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Neha Singh
Student, Department of Biochemistry and Biochemical Engineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Dr. Sushma
Assistant professor, Department of Biochemistry and Biochemical Engineering, Jacob School of Biotechnology & Bioengineering, Allahabad, Uttar Pradesh, India

Dr. Veeru Prakash
Professor, Department of Biochemistry and Biochemical Engineering, Associate Dean (Administration), Jacob School of Biotechnology & Bioengineering, Allahabad, Uttar Pradesh, India

Correspondence
Neha Singh
Student, Department of Biochemistry and Biochemical Engineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Assessment of some antinutritional compounds and minerals in selected seasonal leafy vegetables under soaking and heat treatments

Neha Singh, Dr. Sushma and Dr. Veeru Prakash

Abstract
Leafy vegetables are known for there are nutritional values but at the same time they do contain antinutritional factors because they chelate the nutrients making unavailable them for their absorption. The purpose of the present study is to evaluate the effect of preblanching treatments (with and without NaCl, $NaHCO_3$ and NaCl +$NaHCO_3$) at different temperatures on antinutritional compounds viz. total phenol, tannin, alkaloid, flavonoid and saponin and minerals- magnesium, sodium, potassium and phosphorus of four leafy vegetables such as Cabbage, Amaranth, Coriander and Spinach. The findings suggest that sodium chloride and sodium bicarbonate (in combination) at high temperature can be useful to decrease antiunutritional compounds studied and help to retain the mineral as well.

Keywords: cabbage, amaranth, coriander, spinach, antinutritional factors, blanching, minerals

Introduction
Antinutrients are natural or synthetic compounds that interfere with the absorption of nutrients. These factors/compounds can be divided into protein and non-protein antinutritional factors. Protein antinutritional factors includes trypsin and chymotrypsin inhibitors, lectins and antifungal peptides while non-protein antinutritional factors includes alkaloids, tannins, phytic acid, saponins and total phenols (Arthur, 2012) [3]. Green leafy vegetables such as coriander, broccoli, cabbage, spinach, chenopodium and amaranth have many compounds acting as antinutrients, vitamins and minerals *etc*. Phenolic compounds are major antioxidant of cabbage (Can and Altiokka, 2009) [5]. Spinach is an excellent source of vitamin K, vitamin C, iron, beta-carotene, potassium, magnesium copper and zinc and very low in calories and fats (Harper, 2010) [7]. Cabbage or headed cabbage is a leafy green biennial plant. It is a multi-layered vegetable which belongs to the family of Brassicaceae. Cabbage is a very good source of vitamin K which has the potential role in bone metabolism through promoting osteotrophic activity (Feng *et al*, 2010) [6]. Coriander is a perennial herb that is extensively used around the world as a condiment, garnish or decoration on culinary dishes. Coriander leaves are dark green, hairless and soft. Its leaves and stem posses slightly citrus flavor. Its leaves and seeds contain many essential volatile oils such as borneol, linalool, cineole, cymene, terpinoel and terpinolene. Coriander leaves and stem tips are also rich in numerous anti-oxidants, polyphenolic, flavonoids such as quercetin, rhamnetin, and epigenin. Coriander herb is also a good source of minerals like potassium, calcium, manganese, iron, and magnesium. Amaranth belongs to the family Amaranthaceae. Amaranth contains a huge amount of vitamin K, which is a well known booster for heart health. Amaranth contains numerous flavonoids. Spinach is an edible flowering plant belonging to the family of Chenopodiaceae. It is an annual plant (Boswell, 2010) [4]. Dark leafy green spinach is important for skin and hair, bone health, and provide protein, iron, vitamins and minerals. Spinach is an excellent source of beta-carotene which prevents the Asthma (Juan *et al.*, 2009) [8].

