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THE EFFECTS OF PREPARATION FORM AND COOKING TIME ON THE THIOCYANATE ION CONTENT IN CABBAGE

by

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ABSTRACT

The effects of certain food preparation techniques on the content of one of the goitrogens in cabbage, thiocyanate ion(SCN) were examined. Thiocyanate ion or free SCN is released from indole glucosinolates as a result of enzymatic hydrolysis by myrosinase or by thermal degradation. Free SCN was measured spectrophotometrically following reaction with ferric nitrate and then with mercuric chloride; addition of myrosinase to the assay medium to permit hydrolysis of intact glucosinolates to free SCN allowed measurement of free plus glucoside-bound SCN, which represented total SCN. The arrangement of treatments in a split plot design with four replications permitted a comparison of SCN content in two preparation forms, wedge and shred, and four cooking times, 0, 3, 9 and 27 minutes boiling. Two additional treatments permitted determination of the effect of shredding raw cabbage and the effect of initial water temperature, cold and boiling start in wedges cooked 9 minutes. Raw cabbage and cabbage cooked 3 minutes were assayed for myrosinase activity. Also, a model system was developed using an extract of raw cabbage to isolate thermal degradation of the glucosinolates, independent of enzymatic effects. Myrosinase activity was present in raw cabbage but absent in cooked cabbage. Shredding raw cabbage significantly increased free SCN content from 3.5% of total SCN in the wedge to 12.4% in the shred, but appeared to have no effect on total SCN. Cooking significantly increased the free SCN content and decreased the total SCN content in the combined system of cabbage plus cooking water. The rate of increase in free SCN in the cabbage system was similar to the rate of increase in free SCN in the model system and therefore could be accounted for by thermal degradation of the glucosinolates. The decrease in total SCN in the cabbage system indicating degradation of the glucosinolates by pathways other than those yielding SCN, however was greater than the slight decrease in total SCN in the model system. Considering the cabbage solids alone, cooking led to a significant increase in free SCN over time, but the rate of increase in free SCN was much greater in the shred than the wedge. Cooking significantly decreased the total SCN in the cabbage solids alone and by 9 minutes only 62.2% of initial total SCN in raw cabbage remained in the wedge while only 44.4% of initial total SCN remained in the shred. However, by 27 minutes, the total SCN content in the shred was increased compared to 9 minutes while the total SCN content in the wedge decreased. Initial water temperature had no major effects on free or total SCN. Under the cooking conditions in the present study, it was predicted that the minimum potential SCN intake would occur following consumption of wedges cooked for 3 or 9 minutes.

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INTRODUCTION

Although iodine deficiency is recognized as being the principal etiological factor in endemic goiter (Stanbury, 1973) other factors must be involved since goiter has been found in regions where there are adequate or excessive iodine intakes. The original report from Nutrition Canada (Department of National Health and Welfare, 1973) indicated that moderate enlargement of the thyroid gland appeared to be a significant problem in certain parts of Canada, although it did not appear to be due to iodine deficiency since incidence of goiter was not correlated with urinary excretion of iodine. A recent report however showed that the original 1973 report was incorrect and that the prevalence of goiter was actually much lower, especially in the regions originally reported as having the high incidences (Murray, 1977).

Dietary goitrogens have been investigated as alternative or supplementary factors to iodine deficiency in no less than fifteen countries but their role has not yet been conclusively established (Delange and Ermans, 1976). Some of the dietary goitrogens under investigation are present in plants belonging to the Brassica genus which includes rapeseed and cabbage. Concern in Canada has been raised over the effect on human thyroid function of increasing usage of rapeseed meal as animal feeds and of considering rapeseed protein concentrate as a potential food for humans. Consequently, it would be useful to be able to calculate the potential intake of goitrogens from all Brassica plants commonly consumed by man; one such report is to appear in the literature (Mullin and Sahasrabudhe, in press).

The goitrogenic properties of the Brassica plants are due to the presence of glucosinolates which are precursors of various toxic and goitrogenic compounds. One goitrogen derived from three of the glucosinolates *in vitro* is the thiocyanate ion (SCN), whose goitrogenic nature has been well documented in both man and animals.

The SCN content of raw Brassica vegetables has been measured by a number of researchers, but there is conflicting evidence as to its response to cooking. Since most Brassica vegetables are consumed in the cooked form, estimation of the dietary intake of SCN and other goitrogens is therefore difficult.

The objectives of the present study were to clarify the effects of specific treatments on the SCN content and form (free vs. total) in cabbage and to determine the role of enzymatic and thermal effects in the changes in SCN during preparation for cooking and during cooking. The specific treatments included:

(1) effect of shredding raw cabbage

(2) effect of preparation form during cooking: wedge and shred

(3) effect of length of cooking time: underdone, standard tender crisp and overdone

(4) effect of initial water temperature: cold water start and boiling water start

REVIEW OF LITERATURE

Thiocyanate

A. Sources

Thiocyanate ion (SCN) has been known to be a component of human tissues for over one hundred years. However, its presence in the tissues is considered to be accidental rather than functional (Wood, 1975). It is derived primarily from food sources (Langer and Michajlovskij, 1958) and *in vivo* as a product of various detoxifications; apparently there is also some endogenous synthesis since SCN production has been observed in the absence of exogenous precursors (Wood, 1975).

Data on the SCN content of foods was compiled by Wood (1975). Foods high in SCN were primarily Brassica plants, eg. cabbage, cauliflower and related plants including radish, horseradish, and various mustards. In such plants, the SCN content ranged from 0.1-20 mg/100 g. The content in milk ranged from 0.1-1.0 mg/100 g and such a variation was presumably due to the range in SCN provided by the cows' diet and subsequent transfer to the milk. Foods such as meat, rice and non-Brassica vegetables had a considerably lower content, less than .1 mg/100 g. One study from 1934 found beets to have a high content (10 mg/100 g) but a later study (1961) found no effect on serum SCN following feeding beets to rabbits, while feeding Brassica vegetables did result in an increase. Another study from 1955 considered beer, coffee and tea to be "high" although this has not been confirmed.

i. Glucosinolates

Langer and Michajlovskij(1958) believed that the SCN which appeared in the serum and urine of animals or man fed Brassica vegetables was derived from preformed SCN in the vegetables. Gmelin and Virtanen (1960) however showed that this was incorrect and SCN did not exist in free form in intact plants but rather was a component of a group of compounds known as thioglucosides or glucosinolates.

Over seventy different glucosinolates have been identified in a number of similar botanical families, including the Cruciferae to which the Brassica belong (Wood, 1975; Benn, 1977). Glucosinolates function as precursors of flavour compounds and some may also serve as precursors of insect attractants (Benn, 1977), insecticides (Lichenstein et al., 1964) and plant growth hormones (Kutacek and Kefeli, 1968).

The general structure of the glucosinolates is given in Figure 1 and Table 1 gives the specific R groups involved in some of the more common glucosinolates and the plants in which they are found.

According to Benn (1977), "normal" catabolism of the glucosinolates involves two pathways which are shown in Figure 1. The first step is enzymatic, involving a thioglucosidase commonly known as myrosinase. However, myrosinase is located in idioblasts within the plant cells (Sharma, 1971) and therefore contact between the glucosinolates and myrosinase occurs only after damage to the plant tissue. The second step is nonenzymatic, in which the reaction products depend on the pH of the immediate environment; <u>in vitro</u>, isothiocyanate (ITC) formation occurs at pH 6-7 while a low pH leads to nitrile formation.

The "abnormal" catabolic pathways for certain glucosinolates

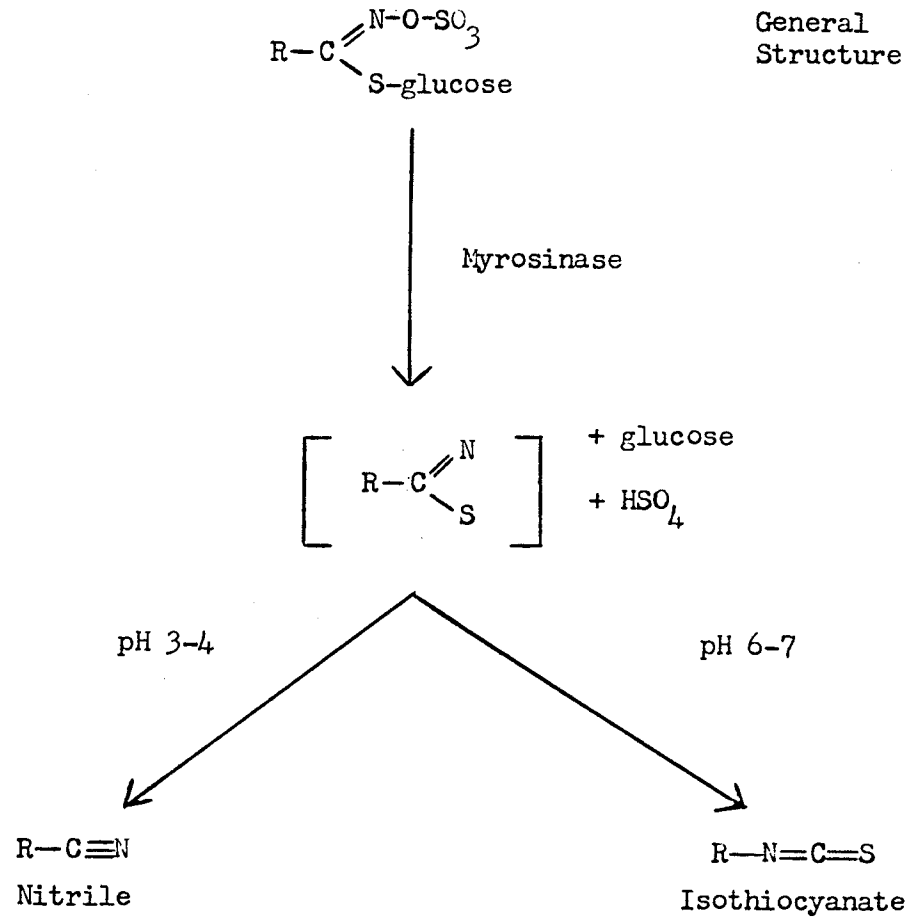
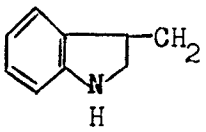
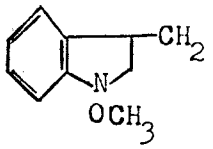


Figure 1. General structure of the glucosinolates and normal catabolism according to Benn(1977)

TABLE 1

SPECIFIC R GROUPS OF SOME COMMON GLUCOSINOLATES AND THE CRUCIFEROUS PLANTS IN WHICH THEY ARE FOUND[1]

Glucosinolate (common name)	R group[2]	Food source
sinigrin	allyl $CH_2=CH-CH_2$	Brassica oleracea species[3], horseradish mustard, rapeseed, crambe seed
progoitrin	2-OH-butenyl $CH_2=CH-CHOH-CH_2$	Brassica oleracea species, turnip, rutabaga, rapeseed
sinalbin	p-OH-benzyl 	mustard, charlock, rapeseed
glucobrassicin	3-indolyl methyl 	Brassica oleracea species, rutabaga, radish
neoglucobrassicin	3-N-methoxy-3-indolyl methyl 	Brassica oleracea species, rutabaga

[1]adapted from VanEtten and Wolff(1973)

[2]R group in Figure 1

[3]includes cabbage, kale, brussels sprouts, cauliflower, broccoli, kohlrabi

lead to formation of organic thiocyanates and cyanoepithiobutanes for which additional enzymes are apparently required (Benn, 1977). If fresh plant tissue is allowed to autolyze at its unadjusted pH (5.6-6.3), nitrile and cyanoepithiobutane rather than ITC formation is observed (VanEtten and Daxenbichler, 1971; Tookey, 1973; Daxenbichler et al., 1977). This was observed in cabbage, crambe seed and rapeseed and was also postulated to be occurring in brussels sprouts (Mullin and Sahasrabudhe, 1978).

Thiocyanate ion formation occurs when the ITC formed is unstable and breaks down spontaneously. Only three glucosinolates present in cruciferous plants form SCN in vitro: sinalbin and two indole glucosinolates, glucobrassicin and neoglucobrassicin (Table 1). Only the two indole glucosinolates were of interest in the present study since sinalbin is not found in Brassica vegetables commonly consumed by man, although it is found in rapeseed.

The environmental conditions for ITC and nitrile formation from the indole glucosinolates differ slightly from the general scheme in Figure 1 and are shown in Figure 2. At pH 7, with myrosinase hydrolysis, glucobrassicin splits quantitatively into SCN, but SCN formation also occurs to some extent under all conditions of hydrolysis including pH 3-4 (Gmelin and Virtanen, 1961). Nitrile formation can occur up to pH 5.2 in vitro (Schraudolf and Weber, 1969) and it has been postulated that nitrile formation may occur at higher pH's in plant tissue in the presence of Fe^{+2} or ascorbate (Mahadevan and Stowe, 1972);

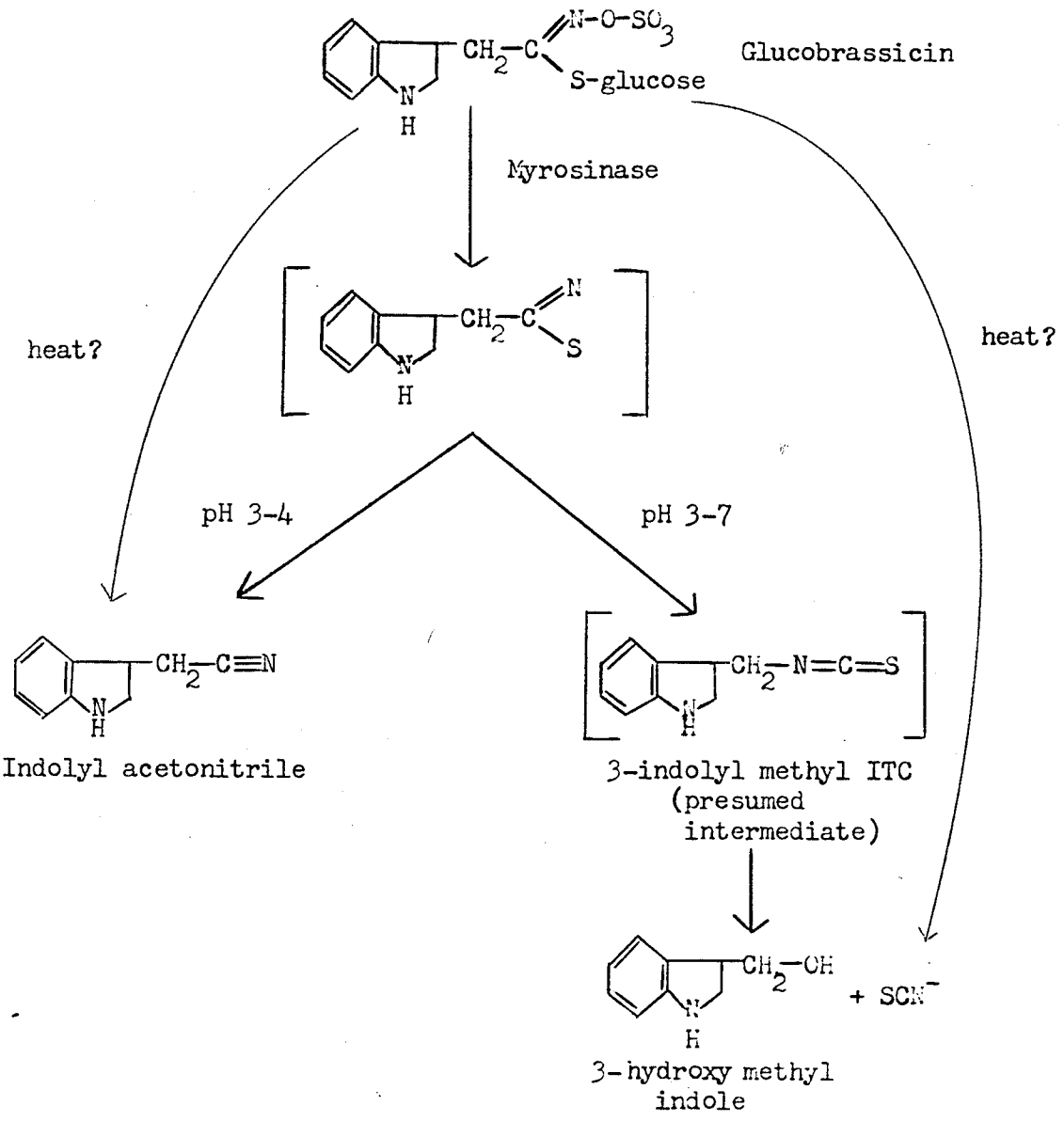


Figure 2. Degradation of glucobrassicin to SCN and nitrile

ii. *In vivo* formation

Besides the three glucosinolates which yield SCN *in vitro*, additional glucosinolates or derivatives yield SCN following metabolism in the animal body. Srivastava and Hill (1975) found that feeding pure sinigrin (Table 1) to rats resulted in an increased urinary excretion of SCN. Similarly Langer (1964a) found that allyl ITC which is derived from sinigrin resulted in increased serum levels of SCN following feeding to rats. This effect was contrary to an earlier report by Jirousek (1956) who found that neither allyl nor phenyl ITC were thiocyanogenetic since no increases in urinary excretion of SCN were found after feeding the compounds to rats. However Jirousek (1956) used a study period of only four days, while Langer (1964a) used a study period of twenty to sixty days.

