

STANDARDISATION OF METHODS FOR THE ESTIMATION OF THIOCYANATE AND IODINE IN FOODSTUFF

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Summary : Thiocyanate content in vegetables has been estimated by passing the water extract of vegetables through an alumina column. The thiocyanate content in the eluate has been measured according to Johnston and Jones. Iodine content in the vegetables has been estimated according to a modified method of Acland. The results show the primarily the Cruciferae family vegetables contain higher concentration of thiocyanate.

Key words : iodine thiocyanate Alumina cruciferae goitrogen

INTRODUCTION

Iodine is an important constituent of thyroid hormone. Iodine deficiency plays an essential role in the occurrence of endemic goitre and cretinism (4,5). Epidemiological evidence suggests that in some instances, it may be additional environmental contribution to the pathogenesis of these diseases (3). Among these may be the presence of goitrogenic substances in food and water, food stuff containing a cyanogenic glycoside (10). Thiocyanate has important effects on thyroid function and iodine metabolism. It inhibits the uptake of radioiodine by the thyroid (6, 11), placenta (8) and mammary gland.

The aim of this work is to standardise methods and to estimate quantitatively iodine and thiocyanate contents in some of the commonly used vegetables in Delhi.

MATERIAL AND METHODS

All the chemicals used in the experiment were of AR grade. Alumina (Al_2O_3) chromatographic grade was obtained from BDH, England.

Determination of thiocyanate in food stuff : Thiocyanate was estimated by a modified method of Johnston and Jones (6).

a) *Thiocyanate in green leafy vegetables* : Deep colouring pigments in the vegetables were separated by Alumina (Al_2O_3) column which retained all the colouring pigments but released thiocyanate quantitatively. Ten gram leafy vegetables (triplicated) was crushed with clean sand (GR, E. Merck), extracted with distilled water and the watery extract was passed through Alumina column (25×1.2 cm). The column was washed repeatedly with glass distilled water and the volume of eluate was adjusted to 50 ml. Five ml eluate was taken separately in two test tubes, mixed with 5 ml of 0.4 M Ferric nitrate in N-Nitric acid and to one, 1 drop of Mercuric chloride (5%) was added to block thiocyanate. Absorbency of both blank and test samples were measured at 460 m μ . Thiocyanate contents in test samples were computed from standard curve.

b) *Thiocyanate in vegetables* : Ten gram vegetables (triplicate) was crushed with clean sand, extracted with distilled water (25 ml) and refluxed for 20 minutes in a conical flask. The substance was cooled and filtered. The residue in the filter paper was repeatedly washed with distilled water and the volume of the filtrate was made upto 50 ml. Five ml filtrate samples were treated as in (a) and thiocyanate content was determined from the standard curve.

c) *Thiocyanate in milk and plasma* : Five ml milk or diluted plasma samples were treated with 5 ml of 20% Trichloro acetic acid, mixed and centrifuged at 10,000 rpm for 15 min. The samples were filtered and the residue in the filter paper was washed repeatedly with distilled water and the volume of filtrate was made upto 15 ml. Five ml samples were treated as in (a) and the thiocyanate contents was determined from the standard curve.

Recovery of thiocyanate added to the samples : Five vegetables samples (10 g) were taken in triplicate. In one of each sample, 10 μ g -SCN solution was added. There were, therefore, five blank, five vegetable and five vegetable with added thiocyanate (Total fifteen samples). The samples were processed as in (b) and thiocyanate contents were determined.

Calculation of recovery (%)

Let A = blank -SCN (μ g)

B = Vegetable sample

C = Vegetable + 10 μ g -SCN

$$\% \text{ recovery} = \frac{(C-A) - (B-A)}{10} \times 100$$

Determination of iodine in food stuff : A modified method as described by Acland (8) was used. AR grade chemicals and I_2 free water were used.

Reagents :—

- i) **Sodium carbonate solution :** Anhydrous sodium carbonate (102 g) was dissolved in 1000 ml of water.
- ii) **Standard I_2 solution :** Potassium Iodide (0.13 g) was dissolved in 1000 ml and the standard solution containing I_2 (0.02 $\mu\text{g/ml}$) was made by diluting 5 ml of the final solution to 250 ml.
- iii) **Ceric Ammonium Sulphate solution :** Ceric ammon. sulphate (8.15 g) low in rare earths was dissolved in water, Conc. H_2SO_4 (80 ml) was added and the volume made up to 1000 ml with water.
- iv) **Arsenious acid solution :** Arsenious Oxide (3.15 g) was dissolved in water containing NaOH (1.65 g); Conc. H_2SO_4 (70 ml) was added and the volume made up to 1000 ml with water.
- v) **Hydrochloric acid solution :** Conc. HCl (63 ml) was added to water and diluted to 1000 ml.