Materials and Methods
Selection and preprocessing of Green leafy vegetables: Four seasonal green leafy vegetables, namely Cabbage (*Brassica oleracea*), Amaranth (*Amaranthus hybridus*), Coriander (*Coriandrum sativum*) and Spinach (*Spinacia oleracea*) were procured from local market. The leaves were separated from the roots and washed under running water to remove the adhering mud particles followed by double glass-distilled water washing and water was

drained completely. Then vegetables were given chemical treatments by soaking them in their respective chemical for 15 mins (e.g. distilled water,T_1-80 °C & T_5-90 °C; 0.1% sodium chloride,T_2-80 °C & T_6-90 °C; 0.1% sodium bicarbonate,T_3-80 °C & T_7-90 °C and combination of 0.1% sodium chloride + 0.1% sodium bicarbonate,T_4-80°C & T_8-90 °C) and then steam blanched for 5 min at 80 and 90 °C after chemical treatments. They were drained after blanching and spread on steel trays for drying in an oven at 60 °C for 2 hr 37 mins. After drying the GLV were used for analysis.

Biochemical analysis

Tannin content was estimated as per the method of Schanderi (1970); Total phenol was estimated as per the method of Bray and Thorpe (1954); Alkaloid content was estimated as per the method of Obadoni and Ochuko (2001) [10]; Saponin content was determined as per the method of Harbone (1973); Flavonoid content was estimated as per the method of Chang *et al*., (2002); Magnesium and sodium content was estimated as per the method of Piper (1950); Potassium content was estimated as per the method of Jackson (1962) and Phosphorus was estimated as per the method of Fiske (1925).

The data recorded during the course of investigation will be subjected to statistical analysis by "analysis of variance" technique for drawing conclusion. The significant and non-significant treatment affect was judged with the help of F (variance ration) table. The significant difference between the means was tested against the critical difference at 5% level.

Estimation of total phenol

Weighed exactly 0.5 to 1.0g of the sample and grind it with a pestle and mortar in 10-time volume of 80% ethanol. Then centrifuged at 10,000 rpm for 20 min, saved the supernatant. Re-extracted the residue with five times the volume of 80% ethanol, centrifuged and pooled the supernatant. Then the supernatant was evaporated to dryness. The residue was dissolved in a known volume of distilled water (5.0ml). Pipetted out different aliquots (0.2 to 2.0ml) in to test tube. Made up the volume in each tube to 3.0ml with water. Then added 0.5ml of FCR reagents. Added 2ml of 20% Na_2CO_3 solution to each tube, after 3.0 min. mixed thoroughly; placed the tube in a boiling water bath for exactly one minute and cooled and measured the absorbance at 650nm against a reagent blank. A standard curve using (0.2, 0.4, 0.6, 0.8, 1.0ml) conc. of Catechol was prepared. The reading of 1.0ml and 2.0ml of sample was taken.

Determination of alkaloids

5g of each sample were weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered to stand for 4 hrs. This was filtered and the extract was concentrated using water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed.

Estimation of Tannin

Weighed 0.5g of powdered material and transferred to a 250ml of conical flask. Added 75ml of distilled water and boiled for 30 min. After that centrifuge it at 2000 rpm for 20 min and collected the supernatant in 100ml of volumetric flask and make up the colume. Transferred 1 ml of the sample extract if Folin-Denis reagent, 10ml volumetric flask containing 75ml distilled water to this added 5ml of Folin-Denis reagent. 10ml of Na_2CO_3 and diluted to 100ml with distilled water. Shaked well and read the absorbance at 700nm after 30 min. Blank was prepared with water instead of sample. The standard graph was plotted. Tannin solution was used as standard.

