Chapter II

GOITROGEN CONTENT IN THE EDIBLE PARTS OF THE SELECTED PLANTS

The dietary goitrogen present in cyanogenic plant foods, their chemical nature, and action on the thyroid gland are briefly reviewed and the goitrogen content of Indian cyanogenic plant foods as observed in the present study have been discussed in this chapter.

Some natural products consumed by man or animals cause hypothyroidism with an enlargement of the thyroid. Among these there are plants from which goitrogens have been isolated and identified (Chesney et al. 1928; Montgomery 1969; Van Etten 1969). Cyanogenic glucosides, glucosinolates (thioglucosides) and thiocyanate are the goitrogenic / anti-thyroid constituents of cyanogenic plants that are often used as foods by men and animals. However, Michajlovskij and Langer (1959) found that goitrogen content of the Brassica plants varied little in relation to the regions where grown but did show a variation from plant to plant within a single field. Extreme differences in the glucosides contents of the plants belong to same family and same taxonomy even within the same geographical area owing to their genetic backgrounds and ecological factors have also been reported (Delange et al. 1982). The goitrogenic / anti-thyroid potential of a plant not only depends on the relative concentrations of cyanogenic constituents found in fresh plants but also on its processing as foods, so in the areas where these plant foods are used, the common measure to reduce goitrogenic potency are soaking, washing, boiling, cooking etc. (de Groot et al. 1991; Oke 1982). The major goitrogenic constituents of cyanogenic plants, their chemical nature and effects on thyroid gland are as follows -

Cyanogenic glucosides

Cyanogenic glucosides are derivatives of 2-hydroxy-nitriles that often form glucosides with β-D glucose. They are optically active. More than 60 cyanogenic glucosides are known which are widely distributed among plants (more than 2600 cyanogenic taxa have been reported) especially among members of Rosaceae, Leguminosae, Graminae and Araceae (Conn 1980). Some specialized herbivores especially insects preferentially feed on cyanogenic plants. Such herbivores have
acquired the ability to metabolize cyanogenic glucosides or to sequester them for use in their predator defence. A few species of Arthropoda (within Diplopoda, Chilopoda, Insecta) are able to de novo synthesize cyanogenic glucosides (Zagrobelny et al. 2004). In addition, some of these species are able to sequester cyanogenic glucosides from their host plant (Zygaenidae). A few insects such as the Zygaenidae (Lepidoptera) feed on plant with cyanogens and sequester those compounds but are also capable of synthesizing the same cyanogens independently themselves (Nahrstedt 1989). In members of the Sapindaceae certain bacteria and fungi produce HCN (Conn 1980). Cyanogenic glucosides are formed in the cytoplasm and stored in the central vacuole of plant cells.

![Cyanogenic glucosides](image)

**Fig 12. General structure of cyanogenic glucosides**

Cyanogenic glucosides are derived from L-amino acids and biosynthesis is catalysed by multienzyme complex (Conn 1980). They are derived mostly from the amino acids L-val, L-ile, L-leu, L-phe or L-tyr and the nonprotein amino acid cyclopentenyl-gly. For synthesis of cyanogenic glucosides, the amino group of L-amino acids is hydroxylated by an L-amino acid N-monooxygenase, upon oxidative decarboxylation amino acid is converted into an aldoxime. Aldoxime is then converted to nitrile catalysed by an aldoxime dehydratase. The nitrile is then hydroxylated by a nitrile monooxygenase to yield the key intermediate cyanohydrin. Finally, from cyanohydrin, β-glucoside is formed by using activated glucose by glucosyltransferase (Conn 1980).

Cyanogenesis, i.e. the ability of living cells to release cyanide under certain biotic and/or abiotic stress conditions, is an old trait (Lechtenberg and Nahrstedt 1999). In case of emergency i.e. when plants are wounded by herbivores or other organisms or during any kind of mechanical damage cyanogenic glucosides come into contact with an active β-glucosidase, which hydrolyses them to yield cyanohydrin. It is further cleaved into the
corresponding aldehyde or ketone and HCN by a hydroxynitrile lyase. HCN is then rapidly detoxified by rhodonase to produce thiocyanate (Conn 1980).

The principal pathway of cyanide metabolism is its conversion to thiocyanate catalyzed by either rhodanese (thiosulfate sulfurtransferase) or by 3-mercaptoppyruvate sulfurtransferase. Both enzymes are widely distributed in the body. Conversion of cyanide to the less toxic thiocyanate by rhodanese is enhanced when cyanide poisoning is treated with the intravenous administration of a sulfur donor such as sodium thiosulfate (ATSDR 1989). The toxicity of thiocyanate is significantly less than that of cyanide, but chronically elevated levels of blood thiocyanate can inhibit the uptake of iodine by the thyroid gland, thereby reducing the formation of thyroxine (Hartung 1982). Other metabolic pathways include the conversion to 2-aminothiazoline-4-carboxylic acid; incorporation into a 1-carbon (formate) metabolic pool; combination with hydroxycobalamine to form cyano-
cobalamine (B12); and combination with cystine to form 2-aminothiazoline-4-carboxylic acid (ATSDR 1989).