The finding that sinigrin was metabolized to SCN was surprising since *in vitro* it is not hydrolyzed to SCN (Srivastava and Hill, 1975). In contrast glucobrassicin which is hydrolyzed to SCN *in vitro* was found to have virtually no effect on serum SCN levels in rats unless myrosinase was fed along with the glucobrassicin (Michajlovskij and Langer, 1967).

Various thiocyanate esters are converted to SCN *in vivo* since they are acted on by transferase enzymes to yield cyanide (Wood, 1975). Similarly, alkyl and arylalkyl nitriles are converted to cyanide by an oxidation and enzymatic process (Wood, 1975). Jirousek (1956) found that indole acetonitrile, which is derived from glucobrassicin *in vitro*, resulted in elevated serum SCN levels when fed to rats. Thiocyanate is a detoxification product from cyanide, and therefore compounds which are metabolized to cyanide eventually yield SCN (Jirousek, 1956).

Thiocyanate may also be derived from non-glucosinolate sources, which also yield cyanide. Sources of cyanide include cyanogenic glycosides which are also natural components of some foods such as cassava, lima beans, kernels from fruits including apples and apricots, and almond kernels (Conn, 1973). An additional source of cyanide is tobacco smoke since it contains various nitriles; the higher serum SCN levels of smokers compared to nonsmokers has been well documented (Dastur et al., 1972; Wood, 1975).

B. Metabolic Action

Astwood (1943), Salter et al. (1945) and McGinty (1949) showed that SCN was goitrogenic in rats only when the iodine level in the diet was low and this property is generally accepted to be true for humans. This has been explained by the finding that SCN inhibited the uptake of iodine by the thyroid (Franklin et al., 1944). Later, Greer et al. (1966) showed that at slightly greater concentrations than those which inhibited iodine transport, organification of iodide that entered the thyroid was also inhibited. Unlike the inhibition of iodide uptake, the inhibition of organification of iodide could not be overcome by the addition of iodide.

Thiocyanate is metabolized by the thyroid (Maloof and Soodak, 1964) and therefore it has been proposed that SCN is a competitive substrate for the thyroidal iodide peroxidase thus explaining both its metabolism by the thyroid as well as its inhibition of organification of iodide (Werner and Ingbar, 1971). However the mechanism of action is still uncertain (Wood, 1975).

i. Studies in Man

The adverse effects of SCN on human thyroid function were first

reported as toxic side effects during the use of SCN as an antihypertensive agent. Barker (1936) reported myxedematous effects and thyroid swellings in 3 of 45 patients but initially questioned whether the effects were due to the SCN therapy. Wald et al. (1939) reported thyroid enlargements in 11 of 246 patients and Blackburn et al. (1951) reported myxedematous effects or thyroid enlargements in 17 patients. Additional case studies have been reported by Fahlund (1942), Foulger and Rose (1943), Rawson et al. (1943) and Estes and Keith (1946).

Generally the oral doses of SCN associated with thyroid dysfunction ranged from 150-450 mg daily for periods of nine days to five years; the average length in the cases reported by Blackburn et al. (1951) was 31 months. Serum SCN levels were found to range from 4.7 to 17.1 mg/100 ml, while normal serum levels in nonsmokers have been found to reach 1.5 mg/100 ml (Mitchell and O'Rourke, 1960). The thyroid swellings subsided following withdrawal of SCN or administration of dessicated thyroid and iodide.

Noting these effects, Mitchell and O'Rourke (1960) and Reinwein and Irmscher (1965) studied the response of the thyroid gland to SCN. Mitchell and O'Rourke (1960) administered 200-800 mg SCN orally to fifteen euthyroid subjects to maintain serum levels of at least 6 mg/100 ml. An inverse relation between serum SCN and radioiodine uptake uptake was found, but complete inhibition of thyroid function did not occur with serum levels of less than 5 mg/100 ml.

Reinwein and Irmscher (1965) also found an inverse relation between serum SCN and radioiodine uptake in 39 euthyroid subjects given varying amounts of SCN. Seven subjects had serum SCN levels greater than 7.2 mg/100 ml, and only 3 of them showed a demonstrable uptake. They

also found that as SCN levels increased, the plasma inorganic iodide levels were increased, such that the absolute iodine intake was actually stimulated at low SCN levels and then inhibited at higher concentrations of approximately 7 mg SCN/100 ml. Contrary to Mitchell and O'Rourke (1960), Reinwein and Irmscher (1965) did not find complete inhibition of thyroid function even when serum SCN levels reached 12.6 mg/100 ml.

Mitchell and O'Rourke (1960) found that following a single oral dose of 1.6 g SCN, the serum level diminished slowly in the ensuing days to weeks. Similarly, Langer (1964b) found that even with a smaller dose of 50 mg serum SCN levels remained elevated for 7 days. Langer (1964b) also showed that while a single dose of 2 mg SCN did not cause a demonstrable increase in serum SCN, if 2 mg was given daily for a period of two weeks, there was a progressive increase in serum SCN from .5 to 1.0 mg/100 ml.

Thiocyanate as a Component of Brassica Vegetables

A. Interactions with Other Goitrogens

Thiocyanate is not the only goitrogen derived from glucosinolates. Progoitrin (Table 1) and related glucosinolates yield unstable isothiocyanates which spontaneously cyclize to form goitrin and related compounds (Greer, 1960). The goitrogenic characteristics of progoitrin and goitrin have been extensively studied by Greer and Deeney (1959), Greer (1964) and Langer et al. (1971). While isothiocyanates, thiocyanate esters and nitriles may also be goitrogenic, this may be due to their conversion to SCN (Van Etten and Wolff, 1973; Wood, 1975), although Langer and Greer (1966) demonstrated antithyroid properties of three

isothiocyanates *in vitro* which were not due to conversion to SCN. Isothiocyanates may also react with free amino acids to form thiourea-type compounds which have been shown to possess goitrogenic properties in the rat (Langer et al., 1964).

Langer reported a series of studies investigating the interactions among the three main goitrogens in cabbage: SCN, allyl ITC and goitrin. Langer (1964c) reported that if rats were fed 1 mg SCN or 100 mg L-5,5' - dimethyl-2-thiooxazolidone (MTO, a compound similar to goitrin) individually for 20 days, or 2 mg allyl ITC for 50 days, no significant effects on thyroid weight were found. However, when the rats were fed a mixture of 0.5 mg SCN, 0.5 mg allyl ITC and 100 μg MTO for 20 days, a significant increase in thyroid weight was found. Langer (1964c) concluded that the effect of the mixture was remarkable and that it was "admissible to assume that they act as synergists."

Later studies were unable to confirm the synergism, but did find similar tendencies: combinations of the compounds led to more significant effects than did the compounds individually (Langer, 1966a) and addition of allyl ITC or goitrin to rats receiving SCN significantly increased the goitrogenic effects (Langer, 1966b).

Langer however found inconsistencies in the effects of SCN and other goitrogens in cabbage in various studies (Langer, 1964c; Langer and Stolc, 1964; Langer and Stolc, 1965). Langer (1964c) reported that of the three goitrogens, SCN, allyl ITC and MTO, only SCN produced hypertrophy of the thyroid when fed at levels similar to those in cabbage, while effective doses of allyl ITC and MTO were much greater than the concentrations in cabbage. However, Langer and Stolc (1964) found that SCN had only a weak effect when administered at levels twice that found in cabbage.

Subsequently, Langer and Stolc (1965) reported a study which indicated that allyl ITC could account, to a great extent, for the goitrogenic activity of cabbage.

Since the literature is unclear, definite conclusions about the role of SCN in the goitrogenic activity of cabbage cannot be made. However, Michajlowskij and Langer (1967) stated that the 1964 study by Langer and Stolc indicated that SCN accounted for 25-50% of the goitrogenic activity of cabbage. Based on the various studies, these percentage values appear to be a reasonable compromise.

B. Brassica Vegetables and Thyroid Function in Man

The role of Brassica vegetables in endemic goiter has never been conclusively established (Delange and Ermans, 1976). One reason for this is that the levels of SCN and other goitrogens are considerably lower than the levels of the goitrogens shown to produce significant antithyroid effects in clinical trials. The clinical trials have shown that 150-450 mg SCN daily (see Part B **i.** Studies in Man, above) or a single dose of 25 mg goitrin (Langer et al., 1971) were the minimum levels required, while 100 g raw cabbage might contain 0.7-1.0 mg SCN and 13 μg goitrin both in precursor form (VanEtten and Wolff, 1973) as well as 0.4-20 mg allyl ITC in precursor form (Langer, 1964c).

i. Experimental Studies

Greer and Astwood (1948) however demonstrated the antithyroid effects of large quantities of specific foods ingested at a single sitting. An antithyroid effect was reflected in a decreased rate of radioiodine uptake after ingestion of the material, compared to a control period. They found that 363 g raw rutabaga, 280 g raw pureed rutabaga and an extract of rutabaga equivalent to 2617 g all depressed

radioiodine uptake. Results with other foods were inconsistent; for example, 412 g cabbage had no effect in one subject, while a slightly smaller quantity (380 g) showed definite effects in another subject.

A similar study was conducted by Langer and Kutka (1964). Nine euthyroid subjects consumed 500-600 g cabbage (raw or cooked) daily for two weeks on a low iodine diet, while six controls remained on a low iodine diet. The uptake curve of radioiodine was measured before and after the two week period in all subjects. The mean rate coefficient of the iodine uptake curve was significantly ($p < .02$) lower in the subjects following cabbage consumption, while there was essentially no change in the control subjects; in 6 trials with the experimental subjects, the decline in the rate coefficient was considered very striking, 40-80% of their initial rate. The percentage of radioiodine in the thyroid was also significantly ($p < .01$) lower in the experimental group.

Langer and Kutka (1964) concluded that these results could probably be taken as proof of the antithyroid action of cabbage in man. They noted that while the average effect on the thyroid was not unduly impressive, the technique that they used was relatively unrefined. Unfortunately, serum levels of SCN, allyl ITC or goitrin were not measured, so whether an individual goitrogen or an interaction among several was responsible is not known.

ii. Endemic and Sporadic Goiter

Suk (1931) attempted to relate the difference in prevalences of goiter between two groups of people in Carpathia to the difference in consumption of cabbage since cabbage was a major dietary component of the people with goiter. However cabbage consumption was not the only difference between the two groups and the conclusions reached by

Suk were based solely on personal observations. More recent studies on this region and surrounding regions have failed to relate endemic goiter conclusively to consumption of Brassica plants although serum SCN levels and urinary excretion of SCN in humans were found to correlate with the incidence of goiter (Silink and Marsikova, 1951; Langer, 1964b).

Similarly, Abbott (1932) attempted to explain the differences in prevalence of goiter among school children in Manitoba to racial origin and diet. It was observed that incidence of goiter was highest among children of Central European nationalities and that cabbage was a major constituent of their diet. Abbott (1932) admitted that the evidence regarding cabbage consumption and incidence of goiter was only suggestive and not conclusive since many other variables were also involved.

Bastenie (1947) reported on diseases of the thyroid gland during occupied Belgium from 1940-44 when consumption of kohlrabi and cabbage markedly increased. Incidence of simple goiter among patients at two medical clinics increased significantly: the incidence prior to 1940 was 16%, and increased to 26% from 1941-1944 reaching a peak of 40.5% in 1942. Greer (1960) noted however that this increase may not have been related to the increase in consumption of goitrogenic foods, but may have been merely coincidental.

Similarly, Means (1947) referred to a report of an "epidemic" of goiter among monks in a monastery in Belgium during the same period when the only food for a considerable time was rapeseed and cabbage. According to Means (1947), the iodine content in the drinking water was adequate for normal purposes and therefore the goiter was

attributed to the consumption of these foods.

Sporadic goiter due to consumption of Brassica plants has been reported by Rawson (cited in Means, 1947) and Fisher et al. (1952). Rawson reported a woman who developed goiter which was apparently due to high consumption of cabbage; when the cabbage was removed from her diet, the goiter subsided and subsequently recurred following re-introduction of the cabbage. Fisher et al. (1952) reported a woman with goiter whose diet included rutabaga twice a day, cabbage three times a week and turnip four or five times a week. Similarly to the woman reported by Rawson, the goiter subsided following removal of the vegetables from the diet.

These studies show the limited evidence relating the role of the consumption of Brassica vegetables to endemic and sporadic goiter. There is however, continued theoretical interest since iodine deficiency is inadequate to explain the pattern of goiter in some endemic areas or the incidence in non-endemic areas (Greer, 1960). It is apparent from the studies reported that the evidence is inconclusive, and more thorough examination of the patterns of goiter is required to establish whether or not Brassica vegetables really do play a role.

Effects of Cooking on SCN in Brassica Vegetables

A. Studies in vitro

At least four different factors which could affect the content of the various goitrogens during cooking have been identified including: enzymatic hydrolysis of glucosinolates, thermal degradation of glucosinolates, disintegration of the goitrogens after their release from the glucosinolates and volatization of the goitrogens (Michajlovskij et al., 1970). The latter two do not apply to (neo)glucobrassicin or SCN since

SCN is not volatile and since both Michajlovskij et al. (1970) and Kozlowska (1971) found total thermoresistance of SCN after boiling pure SCN in aqueous solutions for 30 and 90 minutes respectively.

Myrosinase activity would be expected during preparation for cooking, eg. peeling or shredding, and possibly in the early stages of cooking. Bjorkman and Lonnerdal (1975) found that myrosinases from rapeseed and white mustard seed increased in activity up to 60 C and decreased at higher temperatures with inactivation at approximately 80 C. Eapen et al. (1968) found that inactivation of the myrosinase in rapeseed meal occurred following immersion in boiling water for one to one and one half minutes. In cabbage prepared as thin wedges or shred, inactivation of enzymes would also be rapid since the recommended blanching time is one and one half minutes (Anon., 1971).

Thermal degradation of glucobrassicin was demonstrated by Gmelin and Virtanen (1961). They heated pure glucobrassicin in water for up to three hours and found formation of SCN but only 50% recovery of the glucobrassicin which indicated that glucobrassicin was breaking down by pathways other than those which yielded SCN. One of the pathways results in indole acetonitrile formation (Figure 2) and a considerable amount of this compound was produced during the heating of glucobrassicin. Thermal degradation of neoglucobrassicin to its corresponding nitrile was also demonstrated by Gmelin and Virtanen (1962). However, details as to quantities of the nitriles produced were not provided and therefore it is not clear whether the production of the nitriles accounted for all the decrease in (neo)glucobrassicin levels during heating or whether

additional degradation pathways were also involved. It can also be noted that indole acetonitrile can further be converted to indolylacetamide, indolyl acetic acid, indolyl aldehyde, indolyl carboxylic acid and indole (Gmelin and Virtanen, 1961).

Michajlovskij et al. (1970) found thermal degradation of glucobrassicin within thirty minutes of boiling which resulted in production of SCN and recovery of only 88.7% of initial glucobrassicin. Nitrile production was not monitored however, and whether the less than 100% recovery was correct may be doubted since replications with another goitrogen in a similar system yielded percentage recoveries of 86.0-97.3%.

B. Studies with Brassica Vegetables

i. Effect of Cooking on the Goitrogenic Properties of Brassica Vegetables

Soon after the initial report by Chesney et al. (1928) on the goitrogenic effects of raw cabbage in rabbits, investigations as to the goitrogenic activity of cooked Brassica vegetables in rabbits were conducted; at this time the compounds responsible for the goitrogenic effects were not known. Marine et al. (1929) found that boiling or steaming various Brassica vegetables increased their antithyroid properties. They also found that hashed raw cabbage was not always goitrogenic although cooked hashed cabbage was. Dried cabbage was found to have no goitrogenic properties (Marine et al., 1930). In contrast, Podoba et al. (1957, cited in Michajlovskij et al., 1970), reported a decrease of goitrogenic activity of steamed vegetables in rabbits, to 50-70% of the activity of the raw vegetables. McCarrison (1931) reported inconsistent effects since in one

study, the goitrogenic activity of cabbage was increased by steaming but in another study the activity was decreased.

Greer and Astwood (1948) studied the antithyroid effects of foods in humans. Their original report (Greer and Astwood, 1948) concluded that raw rutabaga had definite antithyroid effects but that cooked rutabaga had no effect; turnip and cabbage had inconsistent effects. Later, Greer and Deeney (1959) modified the conclusion that cooking nullified the antithyroid effects since reexamination of the data showed that both cooked rutabaga and turnip showed antithyroid effects although the response was delayed and had originally been interpreted as an artefact. This reexamination of the data occurred after Greer and Deeney (1959) found that there was a delayed response in the increase of serum goitrin after ingestion of progoitrin in the absence of myrosinase; an analogous situation would occur with cooked vegetables because of the inactivation of the myrosinase during cooking.

ii. Effect of Cooking on the SCN Content in Brassica Vegetables

Relatively few studies have determined the effects of cooking on the SCN content in Brassica vegetables, and the results are in conflict. Differences in cooking procedures could account for some of the variations in the magnitude of the effects, but the reasons for the obviously opposing effects are unclear.

Michajlovskij et al. (1969; 1970) cooked three Brassica vegetables (cabbage, kale and kohlrabi) and measured the effects on free and total SCN. Free SCN referred to SCN in its free, ionic state, while total SCN also included SCN still in precursor form (glucoside-bound), as (neo)glucobrassicin. The vegetables were

cooked for 30 minutes as large pieces started in boiling water, small pieces started in cold water and whole parts or leaves in boiling ethanol. The effects in cabbage are shown in Table 2. They found essentially no change in total SCN content but a considerable increase in free SCN in the samples boiled in water which indicated that the (neo)glucobrassicin was being degraded only by pathways which yielded free SCN. The smaller increase in free SCN in the cabbage boiled in ethanol was credited to the rapid inactivation of myrosinase. Effects with kale and kohlrabi were similar except that the degradation to free SCN in the samples cooked in water was slightly less than the degradation which occurred in cabbage.

