

The Effect of Steaming on the Glucosinolate Content in Broccoli

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Abstract

Total and individual glucosinolates were measured after different duration of steaming broccoli (*Brassica oleracea* L. var. *italica*). During steaming, the temperature profile, cell lysis and inactivation of myrosinase were assessed as well. Steaming resulted in high retention of total aliphatic and indolyl glucosinolates in the cooked product. Only after extensive steaming of broccoli (30 min) substantial losses of total indolyl glucosinolates of 55% and total aliphatic glucosinolates of 8.5% were observed. Steaming broccoli for more than 6 min result in complete inactivation of the hydrolytic enzyme myrosinase. However, steaming of broccoli for less than 6 min may result in a high intake of glucosinolates, in the presence of a residual active myrosinase, allowing the release of health-protective breakdown products of glucosinolates after consumption.

INTRODUCTION

Broccoli (*Brassica oleracea* L. var. *italica*) belongs to the group of *Brassica* vegetables and is a well-recognised health-promoting vegetable because of its high content of dietary fibre, vitamins, flavonoids and glucosinolates. The potential cancer-protective effects of some glucosinolates, a group of thioglucosides naturally occurring in *Brassica* vegetables, are extensively studied (Mithen et al., 2000; Verkerk et al., in press). When plant tissue is chewed or otherwise damaged, glucosinolates are hydrolysed by the enzymatic action of myrosinase (thioglucoside glucohydrolase, EC 3.2.1.147) releasing biologically active products including isothiocyanates, thiocyanates, indoles and nitriles (Verkerk et al., in press). Particularly the isothiocyanates have been shown to act as anticarcinogens by inhibition of phase I enzymes responsible for bioactivation of carcinogens and by induction of phase II detoxification enzymes that affect xenobiotic transformations while the indoles play a dual role acting also as inducers of phase I enzymes (Zhang et al., 1992). The most abundant types of glucosinolates present in broccoli are the aliphatic glucoraphanin, which releases the potent phase II inducing isothiocyanate sulforaphane (4-methylsulfinylbutyl), and the group of indolyls (glucobrassicin, 4-hydroxy-, 4-methoxy- and neoglucobrassicin) (Kushad et al., 1999). A recent epidemiological study has shown that the risk reduction of lung cancer associated with a relatively high intake of broccoli and other *Brassica* vegetables can indeed be linked to the presence of active breakdown products (isothiocyanates) from glucosinolates (Brennan et al., 2005).

Nowadays there is a large amount of evidence from epidemiological studies indicating that diets rich in *Brassica* vegetables can reduce the risk from a number of cancers although there is substantial variation between different studies (Verhoeven et al., 1996). For making these epidemiological studies more accurate it is essential to assess the dietary intake of protective phytochemicals. Previous reports (Dekker et al., 2000; Dekker and Verkerk, 2005) have elaborated to what extent different steps in the production chain of *Brassica* vegetables affect the glucosinolate levels. In this respect, industrial and domestic processing of the vegetables appeared to have the most impact on the

glucosinolate levels. Various studies have shown large effects of cooking *Brassica* vegetables mostly resulting in substantial losses by leaching of glucosinolates into the cooking water (Vallejo et al., 2002; Ciska and Kozłowska, 2001; Rosa and Heaney, 1993; Jiao et al., 1998). However, Vallejo et al. (2002) showed large differences in glucosinolate levels among four cooking processes. Conventional and microwave cooking caused substantial losses of total glucosinolates while steaming had minimal effects on glucosinolates (Vallejo et al., 2002). On the other hand, Verkerk and Dekker (2004) demonstrated high retention of glucosinolates after microwave cooking of red cabbage, even up to levels exceeding the total glucosinolate content of the untreated vegetables. The authors ascribed these higher levels to increased extractability of the glucosinolates (Verkerk and Dekker, 2004). Rungapamestry et al. (2006) showed slight losses of glucosinolates after microwave cooking of cabbage. In this last study, steaming was also investigated, showing no losses of glucosinolates, but this was only studied until a product temperature of 68°C.