Cyanogenic glucosides are toxic to consumers through two possible mechanisms. In some plant tissues (i.e. the seeds of some plants in the Anacardiaceae), cyanogenic glucosides and the enzymes that are responsible for their hydrolysis are present in different cell and tissue compartments. Tissue damage by consumers leads to mixing of cyanogenic substrates (such as amygdalin and prunasin) and to the release of cyanide (this phenomenon is sometimes called the “cyanide bomb”). Other plant tissues (i.e. the pulp of many fruits) contain only the cyanogenic glucosides. Ingestion of the unhydrolyzed glucosides can result in cyanide poisoning when these substances are hydrolyzed by the β-glucosydases of the consumer or of its gastrointestinal microbes. If the animals lack these enzymes, then the cyanogenic glucosides is absorbed and then excreted intact (Struempf et al. 1999).

After ingestion, cyanogenic glucosides are readily converted to thiocyanate by wide spread glucosidases and sulfur transferase enzymes present in the animal body or
plant itself. In this way the inactive precursors are converted to active goitrogenic agents both in the plant and also in the animal body (Gaitan 1990).

In humans and animals, the major route of cyanide elimination from the body is via urinary excretion of thiocyanate. Small amounts of thiocyanate are also eliminated via lung and faeces (EPA 1985). Some free hydrogen cyanide is excreted unchanged in breath, saliva, sweat, and urine (Hartung 1982).

Many studies have shown a correlation between ingestion of cyanogenic plants and development of goitre in various animal species (Tewe et al. 1984) and humans (Adewusi and Akindahunsi 1994). This pathology is related to the main product of transformation of cyanide, thiocyanate that competes with iodide in the Na⁺/I⁻ symporter in the thyroid gland consequently inhibiting the synthesis and clearance of thyroid hormones (Dohan et al. 2000).

Cassava root, a staple food in many tropical regions, contains cyanogenic glucosidess, such as linamarin, which release cyanide (CN⁻) when metabolized endogenously (Sharma 1993; Kamalu 1995). Consumption of insufficiently processed cassava roots over a period of time in combination with a protein deficient diet has been implicated in neurotoxic effects due to endogenous conversion of cyanide to cyanate (OCN⁻). The development of this syndrome is hypothesized to depend on (a) the amount and duration of exposure to dietary cyanide, and (b) the ability of the body to detoxify cyanide, a function that may vary with nutritional status (Kamalu 1995). Other effects associated with cassava consumption include pancreatic diabetes, vitamin B₁₂ deficiency and decreased iodine uptake (Sharma 1993). Prolonged ingestion of cyanogenic plants causes tropical pancreatic diabetes, some studies have suggested that this may be produced by the action of cyanide on acinal portion of the pancreas (Kamalu 1991). Cretinism in children, associated with a deficiency of dietary iodine is worsened by eating cassava (Miller 1974). Excess thiocyanate due to cyanide metabolism results in a depressed uptake of iodine by the thyroid gland that may lead to symptoms of iodine deficiency disorders, including goitre (Adewusi and Akindahunsi 1994). Besides that man is continually exposed to small doses of cyanide not only from the diet but also from the polluted atmosphere and particularly through cigarette smoke (Brunneman et al. 1977)
Glucosinolates / Thioglucosides

Glucosinolates are an important group of phytochemicals, present and often abundant in Brassica vegetables such as broccoli, all types of cabbage, cauliflower, and Brussels sprouts. They are claimed to be the active components responsible for many of the physiological effects proposed for Brassica vegetables in different types of studies, including *in vitro*, animal, human, and epidemiological studies (van Poppel *et al.* 1999). Intact glucosinolates *per se* are relatively biologically inactive.

![General structure of glucosinolates](image)

More than 80 different glucosinolates have been found in higher dicotyledonous plants including Cruciferae, Capparidaceae, Moringaceae, Tropaeolaceae etc. There are about 10-12 distinct glucosinolates found in Brassica family (Stoewsand 1995). These compounds remain intact unless brought into contact with the enzyme myrosinase by pests, food processing or chewing. They may be degraded or leached during processing or preserved by thermal inactivation of myrosinase. Glucosinolates are broken down by plant myrosinase in the small intestine or by bacterial myrosinase in the colon (Johnson 2002). Isothiocyanates are absorbed from the small bowel and colon and metabolites are detectable in human urine two to three hours after consumption of brassica vegetables (Johnson 2002). Glucosinolates are polar molecules formed in the cytoplasm and stored in vacuoles. Glucosinolates can be lost by vegetables during storage. Glucosinolates were totally decomposed in both fermentations during two weeks, and different types of breakdown products were formed. Isothiocyanates, indole-3-carbinol, goitrin, allyl cyanide, and nitriles were determined in the fermented cabbage (Tolonen *et al.* 2002).
Naturally occurring glucosinolates are (Z)-N-hydroximinosulfate esters, possessing a sulfur-linked β-glucopyranose moiety and an amino acid-derived side chain (Fig 15). Side chain and sulfate group have an anti stereochemical configuration across the C-N double bond. The structure of the side chain (R, Fig 15) is highly variable and may possess aliphatic, aromatic or heterocyclic groups (Mithen 2001).