The sampling procedure in this study by Michajlovskij et al. (1969; 1970) was not random, but rather it appeared as though the same part of the vegetable was used for the same treatment in each replication. This was done since preliminary studies showed little variation in glucosinolate content within the vegetable. This was however in conflict with other research. Johnston and Jones (1961) noted the importance of sampling technique since they found considerable variation in SCN content in kale. In one variety the SCN content was 107-111 mg/100 g in younger and middle aged leaves, but only 28 mg/100 g in older leaves. MacLeod and MacLeod (1970) found a similar trend in cabbage, with inside, younger leaves having a higher sinigrin content than the outside older leaves.

The sampling technique used by Michajlovskij et al. (1969; 1970) therefore may have biased the results, although the content of goitrogens other than SCN decreased as a result of cooking to a similar degree as in other studies.

TABLE 2

SUMMARY OF EFFECTS OF VARIOUS COOKING TECHNIQUES ON THE SCN CONTENT IN CABBAGE (mg SCN/100 g)

Cooking Technique	SCN Form		Reference
	Free SCN	Total SCN	
raw	–	2.56	
large pieces from boiling start(30 min)	1.39	3.15	Michajlovskij et al(1969; 1970)
small pieces from cold start(30 min)	1.31	2.72	
boiling ethanol(30 min)	0.20	2.65	
raw		2.6	
cooked(30 min)			Kozlowska (1971)
-cabbage		0.7	
-cooking water		1.2	
-total		1.9	
raw		30[1]	
cooked(15-20 min)			Mullin and Sahasrabudhe (1978)
-cabbage		10	
-cooking water		10	
-total		20	

[1] as µg 3-indolyl methyl ITC/g cabbage

Kozlowska (1971) cooked cabbage and broccoli for 30 and 60 minutes, and measured the effect on total SCN content. A whole head of cabbage weighing approximately 1 kg was added to 3 ℓ of cold water and cooking time was measured once the water started to boil. It can be seen in Table 2 that, contrary to the results of Michajlovskij et al.(1969; 1970), only 73% of initial total SCN was recovered in the cabbage plus cooking water after 30 minutes; 60 minutes of cooking resulted in only a slight further decrease, to 1.8 mg SCN. It can also be seen that of the remaining total SCN, only one third remained in the cabbage and the rest was in the cooking water. Similar effects were found with broccoli.

A decrease in total SCN was found in eight of nine Brassica vegetables in a study by Mullin and Sahasrabudhe (1978), supporting the findings of Kozlowska (1971). Vegetables were cooked from a boiling start for 15-20 minutes, 150 g in 400 ml water. The results for cabbage are shown in Table 2. Only 66% of initial total SCN was found in the cabbage plus cooking water, with the SCN evenly distributed between the cabbage and the water. The seven other vegetables had a range of 33-67% of initial total SCN recovered, while cauliflower showed no decrease.

Mullin and Sahasrabudhe (1978) noted that indole isothiocyanates can degrade to products other than SCN as shown by Gmelin and Virtanen (1961) and therefore this could be responsible for the loss in total SCN. Since Kozlowska (1971) also showed a decrease in total SCN as a result of cooking, it seems likely that such an effect might be expected in the present study.

METHODS AND MATERIALS

Sample Description

Twenty-three kg of cabbage (var. Houston Evergreen) were obtained directly from the Vegetable Producers' Marketing Board and stored at 5 C for twelve to twenty-four days prior to cooking. Ten cabbages weighing between 1,150 and 1,600 g were chosen for uniformity in weight and shape and eight of these were randomly chosen for the study. All cooking was initially done within a two day period, but due to accidental thawing of some of the samples from two of the cabbages during freeze-drying ten days later, two cabbages weighing 1,700 - 2,000 g were prepared as replacements.

Experimental Design

Two preparation forms, wedge and shred, and four cooking times, raw or 3, 9, and 27 minutes from boiling start were incorporated into a split plot design with four cabbages as replications within each preparation form (Table 3). Two additional treatments applied were a cold water start for wedges cooked 9 minutes and raw wedges (Table 3); the raw wedges were included in cabbages 5-8 for comparison against raw shred by paired t-test rather than by the split plot F test with the larger between-cabbage error. A total of 40 cabbage samples were prepared.

The order of cooking cabbages 1-8, the segment of the cabbage used for each treatment and the order of cooking the segments within each cabbage were completely randomized (Table 4).

TABLE 3

EXPERIMENTAL DESIGN FOR ASSIGNMENT OF TREATMENTS TO CABBAGES

Preparation Form	Cabbage Number	Cooking Time (min) raw 3 9 27	Cold Start
wedge	1* 1* 2 3 4		
			Raw Wedge
shred	5 6 7 8		

*cabbages prepared as replacements for original cabbages thawed during freeze drying

TABLE 4

RANDOMIZATION OF WEDGES AND CABBAGES FOR COOKING TREATMENTS

Cooking Order of Cabbages	Cabbage Number	Cooking Order of Treatments 1	2	3	4	5
1	4	B_1[1](6)[2]	F (5)	D_1(3)	C_1(4)	A (8)
2	7	C_2(6)	A (1)	B_2(8)	E (4)	D_2(7)
3	3	A (4)	B_1(5)	F (1)	C_1(3)	D_1(6)
4	8	A (3)	C_2(4)	E (7)	D_2(5)	B_2(8)
5	5	C_2(7)	B_2(4)	A (6)	D_2(1)	E (3)
6	6	B_2(2)	D_2(8)	A (6)	E (4)	C_2(7)
7	1	F (6)	C_1(1)	B_1(8)	A (7)	D_1(5)
8	2	B_1(1)	C_1(4)	A (6)	D_1(2)	F (7)

[1]
A = raw wedge
B_1= 3 minutes, wedge B_2= 3 minutes, shred
C_1= 9 minutes, wedge C_2= 9 minutes, shred
D_1= 27 minutes, wedge D_2= 27 minutes, shred
E = raw shred
F = 9 minutes, cold start, wedge

[2] segment number

Free and total SCN were each measured in duplicate in all 40 cabbage samples. Since 28 of the 40 cabbages were cooked, free and total SCN were also measured in the cooking water, without duplications. A total of 216 measurements were made. In addition, for the 28 cooked samples, values for cabbage plus cooking water were obtained by totalling the mean values in the cabbage and values for cooking water for each of free and total SCN.

Two additional studies were conducted: determination of myrosinase activity in raw and cooked cabbage and determination of heat effects in an extract of raw cabbage. Materials for these studies were randomly chosen from the raw samples after all measurements for free and total SCN had been made. One or two replications were used for determination of myrosinase activity and four replications were used to determine heat effects.

Statistical Treatment of SCN Measurements

For measurements in the split plot design for free and total SCN in cabbage and in cabbage plus cooking water, analysis of variance was performed to identify significant sources of variation.

Homogeneity of variance (s^2) of the eight treatments in each analysis of variance was tested by Cochran's method which uses the ratio $\frac{\text{largest } s^2}{\sum s^2}$ (Winer, 1962). If the test was significant ($P < .05$) the outlying value which contributed to the large variance was identified and all values from the same cabbage were removed from the analysis; data from another cabbage in the other preparation form was then randomly removed and analysis of variance was reconducted using only three replications for each treatment. If no changes in the

significance of effects or interaction were found, the original analysis of variance was used and the lack of homogeneity of variance was not considered critical.

If interaction between preparation form and cooking time was significant ($P < .05$), the effect of cooking time was analyzed separately for wedge and shred; if interaction was not significant, the results for wedge and shred were combined at each cooking time and treated as eight replications. The effect of cooking time was analyzed by linear and exponential regressions or by polynomial regression if linear regression was not significant ($P > .05$). All regression analyses were performed using the mean values of the four or eight replicates. If linear regressions were significant for both wedge and shred, the linear trend of the interaction was isolated to determine whether the slopes were different (Anon., 1973). If no regression was significant, multiple comparisons between time treatments were made by t-tests using the mean square error and 18 df from the split plot analysis of variance.

No statistical analyses on effects of preparation form or cooking time were performed for free or total SCN content in the cooking water alone.

For the two treatments not included in the split plot design, paired t-tests with 3 df were performed to determine significant differences with their counterparts in the split plot design.

No statistical analysis was performed on the data for determination of myrosinase activity. Linear regression analyses using the mean values of the four replications were performed to determine the relationships between heating time and free or total SCN in the experiment to determine heating effects in an extract of raw cabbage.

Determination of Cooking Times

The three cooking times of 3, 9 and 27 minutes boiling were established to represent three stages of doneness: underdone, standard tender crisp and overdone, respectively. The three times were chosen on the basis of preliminary testing which involved cooking cabbage, wedge and shred for 4, 6, 8, 10 and 16 minutes. Cabbage was judged for doneness by visual appearance and apparent texture on cutting with a fork and chewing. Cabbage, either wedge or shred cooked for 8 or 10 minutes was judged to be tender crisp, and 9 minutes was chosen for the experiment. Cabbage cooked 4 or 6 minutes was tougher and bright green, while cabbage cooked 16 minutes was soft and yellow-green. To represent underdone and overdone cooking, 3 minutes and 27 minutes were chosen respectively because of their mathematical relationship to 9 minutes and therefore ease in statistical handling since it was initially believed that an exponential relationship might exist between SCN content and cooking time. It was hoped also that the wide spread in cooking times would ensure that any effects due to differences in cooking time would be evident.

Preparation of Cabbage for Cooking

The weight of each cabbage was recorded and any damaged outer leaves were discarded. The cabbage was cut in half, then into quarters and finally into eight wedge-shaped segments, cutting evenly through the inner core each time. The segments were numbered 1-8 in the order that the cutting was done.

For cabbages 1-4, the five appropriate segments according to the experimental design were selected. The inner core was removed and

discarded. The segment was then weighed and trimmed to approximately 100 g, giving a wedge 3-4 cm thick at the outside edge. The exact weight was recorded.

For cabbages 5-8, the four appropriate segments were shredded after the inner core had been removed and discarded. A Kitchen Aid model 3 CC with slicer attachment # 152-C was used; the blades on the slicer were adjusted to give minimum shredding, but to obtain a uniform shred, each segment was shredded twice. Shredded pieces measured approximately 0.3 cm by 2.0 cm. Exact weight of cabbage that was cooked was determined by measuring the difference in weights of cabbage on wax paper before and after approximately 100 g was added to the cooking water. The fifth appropriate segment, raw wedge, was treated as in cabbages 1-4.

Cooking of the cabbage was started within 15 minutes of the initial cutting into segments.

Cooking Procedure

The order of cooking followed the sequence established in the experimental design (Table 3). Samples were cooked on a Moffat electric range in 3 quart Pyrex pots with 250 ml unsalted tap distilled water; pots were kept covered during the entire cooking period.

For the treatments started in boiling water, water was brought to a boil and the cabbage was added. Timing of the cooking period began when the water returned to the boil which took approximately 15-20 seconds. Water was brought to a boil using maximum heat but after addition of cabbage and return to the boil, the heat was lowered to medium to maintain boiling but minimize bumping. For the single treatment started in cold water, cabbage and water were heated using

maximum heat; when boiling began, the heat was lowered to medium and timing of the cooking period began. For the cold water start, approximately 5 minutes were required to bring the water to a boil.

When cooking was completed, the cabbage solids and cooking water were separated by draining the cabbage in a sieve. Care was taken to collect all the cooking water. The cabbage and cooking water were each placed in weighed, labelled plastic containers suitable for freeze-drying. The cabbage was immediately covered, weighed and placed in a Westinghouse freezer at -18 C and subsequently transferred to an Econaire freezer at -30 C for storage. The water was covered, weighed and subsequently transferred to the Econaire freezer for freezing and storage.

The raw samples were prepared for analysis during the cooking of the other samples. The cabbage was placed in weighed, labelled plastic containers and frozen with liquid nitrogen. The containers were covered, placed in the -18 C freezer and subsequently transferred to the Econaire freezer for storage.

Preparation of Samples for Analysis

A. Cabbage

Frozen cabbage samples were freeze-dried gently for four to five days in weighed plastic containers in a Virtis Freeze-Dryer (Model no. 10-145 MR-BA). Immediately following freeze-drying, the containers were covered, weighed and the weights of the samples recorded. A homogenous sample for analysis was made by grinding each sample in an Osterizer blender using three or four 5-second intervals of power at medium speed. The powdered material was then transferred to a labelled glass jar and covered; a waterproof seal was ensured by

covering the joint between the jar and the cover with masking tape. The samples were stored at -30 C until analysis was performed. Samples were chosen in a random order for total SCN analysis; for free SCN analysis the raw samples were analyzed first.

B. Cooking Water

Frozen samples were freeze-dried gently in the Virtis Freeze-Dryer for three to seven days depending on initial water volume. The samples were immediately rehydrated with 10 ml phosphate citrate buffer (pH 7.0), and stored in 10 ml vials at 8 C. Analysis for SCN was made within 24 hours of rehydration.

Recovery of added SCN in cooking water following separation from cabbage solids was determined in three trials. An average of 95.7% added SCN was recovered, and therefore SCN values as analyzed were multiplied by a factor of 1.045 to account for the less than 100 % recovery.

Chemical Methods

A. In Cabbage

i. Total SCN

Total SCN content was measured by the method developed by McGregor and Mullin (Appendix 1). The only modifications were changes in apparatus as follows: a module heater was substituted for the boiling water bath in Step 2, an automatic pipetter was substituted for the microliter syringe in Step 8 and Whatman #1 filter paper was used instead of #4 in Step 4. In addition, the weight of sample used in Step 1 was decreased to .05 - .06 g so enough filtrate would be ob-

tained in Step 5.

A Pye Unicam SP6 - 300 Spectrophotometer and Hycel certified cuvettes (12 x 75 mm) were used. All reagents were A.C.S. certified except for sodium hydroxide which was reagent grade. The phosphate-citrate buffer (pH 7.0) was prepared according to Gomori (1955) except that the volume of citric acid was increased to 8.2 ml and the volume of dibasic sodium phosphate decreased to 41.8 ml.

Myrosinase was prepared according to the method developed by Jones (Appendix 2). The only modifications were that the weight of mustard seed and volume of 30% acetone in the first steps were halved to enable use of the centrifuge which held only 250 ml tubes (International Refrigerated Centrifuge Model B-20). Samples were centrifuged at 4000 g for 30 minutes.

Since indole acetonitrile reacts with the ferric nitrate reagent (Gmelin and Virtanen, 1961), the procedure for measurement of SCN was tested with indole acetonitrile (Sigma Chemical Company). Reaction and color formation with ferric nitrate was observed, but the colored complex was not cleared by the addition of mercuric chloride in Step 8 and therefore indole acetonitrile was not measured by this procedure.

For each cabbage sample, two .05-.06 g samples were analyzed as duplicates. For the forty samples, the mean SCN content was 1.64 mg/100 g and the difference between duplicates was $\pm$ .07 mg, indicating good precision of the method. Values reported in Table 17 indicate that duplicate error accounted for less than 1% of total variation in SCN content among the treatments.

ii. Free SCN

Measurement of free SCN in raw plants must involve a step to

inactivate myrosinase before the enzyme can act on the glucosinolates to produce free SCN as an artefact of the extraction procedure. The method used by most researchers is immersion of the material into boiling methanol for periods of 2 - 15 minutes (Gmelin and Virtanen, 1960; Kutacek and Kefeli, 1968). In the present study, this method was also used to determine free SCN content in cooked cabbage even though myrosinase was already inactivated by the cooking; this was done so that the statistical comparison of free SCN content between raw and cooked cabbage would be valid.

The procedure used was adapted from the method of Gmelin and Virtanen (1960) so that an aliquot of the extract could be assayed for free SCN by the method of McGregor and Mullin designed for total SCN (Appendix 1). In addition, the lead acetate precipitation of pigments and removal of excess lead by precipitation with hydrogen sulfide were eliminated since initial attempts resulted in low recovery of SCN.

The method used for determination of free SCN in raw cabbage was as follows. The modifications used for determinations in cooked cabbage are given at the conclusion.

1. 1.0 - 1.5 g ground freeze-dried material was weighed into a 250 ml round bottom flask (RBF) with a 24/40 ground glass neck. Exact weight was recorded.

2. 75 ml hot methanol and 6 - 8 boiling beads were added and the mixture was refluxed for 10 minutes once the mixture came to a boil. For this step the 250 ml RBF was fitted into a flask heater (Glas-Col Apparatus Co.) which was connected to a variable auto-

transformer (Staco type 3 PN 1010) to allow regulation of heating.

3. The mixture was allowed to stop boiling and the flask was then removed from the heater. The cabbage was allowed to settle to the bottom of the flask (10-15 minutes) leaving a clear green methanol solution which was then carefully decanted into a 1000 ml RBF.

4. The cabbage was washed with 75 ml methanol and the flask swirled to mix the contents. The cabbage was allowed to settle and the methanol was decanted into the 1000 ml RBF (Step 3).

5. The cabbage was washed with 50 ml 70:30 methanol: glass-distilled water. To speed settling of the cabbage, the mixture was transferred to two 50 ml centrifuge tubes and centrifuged for 5 minutes at 1000 g in an International Centrifuge model CS. The supernatants were added to the 1000 ml RBF.