Estimation of Saponin

1gm of sample were dispersed in 50ml of 20% aqueous ethanol solution. The suspension was heated over a hot water bath at 55 ^{0}C for 90 min with continuous stirring. Then mixture was filter through Whatman filter paper No.40. The residue was re-extracted with another 50ml of 20% aqueous ethanol and both extract were together. The combined extracts were reduced to about 40ml over a water bath at about 90 ^{0}C. The concentrate was transferred into a 250ml separating funnel where 40ml of di-ethyl ether was added and shaken vigorously. The separation was by partition during which the ether layer was discarded while the aqueous layer was reserved. Re-extraction by partition was done repeatedly until the aqueous layer become clear in color. The saponin was extracted with 60ml of n-butanol solution and the extracts washed with 10ml of 5% aqueous NaCl solution and evaporated to dryness in pre-weighed evaporating dish. It was dried at 60 ^{0}C for 30min in the oven (to remove any residue solvent), cooled and reweighed. The experiment was repeated 2 or 3 times to get a constant weight.

Estimation of flavonoid

Plant extract (1ml) in methanol was mixed with 1ml of methanol. The samples (0.2, 0.4) were taken in the test tubes. Volume of each tube was made to 1ml with method. 0.5ml $AlCl_3$ (1.2%) and 0.5ml potassium acetate (120mM) was added in each tubes. And then 1ml mixture was allowed to stand for 30min at room temperature, than the absorbance was measured at 415nm. Quercentin was used as standard.

Estimation of phosphorus

Took 5-10ml of HCl extract into a 50ml volumetric flask and added 5ml ammonium molybdate solution. Added water to make the volume about 40ml. Added 5ml H_2SO_4 and mixed. Added 4ml ferrous sulphate reagent, mixed and made the volume upto the mark. And then absorbance was measured at 660nm. Set the instrument at zero with blank.

Estimation of magnesium

Took 10ml of HCl extract in a 15ml graduated tube. Added one drop of methyl red indicator. Neutralized the solution with NH_4OH. Added 1ml of ammonium oxalate reagent and made the volume to 15ml with water. Mixed and allowed to stand overnight. Centrifuged for 10 minutes and take 1ml of the supernatant liquid in a 15m centrifuge tube. Added 3ml water, 1ml of ammonium phosphate and 2ml of NH_4OH. Mixed and allowed to stand overnight. Centrifuged for 7 minutes and discarded the supernatant liquid. Added 5ml of dilute NH_4OH, mixed, centrifuged for 7 min and discarded the supernatant liquid. Dried the precipitate by placing the tube in a container of hot water, added 1ml of dilute HCl and 5ml of water to dissolved the precipitate. Added 1ml of molybdic acid solution, 0.5ml of hydroquinone solution and 0.5ml of sodium sulphite solution. Mixed and allowed it to stand for 30 min and the absorbance was measured at 720nm.

Estimation of potassium

2gm powdered sample in 500ml conical flask and added 5ml of conc. HNO_3 in the flask. Swirled the flask, placed in a

steam plate for 30 min and then on the electric hot plate at 80-120 ^{0}C. Boil until dryness. Cooled and added 5ml of ternary acid mixture. Digested at 180-200 ^{0}C until dense white fumes of H_2SO_4 and $HclO_4$ are evolved. Continued digestion until the acid liquid is largely volatilized. Stoped the digestion when the residues in the flask are clean and white and only slightly moist with H_2SO_4. The $HclO_4$, at this point is largely removed. Cooled and then added 5ml of conc. HCl and mixed. Transfered to a 25ml calibrated centrifuge tube. Washed the digestion flask with 5ml more HCl, rinse and transfered to centrifuge tube. Made up the volume with 6N HCl to the marked. Centrifuged for 5 min or until clear. Immediately decent the clear supernatant into a dry flask and stopper. Evaporated an aliquot sample (10-15ml of extracted sample) to dryness to reduce the HCl concentration. Added 2 drops of 1% phenolphthalein and 10% NaOH drop wise until phenolphthalein turns distinctly red. Evaporated to dryness of a steam plate. Added 2ml of ammonium- free 1N HCl and 1 small drop of this HCl solution of the sample to a spot plate and test for ammonium with Nesler's reagent. Evaporated the solution to dry out HCl and to extract K completely. Cooled the evaporated container and immediately filter through a dry filter paper. Cooled 15ml of aliquot sample and also cool 10ml of precipitation reagent saparatively and added 10ml of test solution to it. Mixed rapidly during addition of the test solution by keeping on the ice bath for 5h or overnight. Filtered the precipitation (cobaltinitrite precipitation). Washed the precipitate 3 times with 70% ethanol. Washed the precipitate once with absolute ethanol. Dried the precipitate for 5 min at 100-110 ^{0}C. Dissolved the precipitate in 3 or 4 drops of 6N HCl with a little heat. Added 5ml of hot water and place of the steam plate for a few minutes until the precipitate dissolves. Washed the solution through filter into the colorimeter tube and made upto known volume. Added 1.5ml of 6N KOH and added 0.5ml of 3% H_2O_2 and mixed. Added 15ml of saturated $KHCO_3$ and water to a volume of 40ml. After few minutes for bubbles to rise read the color intensity at 620nm against reagent blank.