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Fig 16. Biosynthesis of glucosinolates
The majority of the currently known glucosinolates may be subdivided according to their hydrolysis products into aliphatic and aromatic, terminally unsaturated, β-hydroxy and indolyl glucosinolates. Glucosinolates are classified as aliphatic, aromatic and indole glucosinolates, depending on whether they are derived from methionine, phenylalanine or tyrosine, or tryptophan, respectively (Fahey et al. 2001). They, however, share similar biosynthetic pathways that involve three different stages: the first one is the synthesis of chain-elongated amino acids, the second involves the synthesis of the common aglucone moiety, and the third is the side chain modifications. The presence of the sulfate group in the molecule confers strongly acidic properties on intact glucosinolates (Halkier and Du 1997).

Both protein and non-protein amino acids serve as precursors for the biosynthesis of glucosinolates. Up to the formation of aldoxime is analogous to that of cyanogenic glucosides biosynthesis. Aldoxime is converted to thiohydroximic acid, a key intermediate, using cysteine as a sulphate donor. Thioglucosides is formed by UDP-glucose:thiohydroximate glucosyltransferase. The transfer of sulphate group is catalysed by sulfotransferase using phosphor adenosine phosphosulfate (PAPS) donor. It may be further modified by hydroxylation, oxidation and other substitutions (Mithen 2001).

Glucosinolates are hydrolysed by myrosinase, present in the plant, to D-glucose and an aglycone, spontaneously rearranging to isothiocyanates. Isothiocyanates are responsible for the distinctive pungent flavour and odour of mustard and others (Van Etten 1969). Upon cell damage they undergo hydrolysis by myrosinase (thioglucosides glucohydrolase) to yield glucose, sulfate and aglucones that can undergo fragmentation and/or molecular rearrangement yielding isothiocyanates, thiocyanate, oxazolidine-2-thiones and nitriles, depending on the specific glucosinolates substrate, myrosinase isozyme, presence of protein co-factors, nature of the glucosinolates side chain, reaction pH, temperature, presence of certain ions and also depending upon the environmental conditions, enzymes and other compounds present. Isothiocyanates are formed at pH>7, nitriles are formed at pH<4. Oxazolidine-2-thiones are formed in the presence of β-hydroxylated side chains (Bones and Rossiter 1996).
Fig 17. Degradation of glucosinolates by the enzyme myrosinase

In the intact plant, myrosinase is located in specialized myrosin cells, and thus glucosinolates and myrosinase are physically separated (Duncan 1991). Myrosin cells are dispersed throughout the seedlings, mature plants, and seeds, but during germination, they are present in large proportion in the outermost part of the cotyledons and are most abundant in the cortex area of the radicles. These locations afford myrosin cells a role as a “toxic mine” when the tissue is destroyed (Thangstad et al. 1993). Upon injury and cell rupture, the enzyme and its glucosinolates substrates come in contact, and hydrolysis of these substrates occurs. Processing of Brassica vegetables influences glucosinolates degradation and therefore the biological activity (Verkerk et al. 1997).

Glucosinolates degradation products deter herbivores such as birds, mammals and molluscs and are toxic to micro-organisms. However, many insect pests have become specialist feeders of glucosinolates containing plants and use these compounds and their degradation products to locate their hosts and as egg-laying and feeding stimulants (Mithen 2001).
Comparative studies of glucosinolates distribution and variability between and within groups of the most widely consumed cruciferous vegetables such as broccoli, cabbage, cauliflower, Brussels sprouts, and kale are limited. One of the study reports only total glucosinolates, the data suggests considerable differences in glucosinolates levels between and within the *Brassica* groups (Mullin and Sahasrabudhe 1977). In the more detailed study 13 different glucosinolates have been reported in broccoli, Brussels sprouts, cauliflower and kale (Carlson *et al.* 1987a). In turnip, cabbage, and *Brassica napus* significant variations in glucosinolates are reported from year to year due to differences in climatic conditions (Carlson *et al.* 1987b; Rosa *et al.* 1997). Glucosinolates levels in *Brassica* are also affected by the growing location. Factors that may have contributed to these differences included soil type, sulfate and nitrate fertilizer application, plant spacing, and date of harvest (Rosa *et al.* 1997). The presence of glucosinolates in the seeds of oilseed cruciferous crops significantly reduces the quality of the seed meal left following oil extraction. This is largely due to the presence of certain glucosinolates that degrade to give goitrogenic products (Griffiths *et al.* 1998). In addition to alterations to the size, structure and function of the thyroid, feeding rapeseed meal can lead to damage to the liver and kidneys. The precise cause of this is not fully understood, but may be related either to the presence of intact glucosinolates or the production of nitriles in the digestive tract (Griffiths *et al.* 1998).