6. The methanol- 70% methanol solution was concentrated to approximately 15 ml. A Buchler Instrument Flash Evaporator at 40-45 C was used; because of the low temperature required, the evaporation took at least 1 hour.

7. The concentrate was vacuum filtered through #1 Whatman paper into a 250 ml flask to remove any cabbage solids and some of the pigments. The 1000 ml RBF was rinsed twice with 15 ml of glass distilled water and the rinsings filtered into the 250 ml flask.

8. The 45 ml of filtrate were transferred to a 50 ml volumetric flask. The 250 ml flask was rinsed with approximately 5 ml glass distilled water which was used to bring the filtrate to volume.

9. To measure free SCN, 3 ml of the filtrate were transferred to a test tube. 1 ml of phosphate-citrate buffer (pH 7.0) was added, the solution mixed and then 1.5 ml were transferred to a clean test

tube. Analysis then proceeded with Step 5 of the method of McGregor and Mullin for total SCN (Appendix 1).

Modifications in apparatus were required to analyze cooked cabbage since the boiling beads were not sufficient to prevent bumping. Therefore, the cabbage was weighed into a 250 ml Erlenmeyer flask with a 24/40 ground glass neck. A magnetic stirrer replaced the beads, and the flask was heated on a Corning hot plate-stirrer. All other steps were identical.

The method was tested by determining % recovery of added SCN which was added prior to the refluxing in Step 2. Percent recovery was 99.8 - 105.5 % in 3 determinations, indicating that losses of SCN during the extraction procedure were minimal.

Since both SCN and glucobrassicin are methanol and water soluble, the method was also tested by determining total SCN content, and by comparing values to those determined for the same samples by the method of McGregor and Mullin (Appendix 1). Total SCN was determined by adding 12 mg myrosinase to the 4 ml solution in Step 9 and incubating for 1 hour before the 1.5 ml aliquot was taken for analysis. Total SCN was determined by this method for 4 samples, and was 101.4% (97.8 - 105.4) of the values as determined by the method of McGregor and Mullin.

Duplicate readings for free SCN were made from step 9 of the procedure. For the 40 samples, mean free SCN content was .20 mg/100 g and the difference between duplicates was $\pm$ .04 mg. Values reported in Table 8 indicate that duplicate error accounted for less than 3 % of the total variation in free SCN content among treatments.

B. In Cooking Water

i. Total SCN

Total SCN was measured by the method of McGregor and Mullin (Appendix 1) with modifications as outlined above (see part A i). Other modifications were that steps 1 and 2 were omitted, and 1 ml of the rehydrated sample was added directly to the solutions in Step 3. In addition, the centrifuging in Step 4 was omitted. All other steps were followed as in Appendix 1.

ii. Free SCN

Free SCN was measured as described above for total SCN in cooking water (see part B.i) except that the 2 ml myrosinase solution in Step 3 was replaced by 2 ml glass distilled water.

Calculation of SCN Content

All values for SCN content were calculated and reported as mg SCN/ 100 g raw cabbage to give a common base for statistical comparisons.

For content in cabbage the conversion formula was:

(O.D. due to SCN) x .181 ÷ weight (g) x CF_1 = mg SCN/100 g raw cabbage.

In the formula .181 was the conversion factor from O.D. to mg SCN based on a standard curve of SCN and molecular weight of SCN. Weight (g) was the weight of the sample in the 4 ml of solution; for example if 1.5 g cabbage was used for free SCN determination, the weight of the sample in the 4 ml would be 3 ÷ 50 x 1.5 = .09 g since the extract from 1.5 g was made to 50 ml of which 3 ml was taken for analysis. CF_1 was the conversion factor from freeze-dried weight to 100 g; for example if 101.5 g cabbage weighed 8.0 g after freeze-drying, the CF_1

would be 8.0 ÷ 101.5 x 100 = 7.88. The CF_1 was different for each cabbage sample because of different initial and different freeze-dried weights.

For content in cooking water the conversion formula was: (O.D. due to SCN) x .181 x 10 x CF_2 x 1.045 = mg SCN/100 g raw cabbage. The .181 is the conversion factor as above. The factor 10 was included to convert the SCN value from 1 ml to 10 ml since the O.D. was based on a 1 ml aliquot whereas the total water volume was in 10 ml. The CF_2 was the conversion factor to adjust the weight of the cabbage used to 100 g; for example if 98.5 g cabbage were cooked, the CF_2 would be 100 ÷ 98.5 = 1.015. The CF_2 was different for each water sample because of different initial weights of the cabbage. The factor 1.045 was to account for the less than 100% recovery of added SCN as discussed in Preparation of Samples for Analysis above.

The standard curve was calculated as described by McGregor and Mullin (Appendix 1) except that it was calibrated down to .01 ml SCN instead of just to .1 ml since the O.D. values for free SCN were lower than that for .1 ml. It was found that the standard curve was the same whether or not the values for O.D. between .01 ml and .1 ml SCN were included.

Content in 100 g cooked cabbage would be approximately 94% that of 100 g raw cabbage for wedges and 90% for shredded since the cooked cabbage weighed more than the raw cabbage because of retention of cooking water; shredded cabbage retained slightly more water than did the wedges.

Myrosinase Activity in Raw and Cooked Cabbage

A rough measure of myrosinase activity was obtained by comparing

free SCN content in the freeze dried samples as determined by 2 methods:

A. SCN content was measured without any steps to inactivate myrosinase. The method of McGregor and Mullin (Appendix 1) was used except that the boiling buffer and 10 minutes of heating were replaced by cold buffer, and the 2 ml myrosinase solution was replaced by 2 ml glass distilled water.

B. SCN content was measured according to the method developed for determination of free SCN, using methanol to inactivate the myrosinase.

A higher value for free SCN in samples assayed by method A than by method B would indicate presence of myrosinase activity. The 2 methods were used to measure free SCN content in raw wedges and in cabbage cooked 3 minutes, wedges and shredded.

Isolation of Heat Effects on Glucobrassicin

The method developed for determining free SCN yields an aqueous extract of glucosinolates and other water soluble compounds. Since the solution is free of myrosinase activity, it was used to determine the effect of heat alone on SCN form and content. Heating times of 0, 3, 9, and 27 minutes were used to duplicate the times in the cooking experiment. The procedure was replicated four times.

The extracts were prepared as described under Chemical Methods (Part A.ii), except that the weight of the sample was increased to 2.0-2.5 g and the final volume was increased to 120-150 ml.

Thirty grams of the extract were weighed into each of four 100 ml Pyrex beakers. Three beakers were covered with watch glasses and the solutions brought to a boil on a Corning hot plate; additional glass distilled water (ca. 20 ml) was added to the extract to be boiled 27 minutes to prevent it from boiling dry. Each solution was boiled for

the appropriate time, removed from the heat and allowed to cool. The solution in the fourth beaker was left unheated.

The four solutions were transferred to 50 ml volumetric flasks and rinsed with glass distilled water to bring the contents of the flasks to volume. The solutions were stored at 8 C and analyzed within 24 hours.

Analysis for free SCN was identical to the method outlined in Chemical Methods (Part A.ii) beginning at Step 9. Analysis for total SCN was similar except 12 mg myrosinase were added to the 4 ml solution in Step 9, and the solution was incubated for 1 hour before the 1.5 ml aliquot was taken.

RESULTS AND DISCUSSION

Myrosinase Activity in Raw and Cooked Cabbage

Free SCN content in raw and cooked cabbage samples was measured by two methods and the results are given in Table 5. Method A involved no steps to inactivate myrosinase while method B used boiling methanol to inactivate the myrosinase. A higher free SCN value in samples measured by method A, compared to method B would indicate the presence of myrosinase activity in the sample.

It can be seen that, in raw wedges, the free SCN content was much higher when determined by method A, indicating the presence of myrosinase activity. In wedges or shred cooked for 3 minutes, however, the free SCN content was essentially the same when determined by either method. These results would indicate that heat inactivation of myrosinase was rapid and therefore any effects on free or total SCN which were observed in the present study after 3 minutes of boiling must have been due to nonenzymatic effects.

Effect of Heat on an Extract of Raw Cabbage

Since thermal degradation of the indole glucosinolates was reported by Gmelin and Virtanen (1961; 1962), the effect of boiling an extract of raw cabbage for 3, 9, and 27 minutes was investigated to determine what role heat might play in the effects on SCN formed during cooking of the cabbage. Conditions in this *in vitro* system however may not have duplicated exactly the conditions in the cabbage.

The effect of heat on free and total SCN in the cabbage ex-

TABLE 5

FREE SCN CONTENT IN RAW AND COOKED CABBAGE AS AN INDICATOR OF MYROSINASE ACTIVITY (mg SCN/100 g RAW CABBAGE)

Cabbage Form[1]	Method[2] A	Method[2] B
raw wedge (2)	2.66	.10
cooked 3 minutes- wedge(2)	.03	.02
shred(1)	.13	.16

[1]number of samples analyzed in parentheses

[2]see text for explanation of methods

tract is shown in Figure 3, together with the mean values of 4 replications. Linear regression analyses were performed to determine if there were linear relationships between free SCN and length of boiling, and total SCN and length of boiling.

The increase in free SCN over boiling time was significant ($p < .05$) and the estimated linear relationship $y = .11 + .013 x$ is shown in Figure 3. . Using the estimated relationship it was calculated that the increase in free SCN during 27 minutes of boiling of the extract represented breakdown of 15.6% of initial glucoside-bound SCN. This agrees with Michajlovskij et al. (1970) where it was calculated that free SCN formation represented breakdown of 16.2% of initial glucoside bound SCN following boiling pure glucobrassicin in a buffer solution (pH 6.0) for 30 minutes.

The slight decrease in total SCN during boiling of the extract was not significant ($r^2 = .26$). After 27 minutes of boiling, total SCN was still 96.4% of initial SCN, indicating that there was little degradation to products other than free SCN. These results conflict with Michajlovskij et al. (1970) who found about 88% of initial SCN after 30 minutes of boiling glucobrassicin in buffer solution. However only one trial was reported by these authors and whether the decrease to 88% was real may be doubted since when another goitrogen was boiled under similar circumstances, the range in recovery was 86-97.3% in three trials. Gmelin and Virtanen (1961; 1962) found nitrile formation during heating of the indole glucosinolates which would result in a decrease in total SCN; however, they did not indicate whether substantial nitrile formation would occur within a period of 30 minutes heating.

These results would indicate that thermal effects during cook-

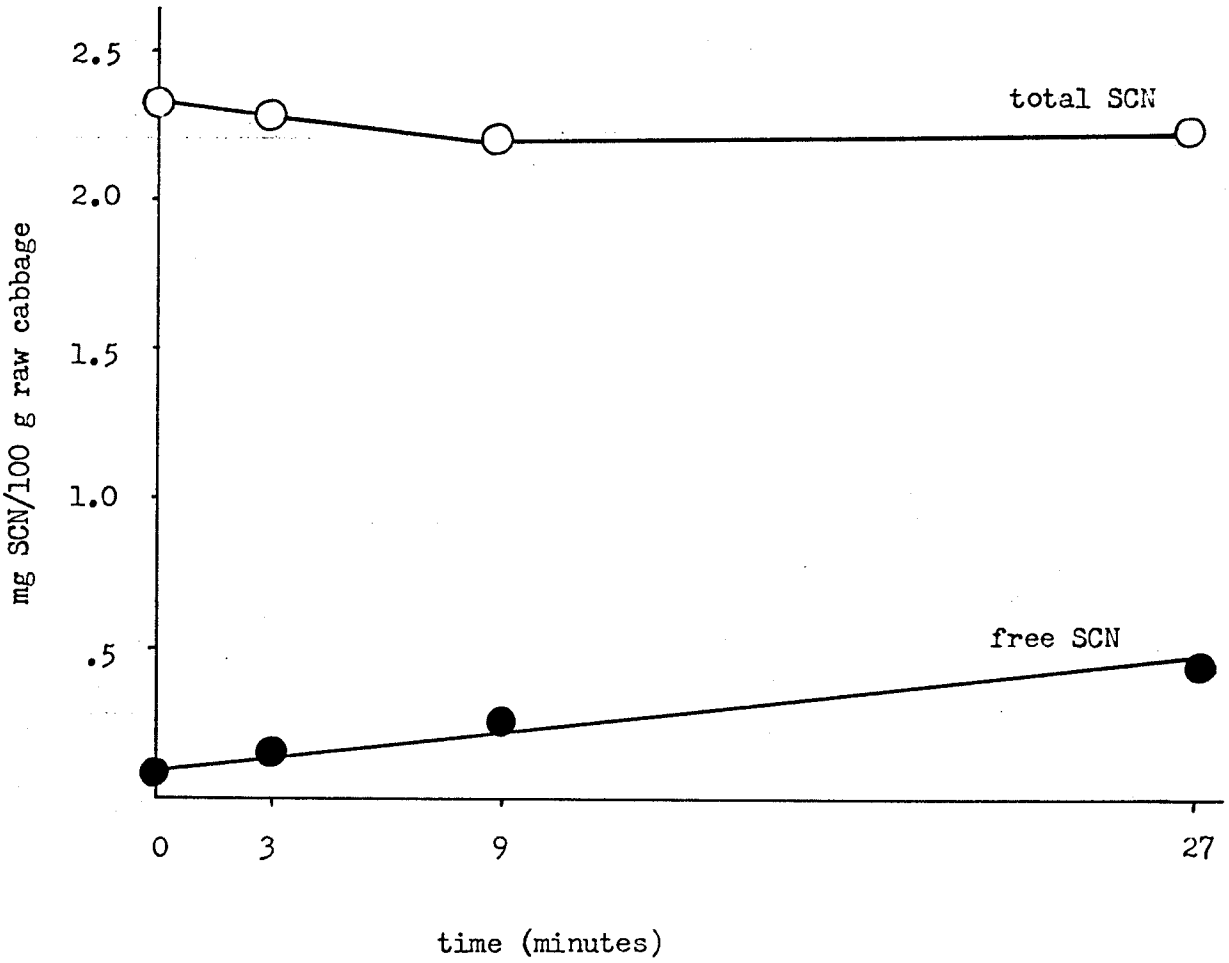


Figure 3. Effect of heat on free and total SCN in an extract of cabbage

ing of cabbage could cause an increased free SCN content compared to raw cabbage, but essentially no decrease in total SCN.

Effect of Shredding Cabbage on Free and Total SCN

Free and total SCN were measured in raw wedges and raw shredded cabbage to determine the effect of shredding since shredding results in disruption of cell structure and permits contact between glucosinolates and myrosinase. An increase in free SCN and no change in total SCN would be expected if only isothiocyanate (ITC) formation occurred as a result, while a decrease in total SCN would be expected if nitrile rather than ITC formation occurred. Daxenbichler et al. (1977) found nitrile formation from several glucosinolates when cabbage was pulped with water and allowed to autolyze, but nitrile formation from the indole glucosinolates was not monitored (Daxenbichler and VanEtten, 1977).

The effect of shredding on free SCN in cabbage is shown in Table 6 and the results were analyzed by a two tailed paired t-test, since both wedge and shred were samples from the same cabbages. It can be seen that the free SCN content increased considerably with shredding and the increase was significant ($p < .002$). In the wedge, free SCN accounted for 3.5% of total SCN, while in shredded cabbage it accounted for 12.4%.

The effect on total SCN is also shown in Table 6 but the results were not compared statistically since the variances were found to be unequal ($p < .01$). Since the mean values are very similar, it would appear that shredding had no effect on total SCN content. This agrees with Kozlowska (1971) who found a less than 5% decrease in total SCN content as a result of slicing cabbage. It

TABLE 6

EFFECT OF SHREDDING RAW CABBAGE ON FREE AND TOTAL SCN (mg SCN/100 g RAW CABBAGE)

Preparation Form	SCN form	
	Free	Total[2]
wedge	.10 ± .01[1]	2.54 ± .08
shred	.31 ± .01	2.49 ± .48

[1] mean ± S.D. of 4 cabbages

[2] F ratio of variances $(.48)^2/(.08)^2$ significant at $P < .01$

should be noted however that in three of the cabbages in the present study, total SCN content in the shred was 88-90% of the content in the wedge, while in the fourth cabbage it was 123% of the wedge indicating that the mean value for that raw wedge may be unreasonably low.

The fact that free SCN formation in raw cabbage was encouraged by shredding suggests that hydrolysis of the indole glucosinolates was catalyzed by myrosinase. Unfortunately the effect of shredding on total SCN was not conclusive. Therefore it remains undecided whether nitrile formation occurred as postulated by Mahedavan and Stowe (1972) and demonstrated for glucosinolates other than the indoles by Daxenbichler et al. (1977). Measurement of indole acetonitrile would clarify this since production of the nitrile would result in a decreased total SCN content.

Effects of Preparation Form and Cooking Time on SCN Content

A. Free SCN

Since free SCN does not exist in intact plant tissue, any free SCN present in the cooked cabbage would be due to degradation of the indole glucosinolates by enzymic, thermal or other effects. Michajlovskij et al. (1970) and Kozlowska (1971) showed that SCN was totally thermoresistant in buffered or unbuffered aqueous solutions so that once SCN is formed from the glucosinolates it remains as such.

i. Free SCN in Cabbage plus Cooking Water

Values for free SCN in cabbage and in cooking water were totalled and are presented in Table 7. It can be seen that the free SCN content was consistently higher in the shred than in the wedge and

TABLE 7

EFFECTS OF PREPARATION FORM AND COOKING TIME ON FREE SCN IN CABBAGE PLUS COOKING WATER (mg SCN/100 g RAW CABBAGE)

Preparation Form	Cooking Time (min)			
	raw	3	9	27
wedge	.06 ± .02[1]	.08 ± .04	.13 ± .06	.25 ± .01
shred	.31 ± .01	.28 ± .05	.36 ± .08	.62 ± .20

[1] mean ± S.D. of 4 cabbages

increased over cooking time for both preparation forms.