Estimation of sodium

Took 1 gm powdered sample and then washed and dry and heat over a gas burner. Allowed to ashing to proceed slowly and when no more growing carbon can be seen, gradually raised the temperature of muffle furnace to a very dull red heat. And after that added 20ml HCl (1:1) in the crucible containing ash. Covered the crucible with watch glass and digest for 20 to 30 minutes or water bath. Removed the watch glass and rinse with water into the contents of the crucible. Filtered through Whatman filter paper no. 42 into a 100ml volumetric flask washed and transferred the residue in the crucible repeatedly with small volumes of 5% HCl solution. Made the volume up to the mark. Mixed the contents thoroughly and preserve this solution for the estimation of different inorganic ingredients. Transferd a suitable aliquot of the solution of the ash to a 100ml volumetric flask. Added 5ml of 5% solution of magnesium acetate, 30ml of conc. ammonia, shake vigorously and leave to stand for some time. When quite cold, dilute to the mark with water and mixed thoroughly. Leaved to stand overnight, filtered through a Whatman no. 44 filter paper. Pipette a suitable volume of the filtrate into a Pyrex evaporating besin and evaporate to dryness on the water bath. When dry added 3ml of water, 5ml of conc. HNO_3, covered the basin with a clock glass and leave on the bath to decompose ammonium salts. After the initial reaction, removed the clock glass, rinse it into the basin and re-evaporate to dryness. Cooled and dissolved the residue in about 6ml of cold water stirring with a glass rod. Added 15ml of uranyl magnesium acetate reagent and stir vigorously for about 15 sec or until a precipitate forms. Covered the basin and allowed to stand for 30 min. filtered through a small Gooch crucible charged with asbestos. Washed the precipitate of sodium uranyl magnesium acetate twice with 2ml of the solution of magnesium uranyl acetate and then five times with 96% alcohol saturated with the triple salt and transfered the precipitate to the Gooch crucible. Dry in an oven at 150 ^{0}C for not more than one hour, cooled in a desicator and weigh.

Result and Discussion

Total phenol, tannin, alkaloid, flavonoid and saponin contents were analyzed in fresh and blanched green leafy vegetables. In the fresh samples, the total phenol content was found to be least in cabbage and highest in coriander, which ranged between 16.78-0.43 mg/g where as blanched leaves, the phenolic content was maximum in coriander followed by amaranth, spinach and cabbage, ranged between 12.89-0.25 mg/g (Figure 3.1). The tannin content was found to be maximum in amaranth followed by cabbage, spinach and coriander in fresh leaves, it ranged between 155.04-28.91 mg/100g where as blanched leaves, the tannin content was found to be high in amaranth and low in coriander 84.20-24.13 mg/100g (Figure 3.2). The alkaloid content was found to be high in amaranth followed by cabbage, spinach and very low in coriander in fresh leaves, ranging between 8.01-7.00 mg/100g where as blanched leaves, alkaloid content was found to be high in amaranth and least in coriander, ranging between 2.10-1.72 mg/100g (Figure 3.4). In the fresh leaves, the flavonoid content was found in very high quantity in amaranth followed by spinach, cabbage and coriander, which ranged between 152.02-10.02 mg/100g while blanched leaves, amaranth has maximum flavonoid content and coriander has least amount, with 94.19-7.22 mg/100g range (Figure 3.3). The saponin content was found to be high in amaranth and least in spinach, ranging between 510.6-4.06 mg/100g in fresh leaves where as blanched leaves, saponin content was found to be high in amaranth followed by cabbage, coriander and spinach, ranging between 361.1-2.97 mg/100g (Figure 3.5). Green leafy vegetables blanched for 10 mins shows the reduction in antinutritional factors. This is because the concentrations of antinutritional factors are highest in the superficial layer of vegetables and blanching ruptures this layer (Albinhn *et al.*, 2004) [1].