### Table 4. Types of glucosinolates present in the selected plants (Van Etten 1969)

<table>
<thead>
<tr>
<th>Name</th>
<th>Part of Plants</th>
<th>Common name of glucosinolates</th>
<th>Name for food</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brassica oleracea</em></td>
<td></td>
<td>Sinigrin</td>
<td>Allyl</td>
<td>Jensen <em>et al.</em> (1953)</td>
</tr>
<tr>
<td><strong>Cabbage</strong></td>
<td>Leaves</td>
<td>Glucobrassicin</td>
<td>3-indolyl methyl</td>
<td>Gmelin and Virtanen (1961)</td>
</tr>
<tr>
<td><strong>Cauliflower</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Raphanus sativus</em></td>
<td>Roots</td>
<td>4-methyl-thio-3 butenyl glucosinolate</td>
<td>4-methyl-thio-3 butenyl 3-indolyl methyl</td>
<td>Friis and Kjaer (1966) Gmelin and Virtanen (1961)</td>
</tr>
<tr>
<td><strong>Radish</strong></td>
<td></td>
<td>Glucobrassicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Brassica juncea</em></td>
<td>Seeds</td>
<td>Sinigrin</td>
<td>Allyl</td>
<td>Jensen <em>et al.</em> (1953)</td>
</tr>
<tr>
<td><strong>Indian or brown mustard</strong></td>
<td></td>
<td></td>
<td></td>
<td>Ettlinger and Thompson (1962)</td>
</tr>
</tbody>
</table>
The presence of glucosinolates in horticultural cruciferous crops is important both for flavour and as potential anticarcinogens. Several studies in cell culture systems and on rodents have shown that certain glucosinolates and isothiocyanates can act as blocking agents, which detoxify carcinogens prior to carcinogenesis, and as suppressing agents, which prevent cellular proliferation and may induce apoptosis (Jongen 1996).

Glucosinolates are iodine antagonist, it causes reduction in thyroidal iodine concentration (Schone et al. 2001) and moreover glucosinolates and their degradation product change the activity of thyroid peroxidase (Kohler et al. 1988).

Glucosinolates hydrolysis products make a significant contribution to the typical flavour of vegetables from the Brassicaceae family. They also possess chemical properties rendering them powerful allelochemical agents against microbes, fungi, and plants (Angus et al. 1994; Teasdale and Taylorson 1986). In contrast to the beneficial properties of glucosinolates hydrolysis products, they can also have deleterious effects on animals, reducing fertility and inducing goitre (Brown and Morra 1997). Although excessive amounts of glucosinolates in animal feed formulations may reduce growth and performance as well as affecting thyroid, liver, and kidney function (Heaney and Fenwick 1995).

Isothiocyanates interact non specifically and irreversibly with sulfhydryl groups, disulfide bonds and amino groups in proteins, thus forming stable products with proteins and amino acids (Fenwick et al. 1983). Isothiocyanates provide the characteristic hot and pungent flavours of many of our cruciferous salad crops and these and other degradation products are important flavour components of cooked cruciferous vegetables. Isothiocyanates are lipophilic and volatile allelochemicals. Isothiocyanates that occur as glucosinolates are released by myrosinase-mediated hydrolysis when raw vegetables are chopped or chewed (Getahun and Chung 1999). A known mechanism of detoxification of isothiocyanate is its conversion to thiocyanate ions (Greer 1950). Their goitrogenic effect may be due to their conversion to thiocyanate ion, a known goitrogen. Other organic isothiocyanates would also acts as goitrogens (Bachelard and Trikojous 1960). Isothiocyanates mainly interfere in the thyroid gland with the organification of iodine and formation of active thyroid hormones (Gaitan 1990).

Nitriles are the major degradation products under acidic conditions. Nitriles formed from the glucosinolates may act as goitrogens. According to Greer (1950) nitriles
cause thyroid enlargement. Similarly like organic isothiocyanates detoxification of nitriles forms thiocyanate ions.

Oxazolidine 2-thiones derivatives (such as progoitrin, gluconringin, glucobarbarin and glucosisymbrin) inhibit the oxidation of iodate to iodine that ultimately leads to the formation of goitre (Kohler 1989). These derivatives have thiourea or thionamide like goitrogens and their action usually cannot be antagonised by iodine. Long-term administration of goitrin to rats results in increased thyroid weight and decreased radioactive iodide uptake and hormone synthesis by the thyroid gland (Ermans and Bourdoux 1989). The thionamide like anti-thyroid effects of goitrin have been confirmed in *in vitro* studies both by marked inhibition of thyroid peroxidase (Gaitan 1986a) and iodide organification. Goitrin possesses 133% potency of propyl thiouracil in humans. Goitrin is unique in that it is not degraded like thioglucosides (Gaitan 1990).

Thiocyanate, the major breakdown product of glucosinolates mainly acts as competitive inhibitor of iodide uptake by affecting iodide efflux into the gland and inhibiting thyroid peroxidase activity ultimately causing inhibition of thyroid hormone formation leading to thyroid dysfunction (Knudsen 2002; Ermans and Bourdoux 1989; Lakshmy *et al.* 1995). Additive anti-thyroidal effects of thiocyanate, isothiocyanate and goitrin occur with combinations of these naturally occurring goitrogens (Gaitan 1990).

Glucosinolates and their degradation products cause growth depression associated with morphological and functional changes in different organs particularly in the thyroid (Spiegel *et al.* 1993).

**Thiocyanate**

The thiocyanate ion involves a linear SCN group in which the double bond character of the S-C reflects the existence of two tautomeric structures –S–C=N and –N=C=S (Gaitan 1989a). Thiocyanate is ambient since the negative charge can be located either on S or N. This tautomerism explains the existence of two series of covalent derivatives, the thiocyanate and isothiocyanates (Ermans and Bourdoux 1989). Thiocyanate is largely of exogenous origin i.e. it is derived through the ingestion of various glucosinolates that release thiocyanate upon hydrolysis (Wood 1975). A common source of glucosinolates is plants belonging to the Cruciferae family. Another important source of thiocyanate is the enzymatic detoxification of cyanide by the enzyme
thiosulfate sulfur transferase (rhodonase). This enzyme is present in most mammalian tissues although the highest concentrations are found in liver, kidney, adrenals, thyroid and pancreas (Reinwein 1961). Cyanide in man is generally produced due to the ingestion of cyanogenic glucosides.