Analysis of variance (Table 8) showed that the effect of preparation form was significant ($p < .001$) as was the effect of cooking time ($p < .0005$) while the interaction between preparation form and cooking time (P x C interaction) was not significant ($p < .05$). Cochran's test for homogeneity of variance was performed to test the assumption of equal variances since the standard errors shown in Table 7 had a wide variation. The test revealed that the variances were significantly different ($p < .01$); however modification of the data to eliminate the significant differences in variances did not alter the results of the original analysis of variance except to reduce the F ratio for the P x C interaction to less than 1.0.

The significant main effect of preparation form indicates that the free SCN content was greater in the shred than the wedge. The lack of a significant P x C interaction indicates that the magnitude of the difference in free SCN between wedge and shred was similar at all four cooking times. These results suggest that the only effect that shredding had was to increase the free SCN content in the raw product, which was then maintained throughout cooking.

Because there was no significant interaction, the results for wedge and shred were combined and treated as 8 replications at each cooking period. Linear regression analysis using the mean values of the 8 replications at each cooking period was performed to determine the relationship between cooking time and free SCN content. The estimated linear relationship, along with the mean values for free SCN is shown in Figure 4; the linear relationship, $y = .17 + .01 x$ was

TABLE 8

ANALYSIS OF VARIANCE: EFFECTS OF PREPARATION FORM AND COOKING TIME ON FREE SCN CONTENT IN CABBAGE PLUS COOKING TIME

Source of Variation	df	SS	MS	F
preparation form (P)	1	.567	.567	43.615*
$error_a$	6	.077	.013	
cooking time (C)	3	.358	.119	24.403**
P x C interaction	3	.031	.010	2.107
$error_b$	18	.088	.005	
total	31	1.121		

* $p < .001$

** $p < .0005$

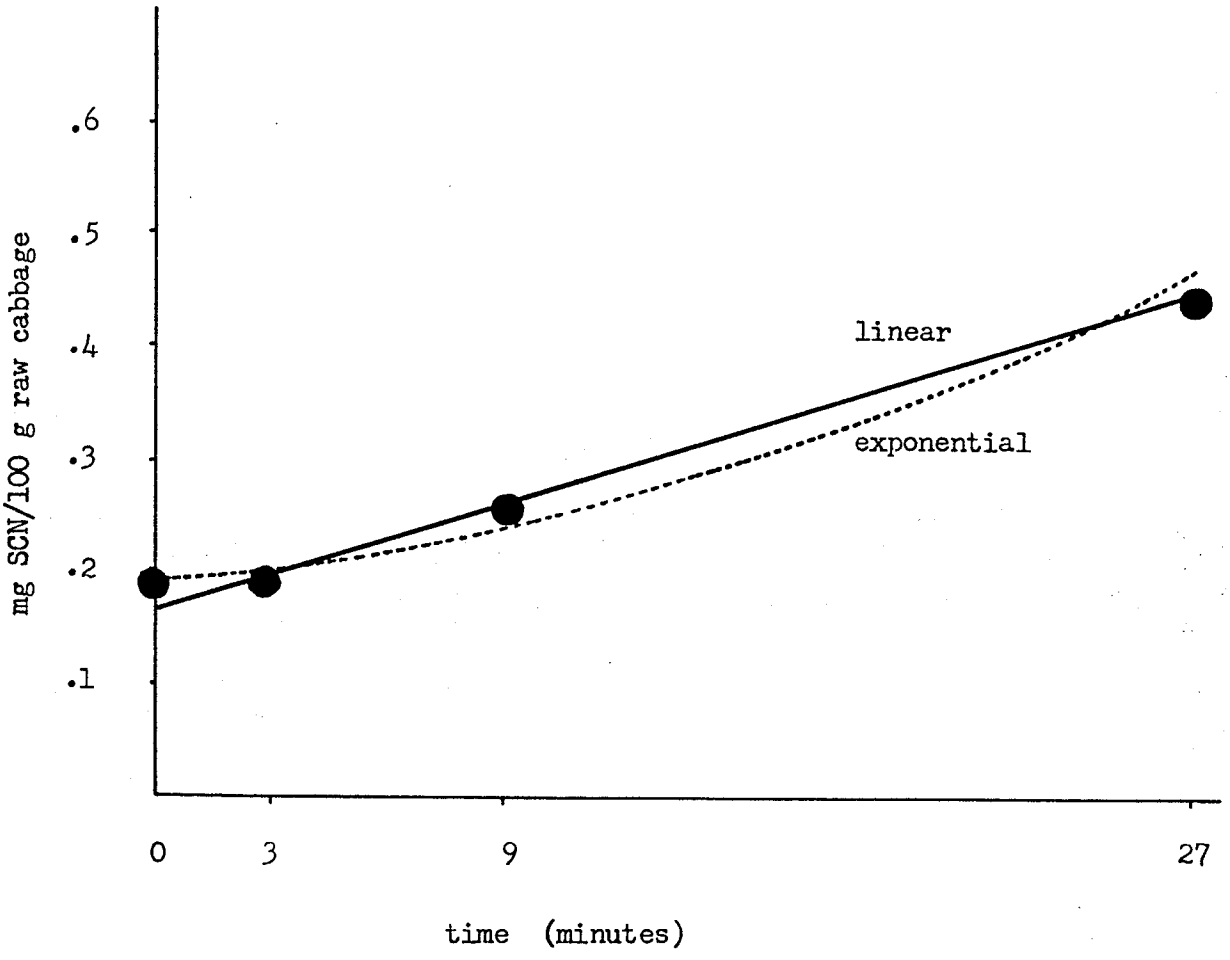


Figure 4. Effect of time on free SCN content of cabbage plus cooking water (mean values of wedge and shred). Fitted regression lines

significant ($p < .01$). It was found that the data also fit an exponential equation of the form $\ln y = \ln a + bx$ where $a=18$ and $b=.03$ ($p < .01$) which is also shown in Figure 4. Because only a few values were used to calculate the regressions, it could not be determined which of the two relationships was more appropriate although Figure 4 shows that there is little difference between the two lines. A longer cooking time, e.g. 60 minutes, would probably clarify whether or not free SCN does increase at an exponential rate. If the equations are extrapolated to 60 minutes, the exponential equation would predict a free SCN content of 1.32 mg while the linear equation would predict a free SCN content of only .72 mg.

Free SCN as a percentage of total SCN in the cabbage plus cooking water (Table 16) was calculated for each cooking period and the values are presented in Table 9. The percentage values increased with length of cooking time. The rate of increase was more rapid than that seen with the values in Table 7 however, since the total SCN content tended to decrease with time while free SCN content increased. By 27 minutes free SCN accounted for 16.1% of total SCN in the wedge, and 30.0% of total SCN in the shred.

The only study in the literature which measured free SCN was that of Michajlovskij et al. (1969; 1970) (Table 2). They found that the free SCN content in large pieces cooked for 30 minutes accounted for 41.6% of total SCN and in small pieces cooked for 30 minutes accounted for 51.1%, considerably higher than in the present study.

It seems likely that the increase in free SCN which occurred during cooking in the present study was due solely to thermal effects and not as a result of myrosinase action. The net increase in free

TABLE 9

EFFECTS OF PREPARATION FORM AND COOKING TIME ON FREE SCN IN CABBAGE PLUS COOKING WATER (AS % OF TOTAL SCN)

Preparation Form	Raw	Cooking Time(min) 3	9	27
wedge	2.7[1] (1.4-3.1)	3.4 (1.2-4.6)	6.5 (3.9-8.4)	16.1 (14.3-18.5)
shred	12.4 (10.1-14.6)	14.5 (12.2-18.3)	18.4 (16.2-20.7)	30.0 (27.6-32.4)

[1]mean and range of 4 cabbages

SCN content after 27 minutes of cooking represented breakdown of 11.5% of initial glucoside-bound SCN which is slightly less than the 15.6% breakdown found after heating the extract (Figure 3). These calculated values indicate that the slopes of the regression equations in Figure 3 for the increase in free SCN in the extract and Figure 4 for the increase in the cabbage plus cooking water are quite similar. It seems unlikely that myrosinase activity was involved since myrosinase activity had ceased by 3 minutes of cooking (Table 5) while the greatest increase in free SCN content occurred after 3 minutes.

This conclusion contradicts Michajlovskij et al. (1970) who suggested that myrosinase activity was important since cabbage boiled in ethanol for rapid myrosinase inactivation had a low free SCN content, while cabbage boiled in water with slower inactivation had a high free SCN content (Table 2). They also found that if pure glucobrassicin was added to the cabbage samples during the cooking, the same pattern of free SCN formation occurred. However, since ethanol boils at 78 C, it would be expected that thermal degradation to free SCN would be slower than with boiling water at 100 C.

The amount of free SCN formed in the study by Michajlovskij et al. (1969; 1970) was greater than that expected to be due to thermal degradation and therefore it may have been that the cooking conditions were such that myrosinase activity was possible in the early stages of cooking. However, in the present study the slower rate of myrosinase inactivation that would occur in a cold water start, compared to a boiling water start was found to have no significant effect on free SCN formation in cabbage plus cooking water (Table 23)

ii. Free SCN in Cabbage

The effects of preparation form and cooking time on free SCN in cabbage alone are given in Table 10. It can be seen that the free SCN content was consistently higher in the shred than in the wedge and increased as cooking progressed. The decrease in free SCN from the raw cabbage to cabbage cooked 3 minutes would reflect loss of SCN from the cut surfaces of the cabbage into the cooking water since this was especially apparent in the shred.

Analysis of variance (Table 11) showed that preparation form, cooking time and the P x C interaction were all significant ($p < .0005$). Cochran's test for homogeneity of variance revealed that the variances were significantly different ($p < .01$); however modification of the data to eliminate the significant differences did not alter the conclusions of the original analysis of variance.

The significant interaction is illustrated in Figure 5 which shows that the four lines between the two preparation forms representing raw cabbage and 3, 9, or 27 minutes of cooking are not parallel. Because the interaction was significant, results for the effect of cooking time were analyzed separately for the wedge and the shred.

The significant main effect of preparation form indicates that the free SCN content was higher in the shred than in the wedge. Free SCN content was consistently higher in the shred than the wedge, but the significant interaction indicates that the magnitude of the difference in free SCN content was not the same in the various cooking periods. The difference in SCN content was greatest at 27 minutes and least at 3 minutes.

TABLE 10

EFFECTS OF PREPARATION FORM AND COOKING TIME ON FREE SCN IN CABBAGE (mg SCN/100 g RAW CABBAGE)

Preparation Form	Raw	Cooking Time (min)		
		3	9	27
wedge	.06 ± .02[1]	.04 ± .04	.07 ± .05	.14 ± .01
shred	.31 ± .01	.12 ± .03	.18 ± .05	.47 ± .13

[1]mean ± S.D. of 4 cabbages

TABLE 11

ANALYSIS OF VARIANCE: EFFECTS OF PREPARATION FORM AND COOKING TIME ON FREE SCN CONTENT IN CABBAGE

Source of Variation	df	SS	MS	F
preparation form (P)	1	.602	.602	61.220*
$error_a$	6	.059	.010	
cooking time (C)	3	.436	.145	33.107*
P x C interaction	3	.170	.057	12.912*
$error_b$	18	.079	.004	
duplicates	32	.032	.001	
total	63	1.378		

* $p < .0005$

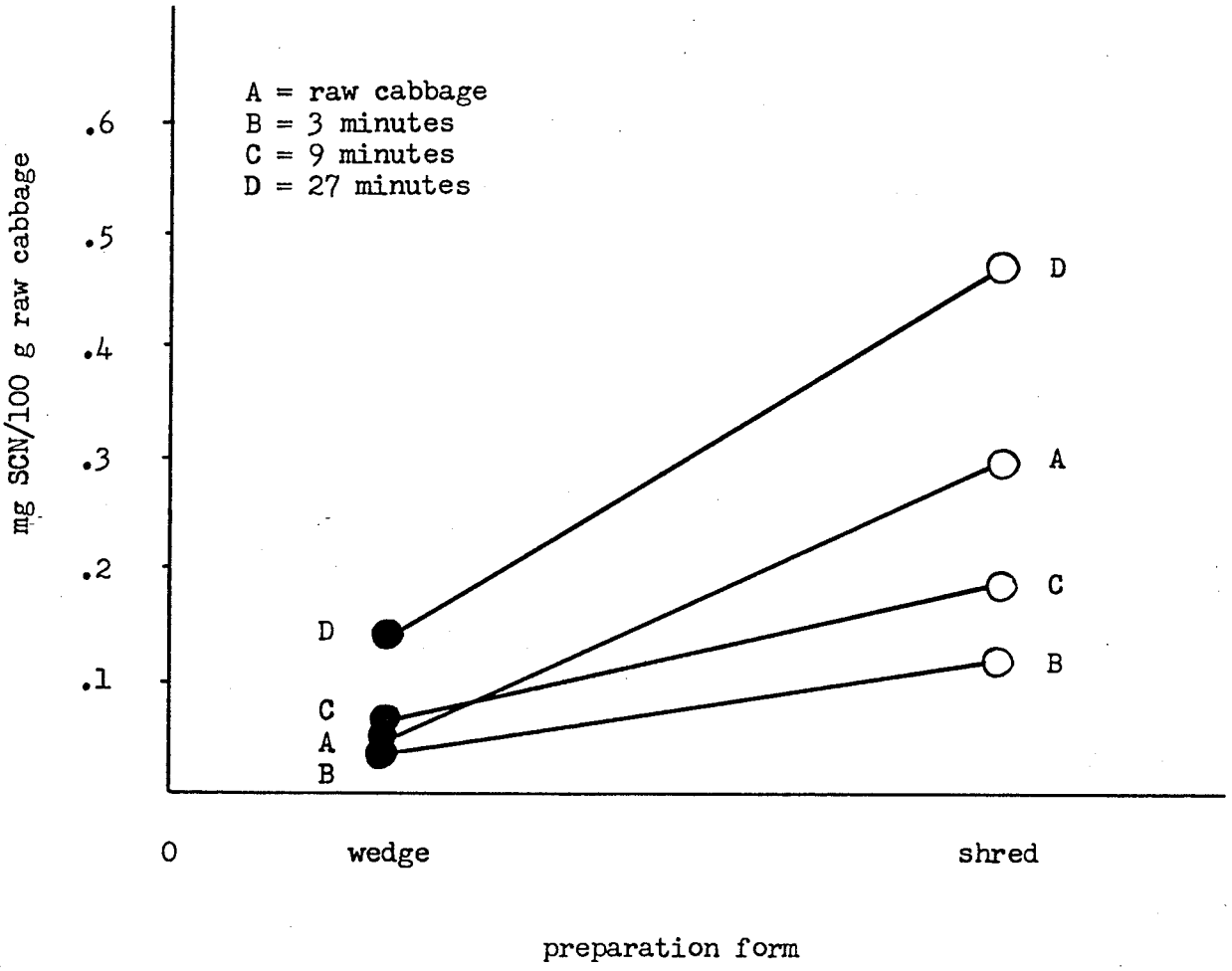


Figure 5. Interaction between preparation form and cooking time for free SCN in cabbage.

Linear regression analysis was performed to determine the effect of cooking time on free SCN content within each of the preparation forms; only the data from 3, 9, and 27 minutes of cooking time were used because of the decrease in SCN between the raw cabbage and 3 minutes. The mean values for free SCN along with the estimated linear relationships are shown in Figure 6. The increases in free SCN over time were significant ($p < .05$) for both preparation forms. Free SCN increased in the wedge with a slope of .004, while the slope in the shred was much greater, .015.

The faster rate of increase in free SCN in the shred was confirmed by reconducting the analysis of variance omitting the data for the raw cabbage and splitting the interaction into its trend components (Table 12). It was found that the P x C interaction was significant in its linear trend indicating that the linear slopes of the wedge and shred were different.

It was also found that the increase in free SCN content in the shred fit an exponential equation $\ln y = \ln a + bx$ with a=.10 and b=.06 ($p < .05$). It seems unlikely that the exponential increase could be maintained since even by 45 minutes it was extrapolated that the free SCN content would be 1.30 mg/100 g cabbage. However since the data for the shred fit an exponential equation, while the data for the wedge did not, it supports the conclusion that the rate of increase in free SCN was greater in the shred than the wedge.