In the fresh leaves, the magnesium content was found to be high in amaranth followed by coriander, spinach and least in cabbage, which ranged between 4.97-0.46 mg/g where as in case of blanched leaves, the magnesium content was high in amaranth and low in cabbage, with 0.81-0.08 mg/g range (Figure 3.6). (Siler *et al.*, 2007) [11] in their study on *Centaurium erythraea* reported that chlorophyll decreased with the increases of salt concentration. Phosphorus content was found to be high in cabbage and low in amaranth in fresh leaves, it ranged between 23.17-0.58 mg/g where as in case of blanched leaves, the maximum phosphorus content in cabbage and minimum in amaranth leaves, ranging between 3.82-0.19 mg/g (Figure 3.9). Sodium content was found in maximum quantity in coriander followed by amaranth, spinach and cabbage, which ranged between 3.61-0.55 mg/g in fresh leaf. Whereas blanched leaves of coriander has maximum sodium content where as cabbage has minimum amount of sodium content, ranging between 1.13-0.12 mg/g (Figure 3.7). In fresh leaf sample, the potassium content was found to be high

in coriander followed by amaranth, spinach and cabbage, which ranged between 41.00-2.08 mg/g where as blanched leaves, coriander has maximum potassium content while cabbage has minimum amount of potassium content, with 8.25-4.08 mg/g range (Figure 3.8). Potassium content was found to be decreasing with increasing in salt stress. These outcomes suggest that there was a competition between sodium and potassium regarding their uptake. Similar findings were reported with green bean cultivars (Yasar *et al*., 2006; Amador *et al*., 2007) [12] in legume.

Table 1: Over all % change in level of antinutrients in leafy vegetables

Antinutrients Studied	Samples	% change (Decrease ↓ or Increase ↑)								
		T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Total phenol↓	Cabbage	100	83.72	74.41	72.09	67.44	74.42	69.76	65.11	58.13
	Amaranth	100	74.00	65.75	65.34	56.84	82.10	58.46	41.37	40.48
	Coriander	100	84.68	83.49	81.94	78.78	81.88	80.39	78.48	76.81
	Spinach	100	78.91	68.42	64.31	52.63	66.66	59.64	57.89	49.12
Tannin↓	Cabbage	100	66.92	64.01	63.80	60.03	62.87	61.44	61.02	57.13
	Amaranth	100	61.93	60.05	59.51	56.75	60.62	58.12	57.98	54.30
	Coriander	100	94.53	93.11	90.66	89.13	93.42	89.58	88.82	83.46
	Spinach	100	97.36	95.99	95.88	93.93	97.14	94.15	93.88	91.80
Alkaloid↓	Cabbage	100	75.37	56.25	50.00	38.75	63.87	37.75	30.62	25.00
	Amaranth	100	75.90	60.79	49.93	37.95	62.79	52.43	46.44	26.21
	Coriander	100	85.85	72.00	61.14	57.14	71.85	57.42	55.14	44.57
	Spinach	100	75.25	52.00	51.37	41.50	66.62	30.25	28.12	25.00
Flavonoid↓	Cabbage	100	81.52	73.71	71.33	63.33	76.47	69.85	63.33	60.61
	Amaranth	100	75.64	67.18	67.09	65.91	64.47	64.38	63.17	61.95
	Coriander	100	92.31	89.82	87.02	81.93	87.62	79.64	73.55	72.05
	Spinach	100	77.42	74.40	74.01	69.07	73.12	70.11	69.91	65.49
Saponin↓	Cabbage	100	88.07	74.36	73.77	56.13	78.99	67.04	65.49	53.54
	Amaranth	100	94.54	92.17	91.80	78.64	94.31	88.43	87.80	70.80
	Coriander	100	82.29	72.43	71.48	59.30	81.48	69.98	67.90	54.41
	Spinach	100	94.08	82.01	79.80	74.63	92.61	80.29	79.06	73.15