Thiocyanate is released to water primarily from discharges of industrial waste waters from coal processing and extraction of gold and silver (Boucabeille et al. 1994). Thiocyanate is also found in mining waste waters where it results from the interaction of free cyanide with sulphur (Boucabeille et al. 1994). Releases of thiocyanate to soil result from anthropogenic and natural sources. Anthropogenic releases occur primarily from direct application in herbicidal formulations and from disposal as by-products from industrial processes. Non-anthropogenic sources include damaged or decaying tissues of plants from the family Brassica (e.g. cabbage, mustard, kale) (Brown and Morra 1993). The environmental fate of thiocyanate has not been thoroughly investigated. Aerobic and anaerobic biodegradations are significant transformation processes for thiocyanate in water (Boucabeille et al. 1994) and soil (Brown and Morra 1993).

Thiocyanate occurs naturally in many edible plants. Vegetables in the family Brassica contain high levels of thiocyanate with concentrations ranging up to 660 µg/g, whereas other commonly consumed vegetables (e.g. spinach, radish, celery) generally contain thiocyanate at concentrations <2 µg/g (Weuffen et al. 1984). Other commonly consumed vegetables (e.g. lettuce etc.) have been found to contain thiocyanate at concentrations ranging from approximately 0.1-5.0 µg/g w/w (Weuffen et al. 1984). Thiocyanate concentrations in milk and other dairy products and in meat have been reported to range from <1 to 9.0 µg/g and 0.5 to 0.7 µg/g respectively (Weuffen et al. 1984). Thiocyanate concentrations in coal plant waste waters (Tuan and Jensen 1993) and mining waste waters (Boucabeille et al. 1994) have been found to range from 100-1,500 mg/L and 300-450 mg/L, respectively.

Previously Stoa (1957) has demonstrated that thiocyanate is present in considerable but variable amounts in green vegetables and milk. Michajlovskij and Langer (1959) found that the thiocyanate content of Brassica plants was highest in spring, varied little in region to region. Salts of thiocyanic acid in Crucifers also interfere with the uptake of iodide in thyroid gland (Gmelin and Virtanen 1960). While Van Etten
(1969) reported that thiocyanate does not occur free in intact plant but form a characteristic group of glucosinolates.

Thiocyanate is secreted by mammary and salivary glands and by the gastric mucosa. Thiocyanate is normally found in mammalian blood (Dahlberg et al. 1984). Studies indicate that cigarette smoke may produce goitre (Gaitan 1988). Similarly goitre and hypothyroidism were documented in patients receiving long-term thiocyanate treatment for hypertension (Gaitan 1990).

Thiocyanate is monovalent anions with a molecular size corresponding to that of iodine. Thiocyanate has an inhibitory effect mainly on the uptake of iodine in the thyroid gland but also on the iodination of thyroglobulin (Greer et al. 1966). Thiocyanate at low concentrations inhibits iodide transport by increasing the velocity constant of iodide efflux from the thyroid gland. At high concentrations the iodide efflux is greatly accelerated where as the unidirectional iodide clearance into the thyroid is inhibited. Thiocyanate at high concentrations also inhibits the incorporation of iodide into thyroglobulin by competing at thyroid peroxidase level (Ermans and Bourdoux 1989). The goitrogenic effect of thiocyanate is more evident in the presence of iodine deficiency. Several observations suggest that thiocyanate crosses the human placenta and may cause both goitre and neonatal hypothyroidism (Roti et al. 1983).

Thiocyanate ion is metabolized by the thyroid and that the sulfur atom is oxidized in part to sulfate (Maloof and Soodak 1959). In the animal organism thiocyanate is formed endogenously from cyanides, nitriles and sulfur containing compounds and that both the SCN' brought exogenously and into the organism and that formed endogenously in it have to be taken into consideration (Gmelin and Virtanen 1960). Dahlberg et al. (1984) reported the thiocyanate has a potent anti-thyroid effect. Langer and Stole (1964) found that goitrogenic action of white cabbage and probably of other plants, are not entirely due to the thiocyanate content and the existence of other substances must be taken into account.

The information on goitrogenic content of cabbage, cauliflower, mustard and radish of Cruciferous family of Indian origin is scanty. Therefore the present study was undertaken to measure goitrogenic constituents viz. cyanogenic glucosides,
glucosinolates and thiocyanate content of these plant foods in both the fresh/uncooked and cooked conditions.

MATERIALS AND METHODS

Processing of plant foods

Described in the methodology section.

Measurement of dietary goitrogens in plant foods

Described in the methodology section.

RESULTS

The goitrogenic principles viz. cyanogenic glucosides, glucosinolates and thiocyanate were measured in selected cruciferous vegetables in the present study in uncooked/fresh and cooked condition because these foodstuffs are generally consumed after cooking (Table 5). Goitrogenic/anti-thyroid principles in cabbage, cauliflower, mustard and radish are glucosinolates and thiocyanate though cyanogenic glucosides present in relatively lower concentration.

