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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

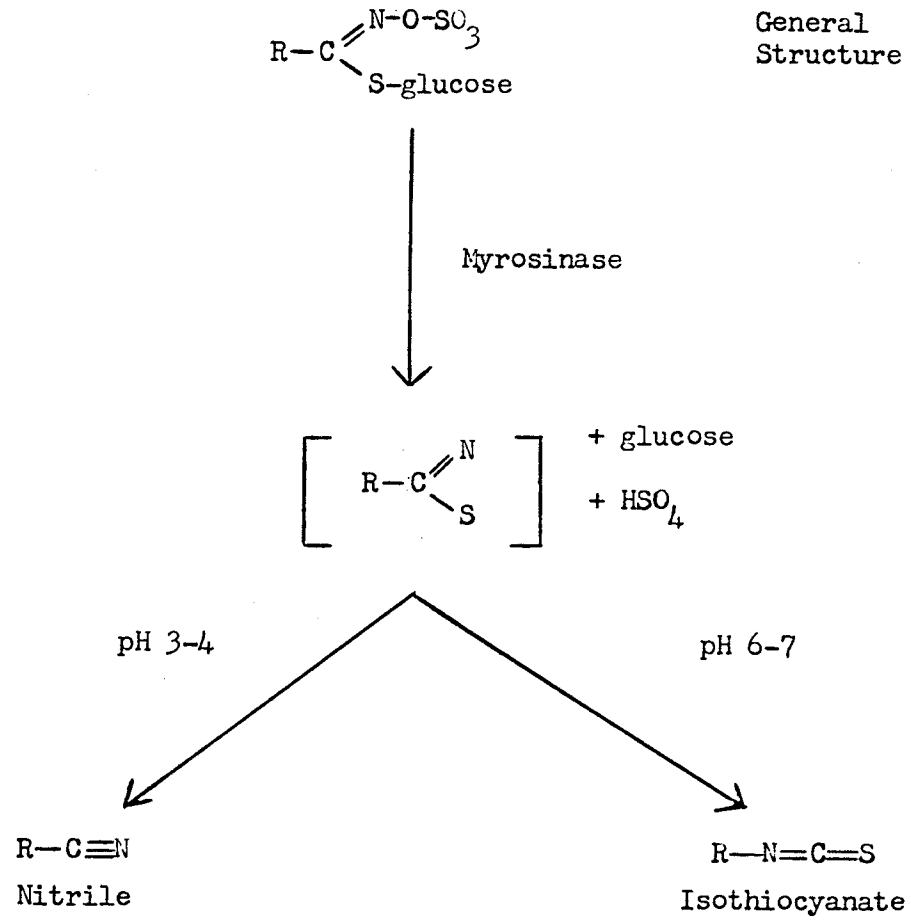
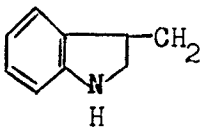
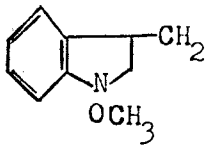


Figure 1. General structure of the glucosinolates and normal catabolism according to Benn(1977)

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);

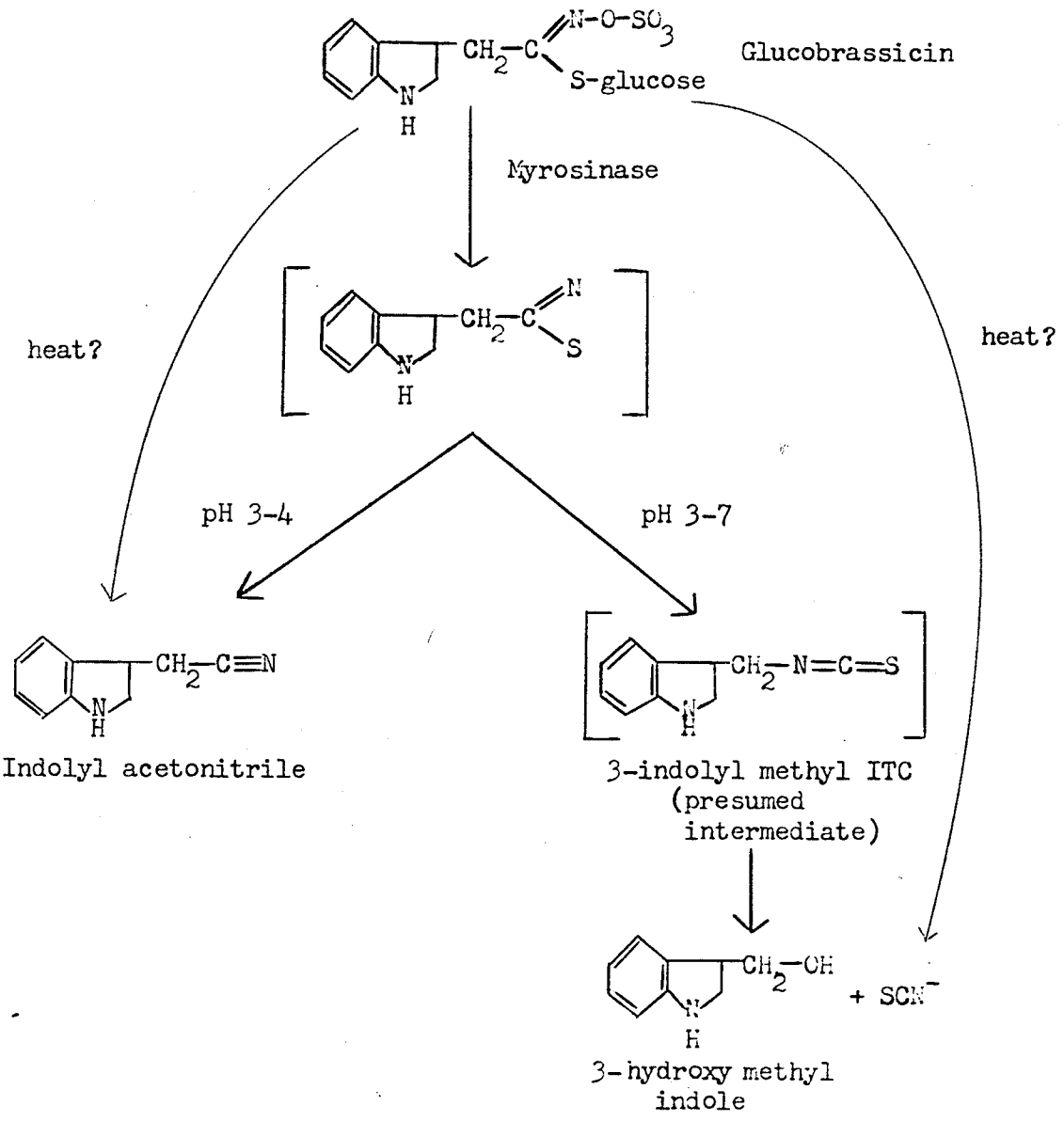


Figure 2. Degradation of glucobrassicin to SCN and nitrile

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