The faster rate of increase in free SCN in the shred could suggest a greater rate of free SCN formation, but the results for free SCN in cabbage plus cooking water showed no difference in the rates of increase between the wedge and the shred. Therefore, the

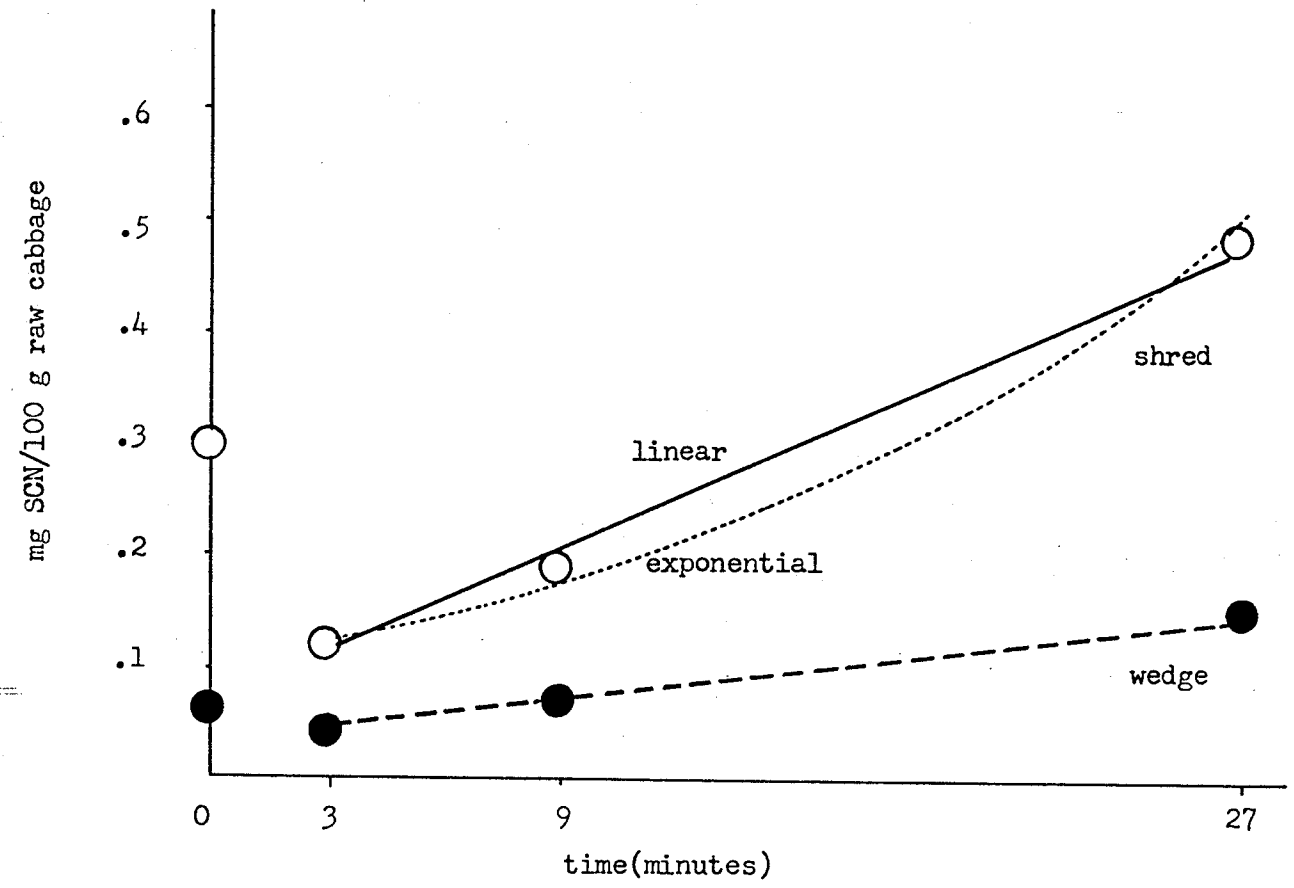


Figure 6. Effect of cooking time on free SCN content in cabbage cooked as wedge or shred. Fitted regression lines

TABLE 12

LINEAR TREND OF THE INTERACTION IN THE ANALYSIS OF VARIANCE FOR FREE SCN IN CABBAGE

Source of Variation	df	SS	MS	F
preparation form (P)	1	.364	.364	
$error_a$	6	.076	.013	
cooking time (C)	2	.433	.217	
P x C interaction				
linear	1	.151	.151	30.200*
quadratic	1	.000	.000	
$error_b$	12	.058	.005	
duplicates	24	.014	.001	
total	47	1.096		

* $p < .0005$

faster rate of increase in the cabbage alone would more likely be due to an increased accumulation in the cabbage with a correspondingly lower SCN content in the cooking water.

iii. Free SCN in the Cooking Water

Free SCN content in the cooking water is given in Table 13. It can be seen that the free SCN in the water in which the wedges were cooked increased as the length of cooking increased, but there was essentially no change in free SCN in the water in which shredded cabbage was cooked.

The difference in distribution of free SCN between the cabbage and cooking water is shown by comparing the values in Table 10 and Table 13 for each cooking period. The ratio of free SCN in cabbage to free SCN in cooking water was approximately 1:1 at all times except for shredded cabbage cooked 27 minutes where the content in the cabbage was approximately three times the content in the cooking water. This could partly be due to the retention of a concentrated solution of free SCN since the initial 100 g shredded cabbage retained 10-20 g of cooking water after draining and by 27 minutes the concentration of free SCN in the cooking water was considerably higher than at any other time (Table 14). Table 14 shows that water from shredded cabbage cooked 27 minutes had a concentration of free SCN approximately twenty five times the concentration in wedges cooked 3 minutes.

B. Total SCN

Total SCN was measured as an estimate of (neo)glucobrassicin content. The range in SCN content in the raw wedges of the eight cabbages used in the experiment is shown in Table 15. It shows that

TABLE 13

EFFECTS OF PREPARATION FORM AND COOKING TIME ON FREE SCN CONTENT IN COOKING WATER (mg SCN/100 g RAW CABBAGE)

Preparation Form	Cooking Time (min)		
	3	9	27
wedge	.03 ± .01[1]	.07 ± .01	.12 ± .02
shred	.17 ± .03	.18 ± .04	.16 ± .07

[1] mean ± S.D. of 4 cabbages

TABLE 14

CONCENTRATION OF FREE SCN IN COOKING WATER
(μg SCN/g COOKING WATER)

Preparation Form	Cooking Time (min) 3	9	27
wedge	.15 [1]	.41	1.35
shred	.90	1.15	3.99

[1] mean (n=4) free SCN content in cooking water/ mean (n=4) weight of water.

TABLE 15

RANGE IN TOTAL SCN CONTENT AMONG EIGHT RAW CABBAGES

Cabbage number [1]	Total SCN [2] (mg/100 g raw cabbage wedge)
1	2.08
2	2.32
3	2.44
4	1.64
5	2.60
6	2.48
7	2.47
8	2.62

[1] cabbages 1-4 were cooked as wedge, 5-8 were cooked as shred.

[2] mean of 2 determinations per wedge.

there was considerable variation, although the variation was less than that reported by Langer (1964c) who found a range of SCN from 0.9-6.8 mg/100 g in 24 cabbages.

Since glucobrassicin is water soluble, a decrease in the cabbage alone as a result of leaching into the cooking water, during cooking would be expected. However, a decrease in the total SCN in cabbage plus cooking water would not be expected unless the (neo)glucobrassicin was breaking down by pathways which did not produce free SCN.

i. Total SCN in Cabbage plus Cooking Water

Values for total SCN in cabbage and in cooking water were calculated and are presented in Table 16. It can be seen that cooking led to a decrease in total SCN, and that the trend appeared to be different between the wedge and the shred. Analysis of variance (Table 17) showed that effect of cooking time was significant ($p < .01$) as was the P x C interaction ($p < .01$); there was no significant main effect of preparation form. Cochran's test was not significant, which indicated homogeneity of variance.

The significant P x C interaction is illustrated in Figure 7 which shows that the lines representing raw cabbage and 3, 9 or 27 minutes of cooking for each preparation form are not parallel. This indicates that the effect of cooking time was not the same in the two preparation forms and therefore the results for the effect of cooking time were analyzed separately in the wedge and the shred.

Linear regression analysis was performed to determine if linear relationships between cooking time and total SCN content existed. The decrease in total SCN content in the wedge was significant ($p < .05$)

TABLE 16

EFFECTS OF PREPARATION FORM AND COOKING TIME ON TOTAL SCN CONTENT IN CABBAGE PLUS COOKING WATER (mg SCN/100 g RAW CABBAGE)

Preparation Form	Raw	Cooking Time (min)		
		3	9	27
wedge	2.12 ± .36[1]	2.18 ± .25	2.03 ± .48	1.52 ± .15
shred	2.49 ± .48[a]	2.00 ± .45[b]	2.03 ± .65[b]	2.11 ± .79[b]

[1] mean ± S.D. of 4 cabbages.

[ab] values bearing same superscript do not differ significantly at $p < .05$ or .01

TABLE 17

ANALYSIS OF VARIANCE: EFFECTS OF PREPARATION FORM AND COOKING TIME ON TOTAL SCN CONTENT IN CABBAGE PLUS COOKING WATER

Source of Variation	df	SS	MS	F
preparation form (P)	1	.312	.312	.382
$error_a$	6	4.963	.816	
cooking time (C)	3	.972	.324	7.200*
P x C interaction	3	.741	.247	5.489*
$error_b$	18	.817	.045	
total	31	7.805		

* $p < .01$

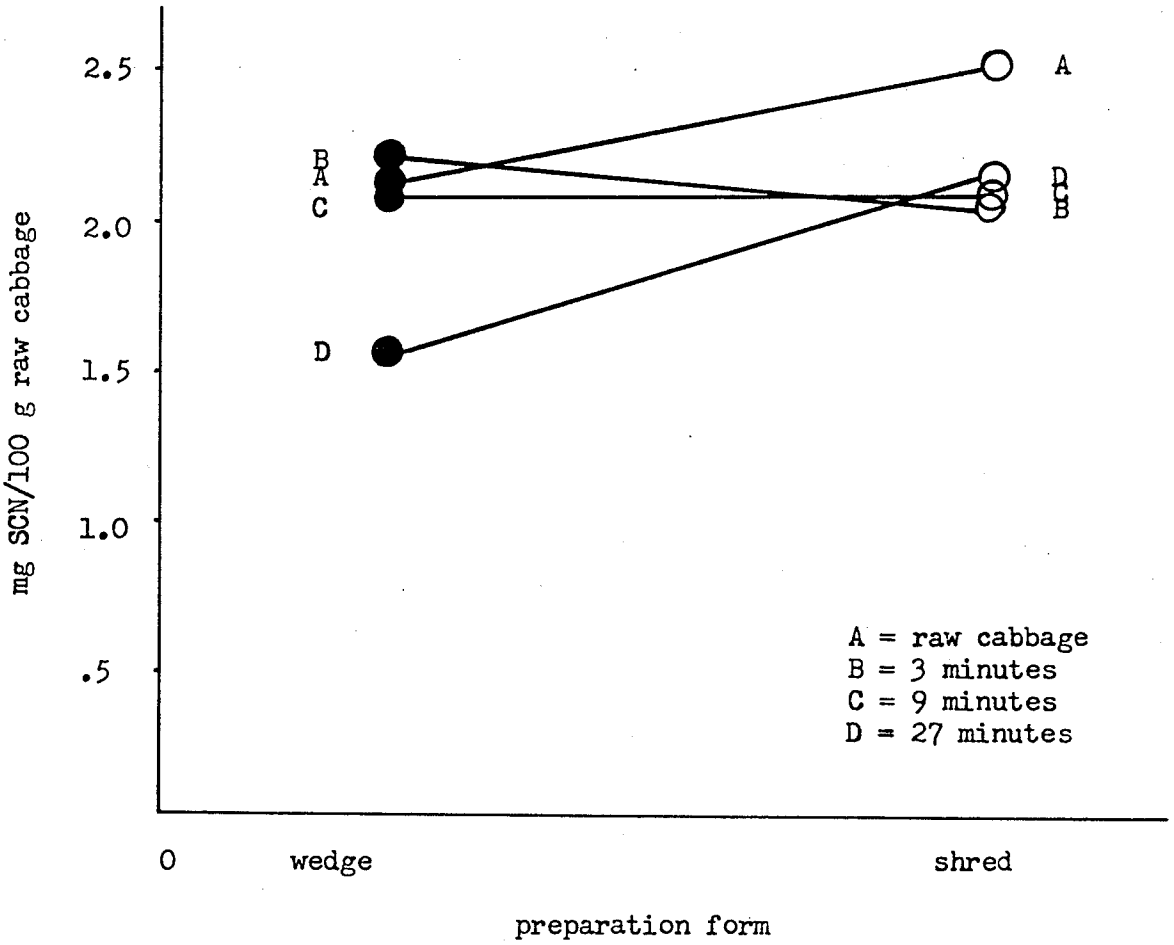


Figure 7. Interaction between preparation form and cooking time for total SCN in cabbage plus cooking water

with an estimated linear equation of $y = 2.20 - .024\ x$. It was also found that the data for total SCN in the wedge fitted an exponential equation at $p < .05$ of $\ln y = \ln a + bx$ where $a = 2.22$ and $b = -.013$. Which of these equations is most appropriate is not known but when both equations are graphed, the lines are essentially identical, so the line shown in Figure 8 represents either the linear or exponential equation. A longer cooking period would distinguish between the two since if the linear equation is extrapolated to 60 minutes, the total SCN is estimated to be .76 mg/100 g while the exponential equation would predict a SCN content of 1.02 mg/100 g.

No regression relationship was significant for the change in total SCN over cooking time in the shred, with r^2 values of .24 and .11 for linear and exponential regressions respectively. Therefore multiple comparisons using t-tests on all pairs of time treatments were made to determine significant differences. Raw shredded cabbage had significantly more SCN than cabbage cooked for 3, 9 or 27 minutes and there was no difference in SCN content among the cooked cabbage at any of the three times (Table 16).

The difference in the relationships between SCN content and cooking time for the wedge and shred demonstrates the significant P x C interaction. In the wedge the SCN content decreased continually with increased cooking time, whereas in the shred the SCN content decreased within the first 3 minutes and then no further decrease occurred as length of cooking increased. However, by 27 minutes the extent of the decrease in SCN content from raw cabbage was similar in both forms. In the wedge the SCN content at 27 minutes was

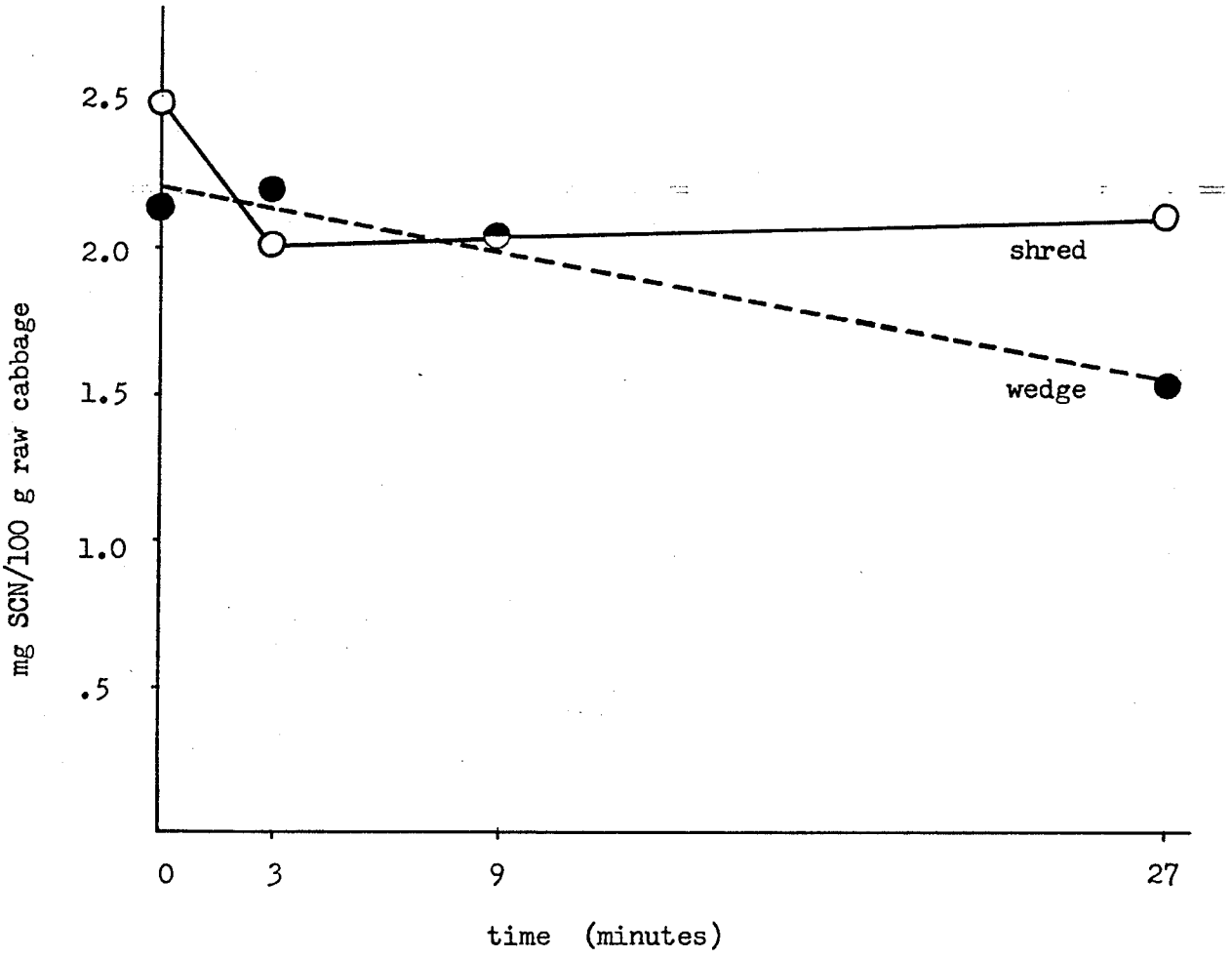


Figure 8. Effect of time on total SCN content in cabbage plus cooking water for cabbage cooked as wedge or shred

73% of initial SCN in raw cabbage with a range in the four cabbages from 66-87%, while in the shred, the SCN content was 83.0% of initial SCN with a range from 67-102% (Table 18). At 3 or 9 minutes however, the mean percentage of initial SCN was considerably higher in wedge and shred, although the ranges were very wide. The wide range in values presumably is the result of variation in SCN content within the cabbage since the cooking procedure was as standardized as possible.