A relative variation in the content of these goitrogenic principles was found between fresh (but incubated) and their cooked counterpart. There was a significant reduction in cyanogenic glucosides; glucosinolates content reduced markedly but thiocyanate remained almost same or slightly increased after cooking.

Of all the studied food plants cauliflower had the highest concentration of cyanogenic glucosides and glucosinolates while thiocyanate content was highest in mustard in both the fresh (uncooked) and cooked condition respectively.

Cabbage had 1.6 mg/kg cyanogenic glucosides, 15.7 mg/kg glucosinolates and 11.6 mg/kg thiocyanate in fresh/uncooked condition and 0.004 mg/kg cyanogenic glucosides, 11.8 mg/kg glucosinolates and 11.52 mg/kg thiocyanate in cooked condition.

Uncooked cauliflower had 1.82 mg/kg cyanogenic glucosides, 17.28 mg/kg glucosinolates and 5.04 mg/kg thiocyanate but after cooking 0.004 mg/kg cyanogenic glucosides, 12.51 mg/kg glucosinolates and 5.25 mg/kg thiocyanate were found.
Table 5. Distribution of cyanogenic glucosides, glucosinolates and thiocyanate content in the edible portion of selected cyanogenic plant foods

<table>
<thead>
<tr>
<th>Plant</th>
<th>Cyanogenic glucosides (mg/kg wet weight)</th>
<th>Glucosinolates (mg/kg wet weight)</th>
<th>Thiocyanate (mg/kg wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uncooked</td>
<td>Cooked</td>
<td>Uncooked</td>
</tr>
<tr>
<td>Cabbage</td>
<td>1.6 ± 0.3</td>
<td>0.004 ± 0.001</td>
<td>15.7 ± 1.3</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>1.82 ± 0.4</td>
<td>0.004 ± 0.002</td>
<td>17.28 ± 1.6</td>
</tr>
<tr>
<td>Mustard</td>
<td>0.24 ± 0.01</td>
<td>0.013 ± 0.005</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>Radish</td>
<td>1.28 ± 0.04</td>
<td>0.005 ± 0.002</td>
<td>2.64 ± 0.2</td>
</tr>
</tbody>
</table>

Values are mean ± SD of six observations.

Fig 18. Distribution of cyanogenic glucosides, glucosinolates and thiocyanate contents in the edible portion of selected cyanogenic plant foods (Values are mean ± SD)
Mustard seeds showed high thiocyanate level (50.5 mg/kg) in uncooked condition and that was slightly higher (50.82 mg/kg) after cooking. But glucosinolates content was reduced markedly (from 4.0 mg/kg to 2.52 mg/kg) during cooking. Cyanogenic glucosides content was quite low in mustard seeds (0.24 mg/kg) as compared to other foods but this was further reduced after cooking (0.013 mg/kg). The concentration of cyanogenic glucosides in radish was 1.28 mg/kg that was reduced to 0.005 mg/kg after cooking. Uncooked radish had 2.64 mg/kg glucosinolates and 13.28 mg/kg thiocyanate but after cooking these were reduced to 1.82 mg/kg and 13.12 mg/kg respectively.

Dietary goitrogens were found in edible parts of all the studied plants in varying proportions. Cyanogenic glucosides and glucosinolates contents were reduced markedly while thiocyanate content was slightly increased or unaltered after cooking.

DISCUSSION

A significant proportion of human diets are composed of cruciferous vegetables that include cabbage, cauliflower, mustard and radish. The goitrogenic content viz. cyanogenic glucosides, glucosinolates and thiocyanate of the selected plant foods were measured in both uncooked and cooked condition.

**Cyanogenic glucosides content**

The available literature shows that cyanogenic glucosides content of cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*Brassica oleracea* var. *botrytis*), mustard (*Brassica juncea*) and radish (*Raphanus sativus*) of Indian origin are not adequately available. The systematic data from other countries are also insufficient.

In the present study cyanogenic glucosides content was found highest in cauliflower followed by cabbage, radish and mustard. After cooking those plant foods, cyanogenic glucosides were measured because these plant foods are generally consumed in India mostly after cooking. In cooked plant the concentration of cyanogenic glucosides was found highest in mustard followed by radish, cabbage and cauliflower. The overall results showed that during cooking cyanogenic glucosides content of all the studied plants were reduced markedly.
All the plants which sequester cyanogenic glucosides also contain the enzyme \(\beta\)-glucosidase that hydrolyses them to yield 2-hydroxynitrile (cyanoxydrin) and hydrogen cyanide and respective aldehyde and ketone (Conn 1980). Cyanogenic glucosides also decompose quickly when placed in boiling water (Cooke 1982).