Ten gram food samples (triplicate), mixed with 2 ml sodium carbonate solution (1N) were dried at 100°C (over night) and then placed in a furnace at 600°C for 90 min. After cooling, iodine was extracted with 3 ml (0.7 N) HCl and diluted with 5 ml water. The samples in hard glass test tubes (10 x 1.5 cm) were centrifuged for 5 min at 3000 rpm and 3 ml of the supernatant was transferred to colorimeter tubes. (Klett Summerson) the colorimeter tubes and tubes containing solution of Ceric ammon. sulphate and arsenious acid were placed in a water bath at 37°C and left for 10 min. Arsenious acid (1 ml) was added to the colorimeter tubes which were left a further 30 min. Ceric ammonium sulphate (1 ml) was added to each tube at intervals of a minute and the transmittance of each sample was measured exactly 12 min later at $415\text{ m}\mu$ Iodine contents in test samples were computed from a standard curve prepared identically.

Recovery of Iodine in the samples : Twentyfive hard glass test tubes (10x1.5 cm) were taken. One was used for blank, 12 for 6 food samples (duplicate) and the remaining 12 for food samples + 0.04 μg iodine solution (duplicate). To each sample 0. μCi carrier free ^{131}I was added. The procedure is the same as in food stuff. Cold iodine contents in samples were computed from the standard curve.

(2) Calculation for recovery (%) of Iodine

Let iodine content in food = A (μg)Iodine content in food + 0.4 μg iodine = B (μg)

$$\text{Recovery (\%)} = \frac{B-A}{0.04} \times 100$$

Recovery of ^{131}I was determined by counting 1 ml samples taken from the colorimeter tubes and comparing them with stock ^{131}I solutions (0.1 $\mu\text{Ci } ^{131}\text{I}$).

RESULTS AND DISCUSSION

The method of Aldridge as (2) modified by Michajlovskij (9) was also tried for thiocyanate estimation in vegetables. The method utilises a pyridine - benzidine complex which develops a pink colouration with thiocyanate and the absorbency was measured at 525 $m\mu$. Although the method yields a good recovery (approx 95%), it could not be

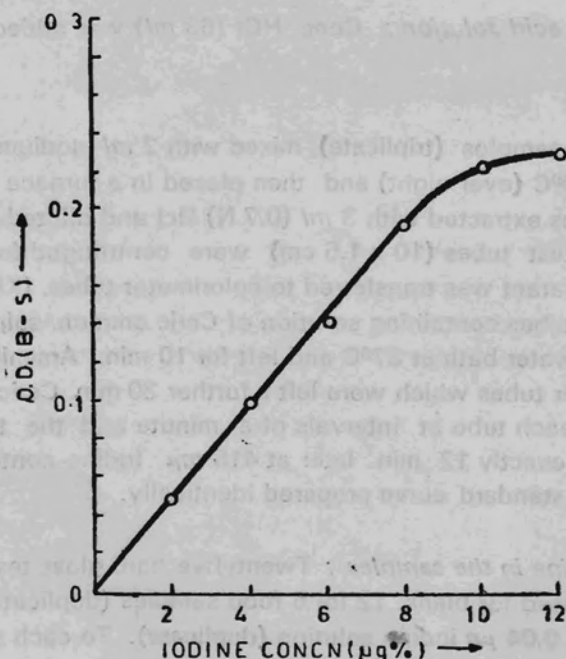


Fig. 1 ; Standard curve of thiocyanate, Concentrations ((μg) against optical density (OD) at 465 $m\mu$.

used as a routine test due to the irritating smell of pyridine and the high toxicity of benzidine. Deep colouring pigments in the leafy vegetables were removed by us by introducing an Alumina column which retained the pigments but thiocyanate was quantitatively eluted with water. The column efficiency was found to be 100%.

Percentage recovery for thiocyanate was found to be 93.5 ± 1.5 (S.D) and that for iodine was 89.4 ± 2.8 (S.D). Thiocyanate and iodine contents in test samples were computed from the standard curve (Figs. 1 and 2). Iodine and thiocyanate contents in food stuff after applying recovery corrections are presented in Table 1. The results show that cruciferae (Brassica group) vegetables e.g. Cauliflower, Cabbage, raddish, turnip etc. have higher thiocyanate content with a relatively lower iodine content. It has been reported that a gradual increase of the plasma thiocyanate level in man may result from prolonged intake of repeated and relatively small doses of thiocyanate (7). Thus regular ingestion of small quantities of naturally occurring goitrogens may affect the human thyroid specially with a low iodine diet.

