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

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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) [5]. 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, *raitha*, *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 A: Ingredients used for making *sag*

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

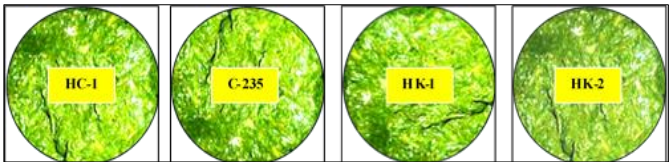

Plate A: *Sags* supplemented with leaves of *desi* and *kabuli* chickpea varieties

Method
1. Rinsed chickpea leaves and chopped.
2. Rinsed spinach leaves and chopped.
3. Added both leaves, boiled and cooked well.
4. Heated oil, added chopped onions till golden brown and then added chopped tomatoes and green chillies.
5. Added cooked *sag* and stirred.
6. Added salt and served with *roti*.

Available minerals (Fe, Ca and Zn)
Available iron
Available iron in the sample was extracted according to the procedure of Rao and Prabhavathi (1978) [6].

Procedure
One gram sample was mixed with 25 ml pepsin HCl (0.5% pepsin in 0.1N HCl) in a conical flask. The pH of the mixture was adjusted to 1.35 with HCl and incubated at 37°C for 90 min in a water bath-cum-shaker. After incubation, pH of the contents was adjusted to 7.5 with NaOH and again incubated at 37°C in a water bath-cum-shaker for 90 min. Contents of the flasks were centrifuged at 3000 rpm for 45 min and the supernatant was filtered through Whatman #42 filter paper. The filtrate was oven dried, digested in the diacid mixture and proceeded for the determination of iron by atomic absorption spectrophotometric method.

Available calcium and zinc
Available calcium and zinc were extracted by the method of Kim and Zemel (1986) [4].

Reagents
1. 0.1% Pepsin in 0.1 N HCl
2. HCl
3. $NaHCO_3$
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 37^0C 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 (Na_2CO_3): 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 1liter.
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.

Procedure
One g of sample was added to 15 ml of methanol (50%) and extracted for three times by maceration of 2 hours. Then it was filtered and volume was made to 50 ml in volumetric flask with methanol (50%). One ml aliquot of the sample was taken in a test tube and diluted with 10 ml of distilled water. Then, 1.5 ml Folin Ciocalteu's reagent was added and allowed to incubate at room temperature for 5 min, then 4 ml of 20% (w/v) Na_2CO_3 was added, adjusted with distilled water up to the mark of 25 ml, agitated and left to stand for 30 min at room temperature Absorbance of the sample was measured at 765 nm. Quantification was done on the basis of a standard curve of gallic acid. Results were expressed as µg gallic acid equivalents (GAE) and percentage w/w.

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 (%) discoloration:

DPPH free radical scavenging activity (%) = $A_{control} - A_{sample} \times 100/A_{control}$

Where,

($A_{control}$) = absorbance for the control

(A_{sample}) = 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 $(NO_3)_3$. $9H_2O$ (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)

Available minerals	*Sag*			
	***Desi* chickpea**		***Kabuli* chickpea**	
	HC-1	**C-235**	**HK-1**	**HK-2**
Iron	19.68 [a] ±0.18	18.66 [a] ±1.9	20.54 [a] ±0.48	20.98 [a] ±0.37
Calcium	30.14 [a] ±1.60	30.29 [a] ±0.80	30.32 [a] ±1.63	30.75 [a] ±0.81
Zinc	48.68 [a] ±0.11	48.78 [a] ±0.23	48.11 [a] ±0.20	49.44 [a] ±0.26

Values are mean ± SE of three independent determinations.

The mean values in same row with same superscripts did not differ significantly ($p \leq 0.05$).

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)

Anti-oxidants	*Sag*			
	***Desi* chickpea**		***Kabuli* chickpea**	
	HC-1	**C-235**	**HK-1**	**HK-2**
Phenolic compounds (mg GAE/100 g)	0.95[a]±0.01	0.94 [a] ±0.02	0.89 [a] ±0.03	0.87 [a] ±0.03
DPPH free radical scavenging activity (%)	32.85 [a] ±0.5	32.53 [a] ±0.7	32.5[a]±1.0	31.9 [a] ±1.31
Flavonoids (mg/g)	8.1 [a] ±0.06	8.3 [a] ±0.09	7.4 [a] ±0.11	7.2 [a] ±0.17

Values are mean ± SE of three independent determinations.

The mean values in same row with same superscripts did not differ significantly ($p \leq 0.05$).

DPPH: 2, 2 – Diphenyl-I-Picrylhydrazyl.

From the above given results, this may be inferenced 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 ($p \leq 0.05$) 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

1. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root; their relative astringency and radical scavenging effects. Chem. Pharmaceut. Bull. 1988; 36:2090-2097.
2. Ibrikei H, Knewtson JBS, Grusak AM. Chickpea leaves as a vegetable green for humans: evaluation of mineral composition. J Sci Food Agric. 2003; 83:945-950.

3. Jia Z, Tang M, Wu J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999; 64:555-559.
4. Kim H, Zemel MB. *In vitro* estimation of the potential bioavailability of calcium from sea mustard milk and spinach under stimulated normal and reduced gastric acid conditions. J Food Sci. 1986; 51:957-963.
5. Nene YL, Reddy MV. Chickpea diseases and their control in: chickpea (Eds. Saxena, M.C. and Singh, K.B.), Wallingford, U.K. CAB International, 1987, 233-270.
6. Rao BSN, Prabhavathi T. An *in vitro* method of predicting the bioavailability of iron from foods. Am. J Clin. Nutr. 1978; 31:169.
7. Singleton VL, Rass JA. Calorimetry of total phenols with phosphomolybdic-phasphotungstic acid reagents. Am. J Enology Viticulture. 1965; 16:144-158.