Cyanide is present in popular cyanogenic plants viz. bamboo shoots, cassava, lima beans, millet etc. in two forms - free form and bound form (Montgomery 1969). During boiling free cyanogenic glucosides are removed rapidly i.e. over 90% is removed within 15 minutes while bound cyanide decreases at a much slower rate i.e. 55% of the bound cyanide are removed at 25 minutes heating (Cooke 1982). Variations in cyanide losses during boiling are reported ranging from 90-100% losses of cyanide, while 50-80% losses and 10% losses had also been reported (Cooke 1982). Bourdoux et al. (1982) found slight heating of cassava (60°C) produced loss of water with concomitant increase in the HCN content. In contrast increasing the temperature above/beyond 60°C reduces the HCN content. The cyanogenic glucosides level decreased up to 88% during the fermentation process while acetone cyanohydrin was retained in the cassava. The pre-fermentation processing that involved crushing, sun drying and milling the cassava into flour reduced the total cyanogen levels by 40%. The process resulted in considerable reduction in the cyanogenic content of the product (Zvauya and Muzondo 1995). The initial total cyanogens of cassava leaves before cooking ranged from 35.9 ± 0.4 to 107.5 ± 0.8 mg HCN (hydrogen cyanide) equivalent kg\(^{-1}\) dry weight. After cooking, the residual cyanogens were significantly reduced ranging from 0.30 ± 0.04 to 1.9 ± 0.2 mg HCN equivalent kg\(^{-1}\) dry weight (Ngudi et al. 2003). Feriera et al. (1995) found that boiling bamboo shoots for 20 minutes at 98°C removed nearly 70% of the HCN and even highest HCN content of bamboo shoots (800 mg/100gm) would be detoxified by cooking for 2 hours. Mpondu, a simple method which includes blanching (10 min), mashing and then boiling for 20-80 min and these processes enhance the detoxification of the cassava leaves, with blanching alone resulting in the loss of 57% of the free (non-glycosidic) cyanide content and of 60% of the bound (glycosidic) cyanide. It is presumed that losses of cyanide during these processes would be accounted for volatile HCN, its derivatives and in the boiling water (Maduagwu and Umoh 1982). The probable process of the rate of loss of free cyanide was described by Cooke and Maduagwu (1978) are as follows

i) loss of volatile hydrogen cyanide
ii) loss and degradation of the less volatile cyanohydrin and
iii) the production by endogenous linamarase of cyanohydrins during the initial stages of drying.

Therefore the decrease in cyanogenic glucosides content in the cooked plants that was observed might be due to hydrolysis of cyanogenic glucosides into HCN and their respective aldehyde or ketone and the evaporation of the produced HCN during cooking.

**Glucosinolates content**

Available literature regarding the glucosinolates content of cabbage, cauliflower, mustard and radish reports a marked variation in their content (Gmelin and Virtanen 1960; Mithen et al. 1987; Slominski and Campbell 1989; Tookey et al. 1980; Sones et al. 1984). Newkirk et al. (1997) reported *Brassica juncea* meals contained more glucosinolates than *B. napus* and *B. rapa*. Again the literatures on the glucosinolates content of these vegetables of Indian origin are not adequate. There is considerable variation in the glucosinolates composition of cruciferous vegetables as shown by the range of values provided by the individual studies. These variations represent true variation due to the measurement of different cultivars of particular vegetables and different growing conditions such as soil, climate and cultivation practices but it may also represent some inter-laboratory variation in methodology. In the present study glucosinolates content was found highest with cauliflower followed by cabbage, mustard and radish.

Processing of Brassica vegetables in domestic food preparation or industrial processing will influence levels of glucosinolates considerably and thus affect their health-protective capacity (Verkerk et al. 2001). Glucosinolates content was also measured in cooked condition as these plant foods are consumed mostly after cooking. In cooked food, concentration of glucosinolates was highest with cauliflower followed by cabbage, mustard and radish. There was reduction in the glucosinolates content after cooking.

Virtanen (1965) demonstrated that following rupture of the cells of plant material glucosinolates is hydrolysed by the endogenous enzyme myrosinase. On myrosinase hydrolysis most glucosinolates form stable isothiocyanates or nitriles as well as glucose and HSO\(_4\) ion (Tookey et al. 1980). Glucosinolates remain intact unless brought into contact with the enzyme myrosinase by pests, food processing or chewing.
Glucosinolates can be gained or lost by vegetables during storage. They may be degraded or leached during processing, or preserved by thermal inactivation of myrosinase (Johnson 2002). Following tissue damage, myrosinase enzymes catalyse the decomposition of glucosinolates to a variety of volatile and non-volatile products (Raybould and Moyes 2001).

Glucosinolates occur with a wide biological variation, both quantitatively and qualitatively. Occurrence and concentrations vary according to species and cultivar, tissue type, physiological age, plant health, environmental factors (agronomic practice, climatic conditions), insect attack, and microorganism intrusion (Rosa et al. 1997; Fenwick et al. 1983). Glucosinolates profiles in vegetative tissues are very different to profiles in flowers and seeds, where the total amount can be 10 times higher and account for up to 10% of the dry matter (Mithen et al. 2000). Processes such as chopping, cooking and fermentation cause cell damage, glucosinolates hydrolysis and the formation of a variety of hydrolysis products. Thus, cooking caused losses of between 30–60%, whereas blanching led to only a minor decrease in total glucosinolates content, indicating that leaching into cooking liquor rather than thermal degradation is the major reason for glucosinolates loss during cooking (Van Etten et al. 1974). The effect of conventional cooking conditions, however, varies widely and does not necessarily cause complete myrosinase inactivation (Wang et al. 1994). Getahun and Chung (1999) reported deactivation of myrosinase enzyme after cooking water cress. Similar type of inhibition in myrosinase activity was reported by Mc Millan et al. (1986) in cooked brussel sprouts. Homogenates of boiled brussel sprouts were devoid of myrosinase activity also reported by Shapiro et al. (2001). Changes in myrosinase activity are likely to be the cause of the apparent differences in the profile of hydrolysis products after processing, and might direct the hydrolysis towards isothiocyanate or nitrile formation (Kushad et al. 1999).