Table 2: Over all % change in level of Minerals in leafy vegetables

Minerals Studied	Samples	% change (Decrease ↓or Increase ↑)								
		T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Mg^{2+}↓	Cabbage	100	43.47	34.78	30.43	21.73	39.13	32.60	23.91	17.39
	Amaranth	100	33.19	18.30	17.00	16.49	25.35	13.68	12.47	10.26
	Coriander	100	66.5	60.5	48.25	31.75	61.00	40.5	31.00	20.25
	Spinach	100	53.59	49.67	44.44	33.33	37.90	30.06	26.14	22.22
Na^{+}↑	Cabbage	100	56.00	65.33	41.38	74.66	50.66	56.00	53.33	62.66
	Amaranth	100	33.68	48.42	45.26	62.10	27.36	43.15	42.10	45.26
	Coriander	100	33.51	38.50	37.95	40.44	24.93	28.25	27.70	31.30
	Spinach	100	80.00	88.57	84.28	97.14	74.28	87.14	82.85	85.71
K^{+}↓	Cabbage	100	87.01	74.03	57.69	51.92	60.09	48.55	40.38	32.21
	Amaranth	100	74.77	70.06	69.61	62.21	65.47	48.31	47.86	45.40
	Coriander	100	33.92	28.90	28.78	26.68	27	22.70	22.63	20.12
	Spinach	100	88.09	87.21	80.91	79.15	81.78	75.13	73.73	65.14
PO_4^{2-}↓	Cabbage	100	37.85	27.23	26.24	21.96	29.13	21.10	18.64	16.48
	Amaranth	100	75.86	67.24	62.06	46.55	68.96	55.17	48.27	32.75
	Coriander	100	34.38	33.85	33.33	28.34	33.85	27.55	25.98	18.89
	Spinach	100	85.44	80.31	74.32	72.03	83.16	62.33	59.77	55.49

From the above results it can be concluded that antinutritional factors studied in selected leafy vegetables were seen low in sodium chloride and sodium bicarbonate (in combination) at 90 ºC, on the basis of results obtained it can be concluded that sodium chloride and sodium bicarbonate (in combination) at high temperature can be useful to decrease some of antinutritional factors as mineral loss is also minimum, it can be show in (Table 1 and Table 2).