Domestic cooking of *Brassica* vegetables can cause different phenomena, which can modify the glucosinolate levels in the vegetables (Dekker et al., 2000):

- enzymatic hydrolysis by myrosinase
- myrosinase inactivation
- cell lysis and leaching of glucosinolates, breakdown products and myrosinase in cooking water
- thermal degradation of glucosinolates and their breakdown products
- increase of the chemical glucosinolate extractability
- loss of enzymatic co-factors (e.g. ascorbic acid, iron)

The present study shows the effect of steaming of broccoli on the levels of individual and total glucosinolates. In order to explain the course of individual and total glucosinolate levels during steaming we have investigated the temperature profile of steamed broccoli and the effects on cell lysis and the hydrolytic activity of myrosinase.

MATERIALS AND METHODS

Sample Preparation

Broccoli (*Brassica oleracea* L. var. *italica*, variety unknown) was purchased from a local supermarket (Wageningen, The Netherlands). The various broccoli plants were chopped into florets (approximately 2–3 cm diameter and 2.5 cm of stalk) and mixed thoroughly. Steam cooking was applied in duplicate using an electrical steamer (Tefal, Steam Cuisine 700 TD, type 6162, 650 W) with portions of 300 g of fresh chopped broccoli for 2, 3, 4, 6, 8, 10, 15 and 30 min. The steamer collects the water that is dripping from the vegetable during steaming in a separate compartment. After each steam treatment samples of 100 g were collected for glucosinolate analysis. The remaining 200 g of broccoli was used for myrosinase activity determination as described by Verkerk and Dekker (2004). The temperature of the broccoli was monitored during the steam treatments with use of 4 thermocouples (HoneyWell Elektronik 15, Fort Washington, PA, USA). Temperatures of cylinder-shaped broccoli stalks were measured at two locations, in the centre of the broccoli stalks and on the outside of the broccoli stalks (surface). Furthermore, changes in weight of the samples were assessed by weighing the broccoli samples before and after the steam treatments.

For glucosinolate analysis of the fresh and steamed broccoli the chopped material was directly frozen with liquid nitrogen. The frozen material was ground in a Waring Blender (Model 34BL99, Dynamics Corp. of America, New Hartford, Connecticut, USA) and stored at -20°C until further analysis.

Glucosinolate Analysis

Individual glucosinolates were analysed using high performance liquid chromatography (HPLC) following on-column desulphation as described by Verkerk et al. (2001).

Myrosinase Activity Determination

The activity of the enzyme myrosinase was determined by measuring its ability to hydrolyze the exogenous glucosinolate sinigrin in juices prepared from fresh and steamed broccoli samples (Verkerk and Dekker, 2004). Samples were diluted 50 times in juice which had been cooked to denature myrosinase. The decreasing sinigrin concentration was fitted to the Michaelis-Menten equation to estimate the enzymatic activity of the samples.

Conductivity Measurement

Cell lysis was determined by measuring the conductivity of the cooking water at 23°C. Broccoli was chopped and cooked in the steamer as described above in portions of 100 g for 3, 6, 10, 15 and 30 min. The samples including drip/condensation water were placed in a beaker filled up to 500 ml with water (submerged). The conductivity of the samples was measured after 60 min with a Microprocessor Conductivity Meter (Type WTW LF 537).

RESULTS AND DISCUSSION

Total and individual glucosinolate levels were evaluated for different steaming duration of broccoli. Analysis by HPLC revealed a total of 8 different glucosinolates, namely 4 aliphatic and 4 indolyl glucosinolates (Table 1). The glucosinolate profiles (Table 2) that were observed in broccoli are in agreement with previous reports (Kushad et al., 1999), with glucoraphanin as the most abundant glucosinolate present in broccoli (37% of total GS). The four indolyl glucosinolates make up 27% of the total glucosinolates.

Total Glucosinolates

The glucosinolate levels in broccoli were significantly affected by the steaming process. In Figure 1 the course of total aliphatic and total indolyl glucosinolate levels at the different stages of steaming is shown. The total levels of aliphatic glucosinolates, mostly dominated by the high level of glucoraphanin, increased during the first 3 min of steaming. Subsequently, a decline took place between 3 and 5 min followed by an increase from 6 till 10 min of steaming. During the last 20 min of steaming the total aliphatic glucosinolate level decreased gradually up to 91% of the initial level in the starting material. The total indolyl glucosinolates behaved in a similar way as the aliphatic glucosinolates but showed a bigger decrease: only 45% of the initial level in the starting material remained after 30 min of steaming.