Two of the studies in the literature reported decreases in total SCN in cabbage plus cooking water and one did not (Table 2). Similar to the present study, Kozlowska (1971) found 73% of initial SCN remaining after 30 minutes cooking. Mullin and Sahasrabudhe (1978) found a much greater decrease in SCN content in wedges cooked 15-20 minutes, with only 66% of initial SCN remaining (Table 2). In the present study, using the linear regression equation shown in Figure 7, it was estimated that the SCN content would still be 78-83% of initial SCN for the same length of time. The reason for the lower value found by Mullin and Sahasrabudhe (1978) is not clear, but since it appears that they had only one cooking trial, the value may not have been representative.

Michajlovskij et al. (1969; 1970) in contrast found no significant decrease in total SCN whether cabbage was cooked 30 minutes as large or small pieces (Table 2). However the sampling period was not random and therefore the results may have been biased.

The reason for the decrease in SCN content in the wedge and the shred is not clear. Myrosinase hydrolysis and subsequent degradation to products other than SCN is unlikely for two reasons. First-

TABLE 18

EFFECTS OF PREPARATION FORM AND COOKING TIME ON TOTAL SCN CONTENT IN CABBAGE PLUS COOKING WATER (AS % INITIAL TOTAL SCN)

Preparation Form	Cooking Time (min)		
	3	9	27
wedge	104.3[1] (91.0-119.3)	95.7 (83.8-111.5)	72.7 (65.9-87.0)
shred	80.0 (74.1-86.2)	80.2 (63.5-90.4)	83.0 (67.2-101.7)

[1]mean and range of 4 cabbages

ly, the decrease in SCN in the wedge was primarily after 3 minutes of cooking and therefore would not have been due to myrosinase activity. Secondly, the decrease in SCN in the shred seen in the first 3 minutes of cooking was not accompanied by a major increase in free SCN (Table 7) as would be expected from myrosinase activity since it was demonstrated that free SCN content increased as a result of myrosinase activity during shredding (Table 6).

Thermal degradation is indicated by the continual decrease in total SCN seen in the wedge. However the lack of a continual decrease over time as seen in the shred in the present study and in the study by Kozlowska (1971) would tend to negate such a conclusion. In addition, no thermal degradation by pathways yielding products other than SCN could be demonstrated in the extract (Figure 3). Gmelin and Virtanen (1961; 1962) found nitrile formation as a result of thermal degradation of glucobrassicin and neoglucobrassicin, but did not indicate whether this would be significant within a period of 30 minutes. Similarly, MacLeod and MacLeod (1970) found nitrile formation from sinigrin during cooking and presumed it to be due to thermal degradation of the sinigrin. However, the nitrile was also found in runner beans, a vegetable not generally regarded as containing glucosinolates and therefore it was suggested that the nitrile could have been derived from compounds other than sinigrin.

Since nitriles were not measured in the present study, no conclusions regarding their possible formation can be drawn. Measurement of indole acetonitrile and other possible degradation products in future studies would clarify the reasons for the decrease in total SCN and might also suggest a reason for the differential effects seen

in the wedge and the shred.

ii. Total SCN in Cabbage

Mean values for total SCN in cabbage are given in Table 19. It can be seen that there was a decrease in SCN following cooking in both the wedge and the shred, but that the pattern of change differed between the two forms.

Analysis of variance (Table 20) showed that the effect of cooking time and the P x C interaction were both significant ($p < .0005$); there was no main effect of preparation form. Cochran's test was not significant, which indicated homogeneity of variance.

The significant interaction is illustrated in Figure 9 which shows that the four lines representing raw cabbage and 3, 9, or 27 minutes of cooking are not parallel. This indicates that the effect of cooking time was different in the two preparation forms, and therefore the results for the effect of cooking time were analyzed separately.

Neither the wedge nor the shred had a significant linear relationship between SCN content and cooking time, the r^2 values being .90 and .08 respectively although the r^2 for the wedge was just below significance at the 5% level. An exponential relationship between SCN content and cooking time was just significant at the 5% level for the wedge. The linear regressions were therefore expanded to the quadratic to improve the r^2 values; for the wedge the r^2 value increased to .999 ($p < .01$) and for the shred, the r^2 value increased to .70, but was still not significant. Figure 10 shows the estimated polynomial relationships for both the wedge and the shred.

TABLE 19

EFFECTS OF PREPARATION FORM AND COOKING TIME ON TOTAL SCN IN CABBAGE (mg SCN/100 g RAW CABBAGE)

Preparation Form	Raw	Cooking Time (min) 3	9	27
wedge	2.12 ± .36[1]	1.77 ± .19	1.32 ± .34	.84 ± .09
shred	2.49 ± .48[a]	1.16 ± .25[c]	1.12 ± .36[c]	1.54 ± .51[b]

[1] mean ± S.D. of 4 cabbages

[abc] values bearing same superscript do not differ significantly at $p < .05$

TABLE 20

ANALYSIS OF VARIANCE: EFFECTS OF PREPARATION FORM AND COOKING TIME ON TOTAL SCN IN CABBAGE

Source of Variation	df	SS	MS	F
preparation form (P)	1	.072	.072	.093
$error_a$	6	4.628	.771	
cooking time (C)	3	13.000	4.330	63.680*
P x C interaction	3	4.078	1.359	20.000*
$error_b$	18	1.224	.068	
duplicates	332	.178	.006	
total	63	23.180		

* $p < .0005$

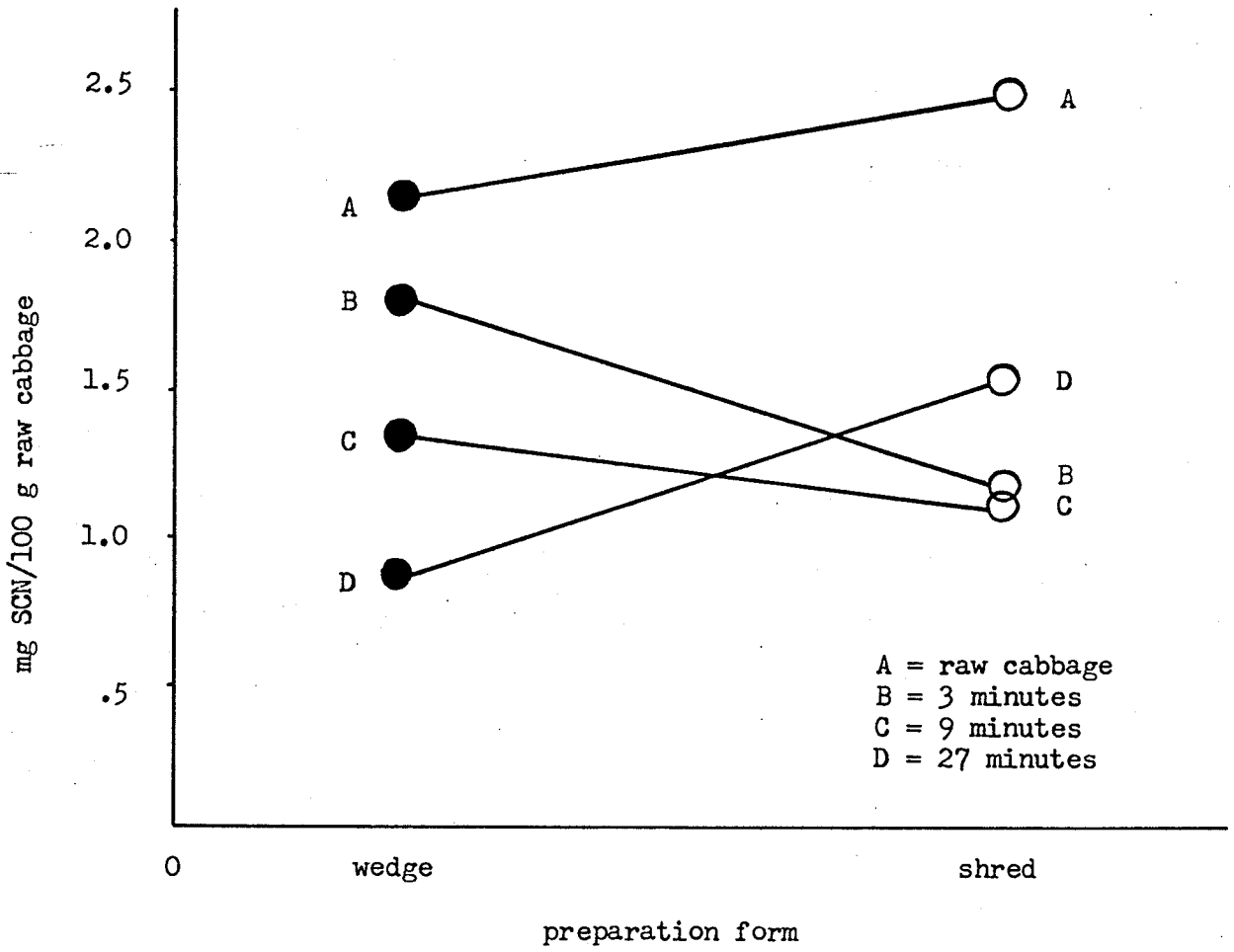


Figure 9. Interaction between preparation form and cooking time for total SCN in cabbage.

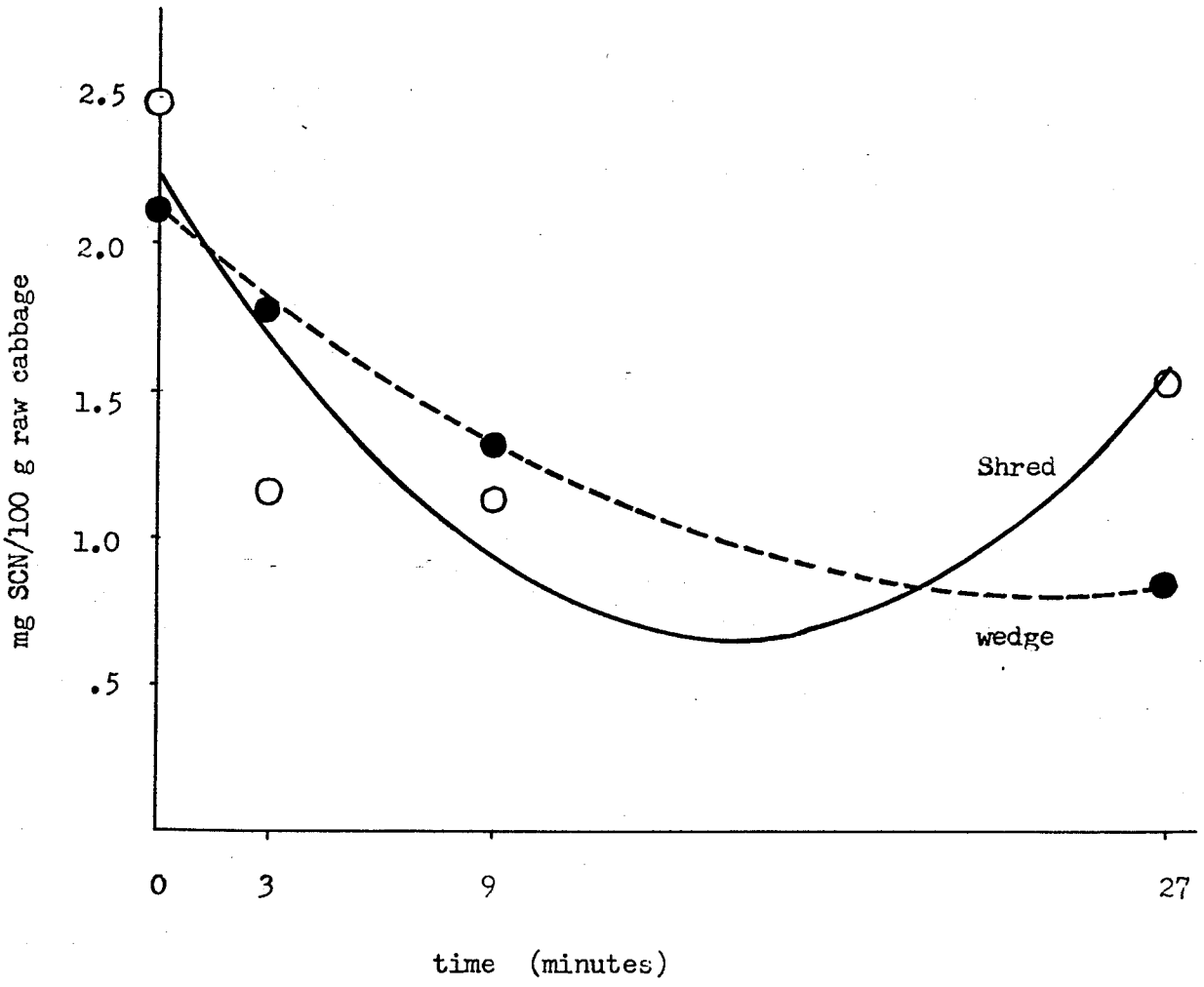


Figure 10. Effect of time on total SCN content in cabbage cooked as wedge or shred

The relationship for the latter is given graphically since, although the relationship is not significant it does describe the best least squares fit of any regression relationship tested.

Since no regression relationship was significant for the shred, multiple comparisons using t-tests were applied to compare all pairs of time treatments. It can be seen in Table 19 that the total SCN content was significantly greater in raw cabbage than in any of the cooked cabbage treatments and that the content at 27 minutes was significantly greater than at 3 or 9 minutes.

The upswing in SCN content especially in the shred, and to a lesser degree in the wedge suggests a similar effect to that with free SCN (see Results part A.ii). The faster rate of increase in free SCN content in the cabbage for the shred appeared to be due to a difference in distribution between the cabbage and the cooking water, and this could also explain the increase in total SCN at 27 minutes, compared to 3 or 9 minutes. It can be noted that Kozlowska (1971) also found a slightly higher content in broccoli after cooking 60 minutes compared to 30 minutes, 1.6 mg/100 g broccoli vs. 1.4 mg respectively; no explanation was provided.

The percentages of initial total SCN remaining in the cabbage at the various cooking times are given in Table 21, which also demonstrates the considerable losses of SCN during cooking. Only two studies in the literature are available for comparison. Kozlowska (1971) found 27% of initial total SCN remaining in the cabbage after 30 minutes of cooking and the same after 60 minutes. The present study found 40% remaining in wedges after 27 minutes and this higher value is probably due to the lower volume of water used. Mullin and

TABLE 21

EFFECTS OF PREPARATION FORM AND COOKING TIME ON TOTAL SCN IN CABBAGE (AS % INITIAL TOTAL SCN)

Preparation Form	Cooking Time (min)		
	3	9	27
wedge	84.6[1] (71.7-96.6)	62.2 (54.0-74.7)	40.6 (34.6-52.0)
shred	46.6 (41.4-53.3)	44.4 (34.2-51.4)	61.1 (48.5-70.8)

[1]mean and range of 4 cabbages

Sahasrabudhe (1978) found 33% of initial total SCN remaining in wedges cooked 15-20 minutes. In the present study 40-47% of initial total SCN would remain in the cabbage for the same period of 15-20 minutes based on the calculated polynomial regression equation in Figure 10. The higher retention in the cabbage in the present study could be due to the lower water volume used, and also to the lower decrease in SCN in cabbage plus cooking water.

iii. Total SCN in Cooking Water

Mean values for total SCN content in cooking water are given in Table 22. It can be seen that there was no direct relationship between SCN content and length of cooking time. In cooking water from the wedge the SCN content increased from 3 to 9 minutes but did not increase with prolonged cooking. In cooking water from the shred, there was little change in SCN from 3 to 9 minutes, but a decrease was found with prolonged cooking.

The effect of initial water volume on the distribution of total SCN between the cabbage and the cooking water is shown by comparing the present study to the results of the literature. It was found that as the ratio of initial water volume to cabbage weight increased, the % of total SCN leached into the cooking water increased. The water volume: cabbage weight ratio was 250 ml: 100 g = 2.5: 1 in the present study and the percentages of total SCN leached into the water in wedges cooked 9 or 27 minutes were 33% and 40% respectively. Mullin and Sahasrabudhe (1978) used a ratio of 4.0: 1 and found that after 15-20 minutes 50% of the total SCN was in the cooking water, considerably higher than what might have been found for a similar

TABLE 22

EFFECTS OF PREPARATION FORM AND COOKING TIME ON TOTAL SCN IN COOKING WATER (mg SCN/100 g RAW CABBAGE)

Preparation Form	Cooking Time (min)		
	3	9	27
wedge	.41 ± .08[1]	.71 ± .14	.68 ± .10
shred	.84 ± .22	.91 ± .29	.57 ± .29

[1] mean ± S.D. of 4 cabbages

time in the present study (ie. between 33 and 40%). Kozlowska (1971) used a ratio of 3.0: 1 and found almost 60% of total SCN in the cooking water, again considerably higher than the 40% found in the present study. The cold water start used by Kozlowska (1971) may also have contributed to the higher percentage of SCN in the cooking water since this trend was found in the present study (Table 23).