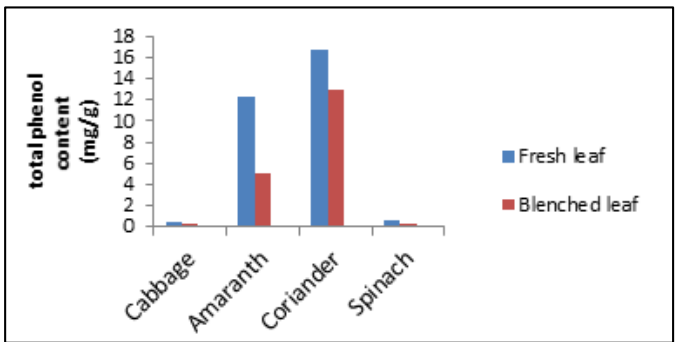

Fig 3.1

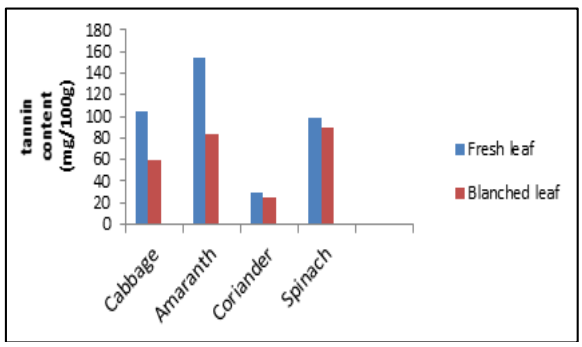

Fig 3.2

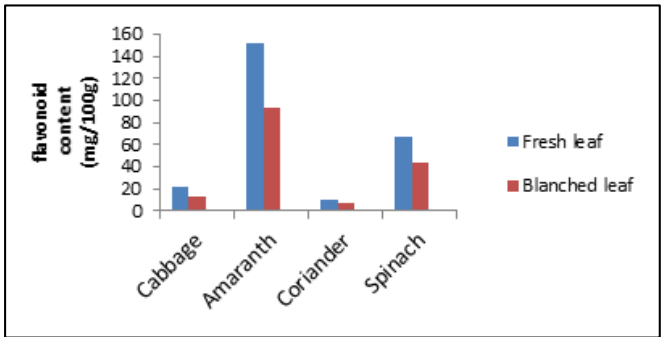

Fig 3.3

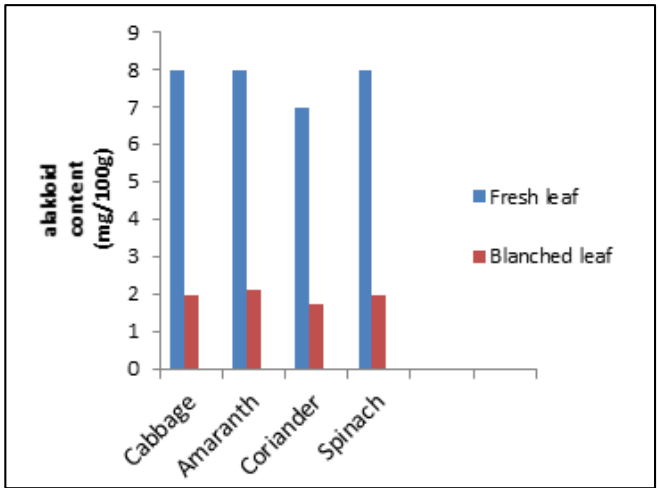

Fig 3.4

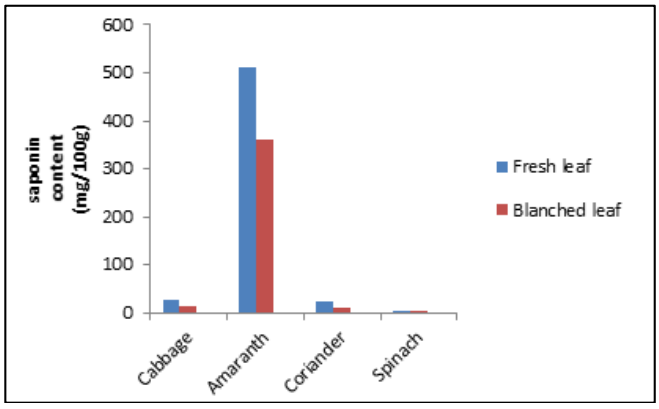

Fig 3.5

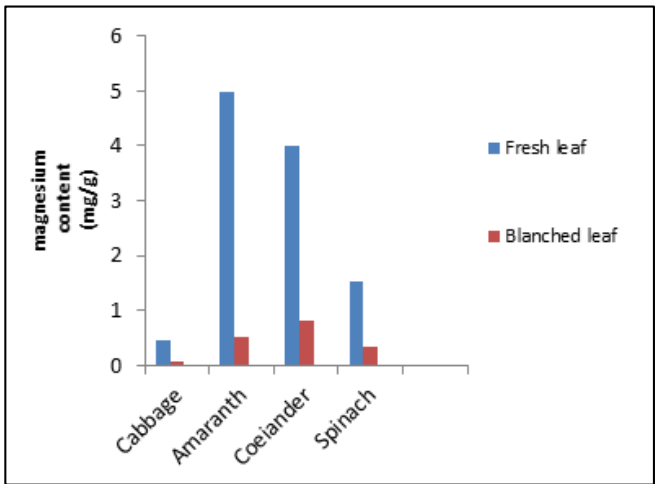

Fig 3.6

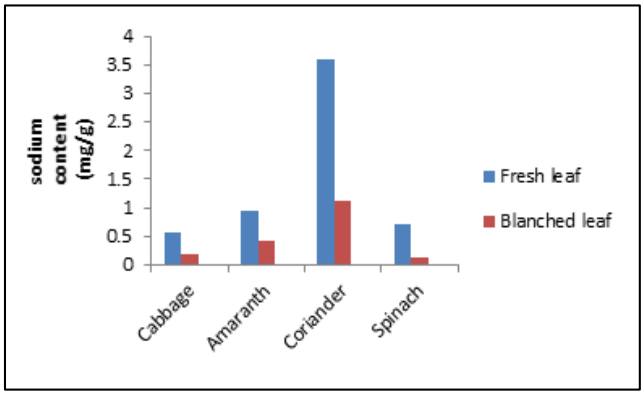

Fig 3.7

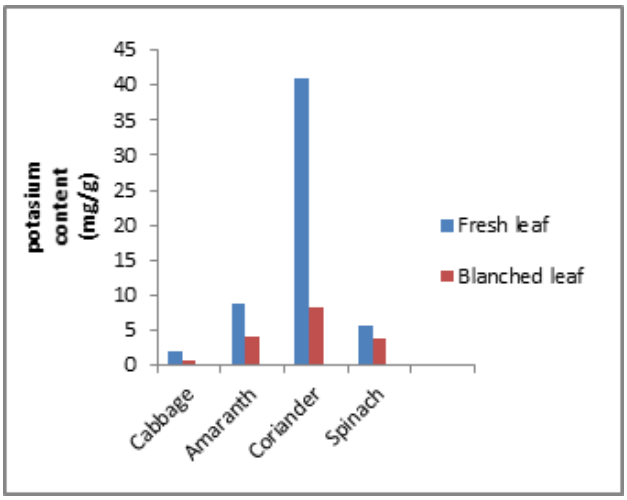

Fig 3.8

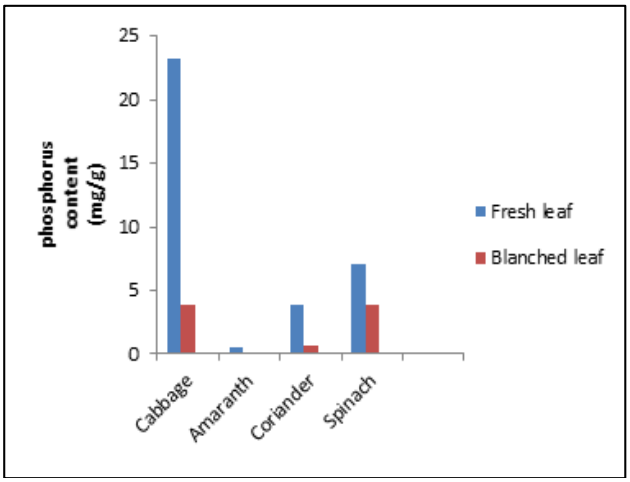

Fig 3.9

Conclusion

From the above results it can be concluded that antinutritional factors studied in selected leafy vegetables were seen low in sodium chloride and sodium bicarbonate (in combination) at 90 °C, on the basis of results obtained it can be concluded that sodium chloride and sodium bicarbonate (in combination) at high temperature can be useful to decrease some of antinutritional factors as mineral loss is also minimum.

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