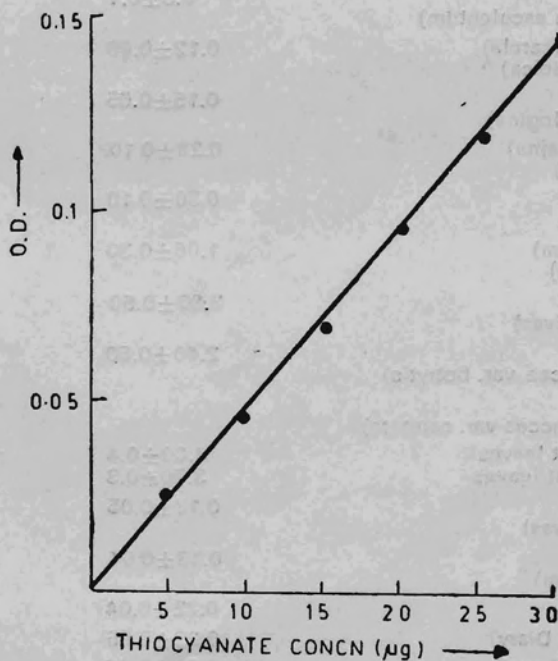


Fig. 2 : Standard curve of iodine. Concentrations (μg) against optical density (OD) at $415 \text{ m}\mu$. B, blank; S, standard iodine.

TABLE I: Thiocyanate and iodine content in vegetables per 100 gm wet weight.*

S. No.	Sample	Thiocyanate (-SCN) (mg/100 gm)	Iodine (μ g/100 gm)
1.	Carrot (Duncus carota)	N.S.	10 \pm 2.0
2.	Potato (Solonom tuberosum)	0.8 \pm 0.3	1.13 \pm 0.2
3.	Gourd (Ghia) Lagenaria siceraria)	0.8 \pm 0.2	0.50 \pm 0.1
4.	Lady finger (Bhindi) (hibiscus esculentum)	1.0 \pm 0.2	0.70 \pm 0.2
5.	Onion (Allium cepa)	0.6 \pm 0.2	0.70 \pm 0.2
6.	Pumpkin (Cucurbita maxima)	0.16 \pm 0.1	0.90 \pm 0.2
7.	Tori Ridge gourd) (Luffa acutangula)	0.5 \pm 0.1	1.20 \pm 0.3
8.	Arbi (Colocasia aniiquorum)	1.0 \pm 0.3	0.50 \pm 0.1
9.	Spinach (Spinacia Oleracea)	0.9 \pm 0.2	4.06 \pm 0.6
10.	Tomato (ripe) (Lycopersicum esculentum)	0.5 \pm 0.1	0.57 \pm 0.1
11.	Bitter gourd (karela) (Memoridica lioica)	0.12 \pm 0.06	0.80 \pm 0.3
12.	Brinjal (Solanum Melogina)	0.15 \pm 0.05	0.70 \pm 0.4
13.	Drum stick (sajna) (Muringaceae)	0.28 \pm 0.10	1.80 \pm 0.6
14.	Mustard (Brassica)	0.30 \pm 0.10	4.50 \pm 0.5
15.	Turnip (Salgam) (Brassica rapa)	1.06 \pm 0.30	0.7 \pm 0.2
16.	Raddish (Raphanus sativas)	3.80 \pm 0.50	0.80 \pm 0.3
17.	Cauliflower (Brassica okracea var. botrytis)	2.40 \pm 0.50	0.90 \pm 0.2
18.	Cabbage (Brassica cleraccea var. capitata)		
	(a) Inner most leaves	4.00 \pm 0.4	0.90 \pm 0.3
	(b) Outer most leaves	3.70 \pm 0.3	1.40 \pm 0.3
19.	Cucumber (Cucumis sativas)	0.18 \pm 0.05	0.56 \pm 0.1
20.	Green pea (Pisium sativum)	0.13 \pm 0.04	2.10 \pm 0.3
21.	Milk (DMS)	0.22 \pm 0.04	4.0 \pm 0.3
22.	Milk (Mother Diary)	0.23 \pm 0.05	4.5 \pm 0.4
23.	Buffalo milk (Local)	0.30 \pm 0.05	5.0 \pm 0.3

*The values are mean \pm S.D of 4 samples obtained from Delhi markets.

*Milk values per 100 ml.

Under these contexts, the assessment of thiocyanate and iodine contents in food stuff have assumed great significance. Survey of iodine and thiocyanate contents in food stuff and water in various goitre belts of the country has been undertaken and will be reported.

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