Effect of Initial Water Temperature on SCN Content

Comparison of a cold water and a boiling water start was made in wedges cooked for 9 minutes' boiling. Different effects on free and total SCN might be expected since inactivation of myrosinase would be slower in wedges cooked from a cold water start, and there would also be greater opportunity for leaching of SCN and glucobrassicin into the water because of the longer period of time in the water. There was essentially no difference in the water volume remaining after completion of the 9 minutes of boiling between the cold water start or the boiling water start.

The design of the experimental treatments permitted comparison of the effect of initial water temperature in cabbages 1-4 and analysis of results by 2 tailed paired t-tests. Mean values of free and total SCN, and various ratios are reported in Table 23. Only 3 of the 4 cabbages were used in the calculations with the raw data since the total SCN content in one of the wedges cooked from a cold start was unreasonably high and calculation of residuals showed that the value was an outlier; therefore the results from this particular cabbage were not included. Data from all 4 cabbages were used when ratio

TABLE 23

EFFECT OF INITIAL WATER TEMPERATURE ON FREE AND TOTAL SCN IN WEDGES COOKED FOR 9 MINUTES' BOILING (mg SCN/100 g RAW CABBAGE)

Distribution	SCN form	n[1]	Initial Water Temperature	
			cold water	boiling water
cabbage	free	3	.07 ± .03[2]	.05 ± .04
	total	3	1.08 ± .06	1.15 ± .09
	free/total (%)	4	7.1%	5.0%
cooking water	free	3	.07 ± .004	.06 ± .004
	total	3	.73 ± .08	.64 ± .09
cabbage plus cooking water	free	3	.14 ± .03	.11 ± .04
	total	3	1.81 ± .11	1.80 ± .18
	free/total (%)	4	8.0%	6.5%
cooking water/ cabbage plus cooking water	total	4	38.4%	35.1%

[1] number of cabbages used in calculation of values

[2] mean ± S.D. of 3 cabbages

values were calculated.

Of the nine measures reported in Table 23, only one difference was significant ($p < .02$) while one approached significance ($p < .10$). Free SCN in cooking water was significantly higher in the cold water start treatment than boiling start, but the magnitude of the difference was small, .07 mg/100 g in the cold start vs. .06 mg/100 g in the boiling start. The measure that approached statistical significance was the percentage of total SCN in the cabbage plus cooking water remaining in the cabbage. In the boiling start, 64.9% of total SCN was in the cabbage, while 61.6% was in the cabbage when cooked from a cold start; this was expected because of greater opportunity for leaching into the cooking water with the cold start. None of the differences in the other seven measures approached statistical significance, showing that the effect of initial water temperature of free and total SCN content was minimal.

There are no comparable studies in the literature. Michajlovskij et al. (1969; 1970) used a cold water and a boiling water start but the preparation form differed for each of these.

Nutritional Implications of the Study

The evidence in the literature on the effect of cooking on the goitrogenic effects of cooked Brassica vegetables is quite incomplete. The early studies which compared the goitrogenicity of cooked Brassica vegetables to the raw vegetable were in conflict; three studies showed that cooking increased the goitrogenicity in rabbits, while two studies showed a decrease. The study by Greer and Astwood (1948) as reinterpreted by Greer and Deeney (1959) would suggest that cooked vegetables would be just as goitrogenic as raw vegetables.

Unfortunately, the study by Langer and Kutka(1964) in which raw and cooked cabbage were found to affect thyroid function in humans did not distinguish between the antithyroid effects of raw and cooked cabbage.

Because of the inconsistencies in these studies it is not possible to correlate the changes in SCN content found in the present study to changes in the goitrogenic properties of the Brassica vegetables as a result of cooking. This is also complicated by a lack of consistent data on the contribution of SCN to the overall goitrogenic effect of the Brassica although the values of 25-50% of the overall effect have been suggested by Michajlovskij and Langer (1967). The effects of cooking on the other goitrogens have also to be considered since they respond differently than does SCN(Michajlovskij et al. 1969, 1970; Kozlowska, 1971; Mullin and Sahasrabudhe, 1978).

However, if the results of the present study are considered in isolation, some tentative conclusions as to the implications of the study can be made. The conclusions would only be tentative since there is little information on the thiocyanogenicity of intact indole glucsinolates and of derivatives of them, e.g. indole acetonitrile, except for the single studies by Michajlovskij and Langer(1967) and Jirousek (1956) respectively. A conservative estimate would consider that both intact (neo)glucobrassicin and its derivatives yield SCN as a result of <u>in vivo</u> metabolism; therefore cabbage with a low free SCN content, a low content of intact (neo)glucobrassicin (glucoside-bound SCN) and minimum degradation to products other than SCN would be least goitrogenic. Wedges cooked 9 minutes would meet these requirements

best(Table 24). Conversely if neither glucoside-bound SCN nor non-SCN derivatives yield SCN *in vivo*, then only the free SCN present in cabbage would be of concern and therefore wedges cooked 3 minutes would be best.

The tentative conclusion therefore would be that minimum cutting before cooking and relatively short cooking periods would result in the least goitrogenic cabbage, in regards to the contribution by SCN alone.

TABLE 24

ESTIMATE OF TOTAL POTENTIAL SCN CONTENT IN CABBAGE FOLLOWING CONSUMPTION AND IN VIVO METABOLISM (mg SCN/100 g RAW CABBAGE)

Preparation Form	Cooking Time (min)	Free SCN[1] (A)	Glucoside-bound SCN[2] (B)	Degradations other than SCN[3] (C)	Total Potential SCN[4]
wedge	3	.04	1.73	0	1.77
	9	.07	1.25	.09	1.41
	27	.14	.70	.60	1.44
shred	3	.12	1.04	.49	1.65
	9	.18	.94	.46	1.58
	27	.47	1.07	.38	1.92

[1] from Table 10

[2] total SCN(Table 19) - free SCN(Table 10)

[3] SCN in cabbage plus cooking water - SCN in raw cabbage(Table 16)

[4] total of columns A, B and C

SUMMARY AND CONCLUSIONS

Free and total SCN were measured in raw cabbage, wedge and shred and in cabbage cooked for 3, 9 or 27 minutes. Myrosinase activity was measured in raw and cooked cabbage and thermal degradation of the indole glucosinolates was determined in an *in vitro* system to determine their roles in the changes in SCN as a result of cooking.

From these studies it may be concluded that:

1. Myrosinase activity was present in raw cabbage but was absent in cabbage cooked 3 minutes as either wedge or shred. Effects on SCN content that occurred after 3 minutes were therefore due to nonenzymatic effects.

2. Boiling an extract of raw cabbage, free of myrosinase activity, resulted in a significant linear increase in free SCN as length of boiling time increased. There was virtually no effect on total SCN since 96.4% was recovered after 27 minutes of boiling.

3. Shredding of raw cabbage resulted in an increase in free SCN from 3.5% of total SCN in the wedge to 12.4% of total SCN in the shred. Shredding appeared to have no effect on total SCN content.

4. Free SCN content in cabbage plus cooking water increased with length of cooking time, to account for 16.1% of total SCN in the wedge and 30.0% of total SCN in the shred after 27 minutes. The increase was significant and fit either linear or exponential regressions. The rates of increase in free SCN in the wedge and shred were similar and only slightly less than the rate of increase in free SCN found during

boiling of the extract. Therefore, the increase in free SCN in the cabbage plus cooking water could be accounted for solely by thermal degradation of the indole glucosinolates.

5. Free SCN in the cabbage solids alone increased as length of cooking increased. However, the rate of increase was greater in the shred than in the wedge; this was apparently due to retention of free SCN present in the cooking water by the cabbage rather than due to an increased rate of formation.

6. Free SCN in the cooking water was not directly related to cooking time. Free SCN increased as length of cooking increased in the cooking water in which wedges were cooked, but there were no changes after 3 minutes in free SCN in the cooking water in which shredded cabbage was cooked.

7. Total SCN in cabbage plus cooking water was significantly lower than total SCN in raw cabbage, indicating degradation of the indole glucosinolates by pathways other than those yielding free SCN. The decrease in total SCN in the wedge fit a linear regression, but in the shred, total SCN was significantly decreased by 3 minutes and additional cooking caused no further decrease. By 27 minutes of cooking only 72.7% of initial total SCN in raw cabbage was recovered in the cabbage plus cooking water for the wedge, and 83.0% of initial total SCN was recovered for the shred.

8. Total SCN in cooked solids alone was significantly lower than total SCN in raw cabbage due to leaching into the cooking water and the degradation to products other than free SCN. Wedges cooked 9 minutes had 62.2% of initial total SCN in the raw cabbage remaining in the cabbage solids, while shredded cabbage had only 44.4% of

initial total SCN remaining.

9. Extended cooking of shredded cabbage to 27 minutes led to an increase in total SCN in the cabbage solids compared to cabbage cooked 3 or 9 minutes, although total SCN was still only 61.1% of initial total SCN in the raw cabbage. Extended cooking of the wedge to 27 minutes led to further decrease compared to 3 or 9 minutes, to 40.6% of initial total SCN.

10. Total SCN in cooking water was not directly related to cooking time. In cooking water from wedges, the content increased up to 9 minutes and then remained the same at 27 minutes. In cooking water from shredded cabbage the total SCN was unchanged between 3 and 9 minutes, and then decreased after 27 minutes.

11. Initial water temperature had only a small effect on free or total SCN or distribution between cabbage and cooking water. The only significant difference between cold and boiling water start was a higher free SCN content in cooking water when a cold water start was used.

LIMITATIONS AND RECOMMENDATIONS

Some future research needs are indicated by the results of the present study. The most obvious research would be determination of the compound or compounds formed as a result of degradation of the indole glucosinolates, instead of free SCN. Gmelin and Virtanen (1961;1962) found thermal degradation to the indole acetonitriles and therefore measurement of these compound would be of interest. Determination of the mechanism of the decrease in total SCN in the cabbage plus cooking water would also be of interest, i.e. thermal, enzymatic or other. This could also clarify why the pattern of decrease was different in the wedge and the shred.

One limitation of the present study is that the tentative conclusion as to the methods of cooking to minimize the goitogenicity of cabbage due to SCN were based on studies conducted using rats to determine the thiocyanogenicity of various indole glucosinolate derivatives. The differential response of rats and humans to various goitrogens other than SCN has been noted. Therefore it is possible that there also may be a differential response to SCN and derivatives of the indole glucosinolates and the rat studies may not be applicable to humans.

The major limitation of the study is its nutritional implication specifically the importance of the changes in SCN content as as result of cooking in the total picture of the role of Brassica vegetables in endemic and sporadic goiter. At the present time, no conclusions in this regard can be drawn because of the general lack

of information on the role of SCN in the antithyroid properties of Brassica vegetables as well as on the general role of Brassica vegetables in endemic goiter and sporadic goiter.

Further research which could clarify the role of SCN could include:

1. extension of the studies by Langer(1964c; 1966a; 1966b) on the interactions of the various goitrogens in cabbage
2. investigation of the in vivo metabolism of the derivatives of the indole glucosinolates and intact indole glucosinolates to SCN
3. extension of the study by Langer and Kutka(1964) which showed the antithyroid effects of raw and cooked cabbage in man. The study could be extended to include lower quantities of cabbage cooked by various methods for extended periods of time. Measurement of serum levels of the goitrogens would clarify which goitrogen or goitrogens were responsible. The minimum levels therefore could be related to epidemiological evidence relating goiter to consumption of Brassica vegetables and determine whether or not consumption of the vegetables was really a factor in the goiter.

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APPENDICES

APPENDIX 1

Thiocyanate Analysis[1]

Reagents

1. Thiocyanate standard solution (Fisher Scientific $\frac{N}{10}$ Thiocyanate SO-T-18) diluted to 0.001 N. Store solution in dark.
2. Ferric nitrate 16g /100 ml 1.0 N HNO_3. Prepare daily. Store solution in dark.
3. Mercuric chloride 5 g/100 ml water. Keeps indefinitely.
4. 1.0 N HNO_3 9 ml concentrated made to 100 ml
5. 0.1 N HNO_3 dilute 1.0 N HN_3 1:10
6. Myrosinase

Apparatus

Test tubes and racks

Spectrophotometer and cuvettes (10 mm pathlength)

Pipettes (3 ml volumetric, 2 ml graduated to tip for transferring 1.5 ml filtrate)

Water bath

Microliter syringe (50 ul)

Burette (for dispensing ferric nitrate solution; could use pipette if needs be)

Thiocyanate Analysis

1. Weigh out 0.100 g freeze dried material into test tubes(a).
2. Add 1 ml boiling buffer solution, pH 7.0 phosphate-citrate; place in water bath @ 95 C for 10 minutes. Cool in air or by dipping in cold water.
3. Add 2 ml myrosinase solution(6 mg/ml) and 1 ml water. Incubate at

room temperature for 1 hr., shake frequently.

4. Centrifuge @ 5000 r.p.m. for 10 minutes to clear soln. Filter supernatant if necessary (Whatman #4, small funnel to avoid loss of liquid on filter paper).

5. Transfer 1.5 ml of filtrate into clean test tube, add 0.5 ml of 0.1 N NaOH, mix and allow to stand 15 minutes.

6. Add 0.5 ml 0.1 N HNO_3 and 2.5 ml of $Fe(NO_3)_3$ solution within 3 minutes.

7. Pipette 3 ml into a cuvette measure O.D. at 470 nm 5 minutes after addition of $Fe(NO_3)_3$ solution (This timing is important).

8. Add 50 μl of 5% $HgCl_2$ solution to contents of cuvette, cover with Teflon cap (b), invert several times to mix, read immediately at 470 nm. Subtract this 'background' reading from first reading in step #7 to give O.D. due to SCN^-.

(a) 16 x 75 mm pyrex tubes with Teflon lined screw caps (Canlab Catalogue number T 1356-2)

(b) Wash out syringe with water then methanol and dry thoroughly after use. If you do not take care the iron comes out of the plunger and causes serious problems in analysis also the needle will plug.

Calibration

Tube	pH 7.0 Buffer	0.001 N KSCN	H_2O
1	1.0 ml	0.0 ml	3.0 ml
2	"	0.1 "	2.9 "
3	"	0.3 "	2.7 "
4	"	0.5 "	2.5 "
5	"	1.0 "	2.0 "
6	"	1.5 "	1.5 "
7	"	2.0 "	1.0 "
8	"	2.5 "	0.5 "
9	"	3.0 "	0.0 "

Make up in duplicate, i.e. 18 tubes each containing a total of 4 mls. Starting at 5) of SCN^- analysis, go through the same steps as for dry sample. Plot O.D. on vertical axis and mls KSCN on the horizontal. You should end up with an O.D. of about 0.95 for the 3.0 ml KSCN tube and going in a straight line through the origin. You can read directly from the graph the amount of SCN^- in 0.100 gms of starting material; this is in moles x 10^{-3} so moles/g would be 10 x this amount. To get mg or g/gm you multiply by the average molecular weight of the three main glucosinolates which form SCN^- i.e. 3-indolylmethyl-, N-methoxy-3-indolylmethyl- and p-hydroxy-benzyl-glucosinolates.

[1] unpublished method of D.I. McGregor(Agriculture Canada, Saskatoon) and W.J. Mullin(Food Research Institute, Ottawa)

APPENDIX 2

Improved Method for the Preparation of Myrosinase[1]

200 g of clean yellow mustard seed (Sinapis alba) are cooled overnight in a refrigerator at 0 to -5 ° C.

30% v/v acetone in distilled water is pre-cooled overnight at 1-2 ° C.

Mustard seed in 50 g proportions is macerated in a polythene centrifuge bottle (500 ml capacity) with the cold 30% v/v acetone (200 ml). Maceration is accomplished with a Polytron PT macerator (Brinkman) using short spurts at high speed. Heating of the macerate must be avoided and maceration is complete when a smooth paste is formed. Maceration of 50 g seed is completed in less than 5 minutes. The macerate is transferred to a 1000 ml polythene centrifuge bottle.

The macerate from 200 g of seed is combined in one 1000 ml centrifuge cup. The macerate is held in a refrigerator at 1-2 ° C for 60 minutes and then centrifuged in an International Refrigerated Centrifuge (+5 ° C) Model PR-2 at 2500 rpm for 50 minutes.

The supernatant is poured off into a measuring cylinder and the volume noted. The clear orange colored supernatant is then transferred to a 2 litre polythene beaker and acetone pre-cooled to -20 ° C added so as to give a final acetone concentration of 70% v/v. (Approximately 140 ml 100% acetone for each 100 ml of 30% v/v acetone supernatant solution). The mixture is stirred and left to stand for 30 minutes in the cold (1-2 ° C). A copious sticky precipitate is formed on adding the acetone. The precipitate is gathered by means of a stirring rod. After 30 minutes, the acetone solution is poured off, and 500 ml of cold acetone (100%) added and the precipitate broken up with a glass rod. After about 15 minutes the precipitate is dehydrated and can be broken up and transferred to a Buchner funnel with a Whatman No. 1 filter paper. All the precipitate is transferred to the Buchner funnel with acetone and washed well with further quantities of cold acetone. The precipitate is then transferred to a large piece of filter paper, broken up with a spatula, transferred to a glass beaker and dried in a vacuum desiccator over sulphuric acid overnight. The resulting powder

has a high myrosinase activity and stores well in the dry state at -5 to -10° C.

[1] unpublished method of J.D. Jones(Food Research Institute, Ottawa)