectrophotometric method.

Antioxidant activity

Total phenolic contents

Total phenolic contents were determined by the method of Singleton and Rass (1965) [7].

Reagents

1. Folin-Ciocalteu reagent (1N)
2. Sodium carbonate (Na₂CO₃): Dissolved 200 g anhydrous sodium carbonate in 800 ml water and brought to a boil. After cooling, added a few crystals of sodium carbonate and let it sit for 24 h at room temperature. Filtered through Whatman # 1 filter paper and added water to make the volume to 1 liter.
3. Standard gallic acid

Preparation of calibration curve using gallic acid as standard

Ten mg of standard gallic acid was accurately weighed and dissolved in 100 ml distilled water in a volumetric flask (100 μg/ml of stock solution). From the above stock solution, 0.1 to 1 ml aliquots were pipetted out into 25 ml volumetric flasks. Ten ml of distilled water and 1.5 ml of Folin Ciocalteu reagent were added and diluted according to the label specification to each of the above volumetric flasks. After 5 min., 4 ml of 20 percent sodium carbonate solution was added and volume was made up to 25 ml with distilled water. Absorbance was recorded after 30 min. at 765 nm and a calibration curve of absorbance verses concentration was plotted.

Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (RSA)

The DPPH free radical scavenging activity of sample extracts was evaluated by the DPPH method of Hatano et al. (1988) [1].

Reagents

DPPH: 2.5 mg/l in methanol
Method
An aliquot of (0.1 ml) methanolic solution containing 20-100 µg of crude phenolic extract of sample was mixed with 2 ml of methanol and then added to a methanolic solution of DPPH (1 mmol/l, 0.25 ml). The mixture was vortexed for 10 s, left to stand at room temperature for 30 min and then its absorbance was recorded at 517 nm against methanol blank. A control was measured using the same procedure except that methanol was used instead of extracts (at zero min). The percent of DPPH radical discoloration of the sample was calculated according to the equation (%):}

\[
\text{DPPH free radical scavenging activity} (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

Where,

\[
(A_{\text{control}}) = \text{absorbance for the control}
\]

\[
(A_{\text{sample}}) = \text{absorbance for the sample}
\]

Flavonoid content
Total flavonoid content was determined using a method described by Jia et al. (1999), 0.5 ml of each methanol extract was mixed with 1.5 ml of de-ionized water, 0.1 ml of 1 mg/ml Al (NO₃)₃, 9H₂O (Wako), and 0.1 ml of 1 M CH₃COOK (Wako). After 40 min at room temperature in the dark, the absorbance of the mixture was determined at 415 nm against a blank. A higher absorbance indicates higher flavonoid content. Content of total flavonoid was calculated on the basis of the calibration curve of quercetin (Sigma).

Results
Available mineral content in sag
Data related to available mineral in sag containing 20 percent fresh leaves at 45 days after sowing of desi and kabuli chickpea varieties is depicted in Table 1. The highest available iron content was observed in sag containing the 20 percent fresh leaves of kabuli chickpea variety HK-2 (20.98%) and lowest in sag containing 20 percent fresh leaves of desi variety C-235 (18.66%) at 45 days after sowing. The highest available calcium content (30.75%) was observed in sag containing leaves of HK-2 while the lowest was observed in sag containing leaves of kabuli chickpea variety HC-1 (30.14%). The highest zinc content was recorded in sag containing 20 percent fresh leaves of kabuli chickpea variety HK-2 (49.44%), while lowest in sag containing the leaves from kabuli chickpea variety HK-1 (48.11%). None of the variety showed significant differences in available mineral contents.

Table 1: Available mineral (%) contents of sag incorporating 20 percent fresh chickpea leaves at 45 days after sowing (on dry matter basis)

<table>
<thead>
<tr>
<th>Available minerals</th>
<th>Sag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desi chickpea</td>
</tr>
<tr>
<td>Iron</td>
<td>HC-1</td>
</tr>
<tr>
<td>Calcium</td>
<td>19.68 ± 0.18</td>
</tr>
<tr>
<td>Zinc</td>
<td>30.14 ± 0.16</td>
</tr>
</tbody>
</table>

Values are mean ± SE of three independent determinations. The mean values in same row with same superscripts did not differ significantly (p ≤ 0.05).

Anti-oxidant contents in sag
The phenolic compounds ranged from 0.87 to 0.95 mg GAE/100 g and DPPH free radical scavenging activity were (31.9 to 32.85%) present in the sags incorporating 20 percent fresh chickpea leaves at 45 days after sowing of desi and kabuli chickpea varieties i.e. HC-1, C-235, HK-1 and HK-2. There were no intervarietal differences in desi and kabuli chickpea when their effect on flavonoid contents of sag containing their 20 percent fresh leaves at 45 days after sowing was studied. The flavonoid contents in sag ranged from 7.2 to 8.3 mg/g (Table 2).

Table 2: Anti-oxidant contents of sag incorporating 20 percent fresh chickpea leaves at 45 days after sowing (on dry matter basis)

<table>
<thead>
<tr>
<th>Anti-oxidants</th>
<th>Sag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desi chickpea</td>
</tr>
<tr>
<td>Phenolic compounds (mg GAE/100 g)</td>
<td>0.95 ± 0.01</td>
</tr>
<tr>
<td>DPPH free radical scavenging activity (%)</td>
<td>32.85 ± 0.5</td>
</tr>
</tbody>
</table>

Values are mean ± SE of three independent determinations. The mean values in same row with same superscripts did not differ significantly (p ≤ 0.05).

DPPH: 2, 2 – Diphenyl-1-Picrylhydrazyl.
From the above given results, this may be inferred that sag fell in the category of ‘liked moderately’. Highest mean scores of overall acceptability for sag were observed with 20 percent fresh leaves (45 DAS). Moisture content in sag was found non significant having leaves of desi and kabuli chickpea varieties. Crude protein sag was not affected by chickpea varieties. Sag ash content was significantly (p ≤ 0.05) affected by the supplementation levels of chickpea leaves. β-carotene content was maximum in sag (3.24 mg /100 g, HK-1, 45 DAS). Varietal differences affected the oxalic acid and phytic content in sag and these were present low quantity.

Discussion
Sag had iron, 19.68 to 20.98, calcium 30.75 to 30.14 and zinc content in the range of 49.44 to 48.68 percent. Phenol compounds were 0.87 to 0.95 mg GAE / g. The DPPH free radical scavenging activity of sag ranged from 31.90 to 32.85 percent. The highest flavonoid content was recorded in sag (7.2 to 8.3 mg / g).

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References