During steaming, the weight of broccoli samples increased by the condensation water. This rise in weight during steaming was 8.5% (3 min), 14.9% (6 min), 18.4% (10 min), 19.2% (15 min) and 22.1% (30 min). Figure 2 shows the relative changes in time of the aliphatic, indolyl and total glucosinolate content (as percentage of GSL in fresh broccoli) corrected for the weight increase. It appeared that the total glucosinolate content measured in the steamed broccoli showed, as compared with the untreated broccoli, up to 55% higher levels after about 10 min (50% higher for indolyl-GSL and 61% higher for aliphatic-GSL) and approximately 4% lower levels after 30 min of steaming (45% lower for indolyl-GSL and 13% higher for aliphatic-GSL).

Individual Glucosinolates

Steam cooking of broccoli up to 15 min has led to high levels of all aliphatic glucosinolates which is in accordance with previous results of Vallejo et al. (2002). The aliphatic glucosinolates were stable or increased in levels, while glucoraphanin was mostly reduced after 30 min (34% loss). In general, the indolyl glucosinolates followed the same trend as the aliphatic glucosinolates. Though, 4-hydroxy- and 4-methoxyglucobrassicin appeared to show higher losses after 30 min of steaming (82 and 53%, respectively).

Processes during Steaming

In order to be able to understand the course of glucosinolate levels it was necessary to investigate the temperature profile of the steamed broccoli and the effects on cell lysis and hydrolytic activity of myrosinase. The temperature of broccoli samples was measured inside and outside of cylinder-shaped broccoli stalks. As expected, initially (first 7 min) the outside of broccoli samples showed a faster increase in temperature than the inside (Fig. 3). However, after 8 min a temperature of above 90°C was reached both on the outside and inside of the stalks, gradually rising to 100°C. Cell lysis was determined by measuring the change in conductivity as an indication of the release of cell material. The myrosinase activity is crucial for the production of health-protective breakdown products (e.g. isothiocyanates) from glucosinolates. Therefore, it is important to assess myrosinase activity during the steaming treatment of broccoli.

The processes of cell lysis, myrosinase activity and the temperature profile of the broccoli during steaming are depicted together with the course of the total glucosinolate concentration (Fig. 4). The graph is subdivided into 4 different segments allowing the explanation of the different processes that took place. In segment I the temperature rise from ambient temperature to up to 60°C which causes limited cell lysis (18%) and partial inactivation of myrosinase. In segment II the temperature is between 60 and 80°C, causing a sharp increase in cell lysis (18–65%) and considerable loss of myrosinase activity. The temperature rises further to 95°C in segment III resulting in 80% cell lysis and complete inactivation of the enzyme myrosinase after 10 min.

The higher levels of glucosinolates measured in these first 3 segments (up to 32% more compared with untreated broccoli) could be ascribed to the conditions. The initially limited cell lysis (segment I) is not optimal for leaching of glucosinolates and enzymatic hydrolysis since only a small percentage of the myrosinase can get into contact with a small percentage of the glucosinolates. This high retention of glucosinolates is in agreement with previous findings on microwave cooking of red cabbage (Verkerk and Dekker, 2004) where leaching of glucosinolates was prevented by absence of direct contact with water. Glucosinolate levels exceeding the content in raw broccoli (in segment I, II and III) are in agreement with findings reported by Vallejo et al. (2002) who steamed broccoli florets for 3.5 min. These higher levels could be explained by an increase in chemical extractability caused by the heat treatment (Verkerk and Dekker, 2004). An increase in glucosinolate content is also reported by Ciska and Kozłowska (Ciska and Kozłowska, 2001) during cooking of white cabbage, who suggested that strong disintegration of cell structures (e.g. intense heat treatment) release glucosinolates bound to cell walls.

The small decline of GS content in segment II could be caused by the sharp increase in cell lysis (18–65% in segment II); in combination with, as yet, approximately 50% of remaining myrosinase activity present, hydrolysis of part of the glucosinolates could take place. In segment IV (from 10 till 30 min steaming), the temperature of the broccoli is about 100°C and cell lysis reaches its maximum. Myrosinase is now completely inactivated. The glucosinolate content declines gradually to about 80% of the total content in untreated broccoli, probably due to leaching and/or thermal degradation of the glucosinolates.

Thermal Degradation

One of the possible causes of glucosinolate losses during longer steaming duration could be thermal degradation of glucosinolates. Oerlemans et al. (2006) have studied thermal degradation of individual glucosinolates in red cabbage at different time-temperature profiles. They reported degradation of all glucosinolates when heated at temperatures above 100°C. However, the indolyl glucosinolates showed a higher degree of degradation than the aliphatic glucosinolates. Also, amongst the individual indolyl glucosinolates differences in susceptibility for high temperatures was found (Oerlemans et al., 2006).