Total and individual glucosinolates levels showed very high significant differences between the aerial and root of cabbage. Despite the constant temperature, light and relative humidity, glucosinolates varied within a 24-h period (Rosa and Rodrigues 1998). Limited breakdown of aliphatic glucosinolates in cabbage was found, whereas unexpected increased levels of indolyl glucosinolates were detected after chopping and storage of cabbage and broccoli under ambient conditions (Verkerk et al. 2001). Jensen (1999) reported that heating the rapeseed at 107°C for 25 minutes reduced the level of intact glucosinolates one-third. Another report by Jensen et al. (1995) showed after
heating at 100°C for 15 minutes, 30 minutes, 60 minutes and 120 minutes, the total content of glucosinolates decreased 24%, 46%, 70% and 95% respectively. Slominski and Campbell (1989) further showed that heat treatment resulted in substantial decomposition of glucosinolates with thiocyanate ion and nitriles accounting for 50% and 30%, respectively of the degraded glucosinolates in cabbage, cauliflower, broccoli and brussel sprouts. de Groot et al. (1991) found that boiling the brussel sprouts in water reduced the level of total glucosinolates up to 40% with considerable difference in reduction of the various glucosinolates but the level of intact glucosinolates in the diet with the cooked vegetable were considerably higher than in those with the uncooked (but incubated) vegetable that had been submitted to extensive enzymatic degradation. During heating the glucosinolates are degraded and partially vaporized as pungent flavour and partially converted to oxazolidine, isothiocyanates and nitriles (Fenwick et al. 1983). Srisangnam et al. (1980) showed that glucosinolates are susceptible to thermal degradation and degradation causes the production of break down products similar to those observed in the autolysis but with a relatively high level of the nitriles (Slominski and Campbell 1989). But on the contrary Stoewsand (1995) reported that nitriles are more likely to be formed in fresh tissues than after heating.

Glucosinolates and their breakdown products are water soluble compounds and it has been suggested that loss of glucosinolates during cooking is due to leaching into the cooking water (De Vos and Blijleven 1988; Verkerk et al. 1997), although at least some of the loss of glucosinolates is due to degradation (Heaney et al. 1985). It has been shown that the level of leaching into the cooking water is more strongly related to the amount of cooking water used rather than the cooking time or method (Dekker et al. 2000).

Therefore the reduction in the glucosinolates content in cooked plant might be due to the inhibition of myrosinase activity during cooking or might be degradation of glucosinolates into thiocyanate, isothiocyanates, nitrile, oxazolidine etc. which are considered to be more harmful than the intact glucosinolates.

**Thiocyanate content**

Thiocyanate content of cabbage, cauliflower, mustard seeds and radish were measured by earlier workers (Rao 1995; Basu et al. 1986; Gmelin and Virtanen 1960, Michajlovskij and Langer 1959). Available data showed marked variation in thiocyanate
content in same plant that might be due to differences in genetic background, ecological factors and also for the presentation of data.

Thiocyanate content of cabbage, cauliflower, mustard and radish of Indian origin were measured both in the fresh / uncooked and cooked plants because these plants are often consumed after cooking. The study showed thiocyanate content was highest in cooked mustard followed by radish, cabbage and cauliflower as compared to their uncooked counterparts indicating that after cooking thiocyanate content was almost same or slightly increased.

Cyanogenic glucosides are detoxified to thiocyanate, moreover glucosinolates release thiocyanate upon hydrolysis (Hershman et al. 1985) and so thiocyanate is largely of exogenous origin (Dahlberg et al. 1984). Moreover isothiocyanates degradation product of glucosinolates and cyanogenic glucosides act on the thyroid mainly by their rapid conversion to thiocyanate (Gaitan 1989a). The chief cause of thiocyanate content in the organism is the thiocyanate already present in foods (Michajlovskij and Langer 1958).

The literature on the effect of heat treatment on the cyanogenic plant foods on thiocyanate content is very scanty. The present study showed that after cooking the plants there was a slight increase in the thiocyanate content. This observation might be due to the conversion of other goitrogenic / anti-thyroid precursor of the plants (viz. cyanogenic glucosides, glucosinolates) to thiocyanate to a considerable amount during heating and due to hydrolysis of those precursors.

A systemic quantification of different goitrogenic / anti-thyroid components of the commonly consumed India foods of cyanogenic origin were made in the present study. Among all the Brassica vegetables, cabbage and cauliflower were rich in glucosinolates; mustard and radish were rich in thiocyanate while cyanogenic glucosides were present in all the studied plants. The goitrogenic or anti-thyroid potential of a plant not only depends on the relative concentrations of these constituents found in fresh plants but also on the processing as foods. Therefore in this study goitrogenic / anti-thyroid constituents were also measured in cooked plants that showed a reduction in the content of cyanogenic glucosides and glucosinolates although thiocyanate almost same or slightly elevated as compared to their uncooked / fresh counterparts.