Myrosinase Activity

Myrosinase plays a critical role in the conversion of glucosinolates to the biologically active breakdown products. Hydrolytic conversion can, in principle, take place in two different ways: 1) by the plant myrosinase during processing (e.g. industrial or food preparation) or mastication of the raw or prepared *Brassica* vegetables; or 2) when glucosinolates are ingested intact, by intestinal microbial enzymatic activity in the human body (Getahun and Chung, 1999; Elfoul et al., 2001). This study has shown that steaming for less than 6 min preserve a partial active myrosinase, possibly capable to convert glucosinolates during mastication of the vegetables. However, steaming longer than 6 min completely inactivates the enzyme. Rungapamestry et al. (2006) found myrosinase as remaining partially active after steaming cabbage for up to 7 min, although in this study only product temperatures of 68°C were reached. Conaway et al. (2000) showed complete inactivation of myrosinase after steaming broccoli for 15 min. Their study emphasized the significance of plant myrosinase mediated conversion; the bioavailability of isothiocyanates from fresh broccoli was about three times greater than that from steamed broccoli, in which myrosinase was inactivated (Conaway et al., 2000).

From this study it can be concluded that during steaming large amounts of glucosinolates remained in the edible broccoli, mainly as a result of limited opportunity for enzymatic breakdown in relation to the relative timing of myrosinase inactivation and increased cell lysis during the course of steaming. Glucosinolates are water-soluble and during conventional cooking most of the loss is found in the cooking water. Non-direct contact with water during steaming prevents leaching and solubilisation of glucosinolates in the cooking water, only after extended steaming some leaching may occur in the condensation water that is dripping from the product. Therefore, steaming for less than 6 min produces broccoli with high levels of glucosinolates and yet, residual active myrosinase enabling a more efficient conversion of glucosinolates during mastication into the health-protective isothiocyanates.

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Tables

Table 1. Glucosinolates identified by HPLC in broccoli.

Compound no.	Common name	R group
A1	Glucobrassicin	3-Methylsulphinylpropyl
A2	Progoitrin	2-Hydroxy-3-butenyl
A3	Glucoraphanin	4-Methylsulphinylbutyl
A4	Gluconanapin	3-Butenyl
I5	Glucobrassicin	3-Indolylmethyl
I6	4-Hydroxyglucobrassicin	4-Hydroxy-3-indolylmethyl
I7	4-Methoxyglucobrassicin	4-Methoxy-3-indolylmethyl
I8	Neoglucobrassicin	1-Methoxy-3-indolylmethyl

A: aliphatic glucosinolate; I: indolyl glucosinolate.

Table 2. The concentration of individual and total glucosinolates in raw and steamed broccoli florets. Values represent the mean of two replicates ($\mu\text{mol}/100\text{ g F.W.}$).

GS	Steaming time (min)									
	0	2	3	4	6	8	10	15	30	
Glucoiberin	17.1	23.6	24.3	21.9	22.5	26.0	29.5	31.5	20.3	
Progoitrin	12.5	14.6	15.1	15.7	14.8	15.9	17.9	18.6	15.2	
Glucoraphanin	40.5	50.0	51.6	43.4	38.3	44.4	51.9	53.4	26.9	
Gluconapin	10.7	8.4	10.1	10.9	16.6	11.2	8.6	6.7	11.7	
Glucobrassicin	4.9	7.3	10.1	8.9	5.9	7.2	6.7	7.5	4.4	
4-Hydroxyglucobrassicin	11.9	16.1	13.2	14.1	13.8	18.5	16.9	10.4	2.1	
4-Methoxyglucobrassicin	9.4	11.7	10.5	11.2	11.0	11.0	11.5	10.5	4.4	
Neoglucobrassicin	3.7	3.7	3.8	3.8	3.2	3.6	2.8	2.7	2.5	
Total aliphatic	80.8	96.6	101.0	91.9	92.1	97.6	108.0	110.3	73.9	
Total indolyl	29.9	38.8	37.6	38.0	33.9	40.4	37.9	31.1	13.4	
Total GS	110.7	135.4	138.5	129.9	126.0	137.9	145.9	141.4	87.3	

Figures

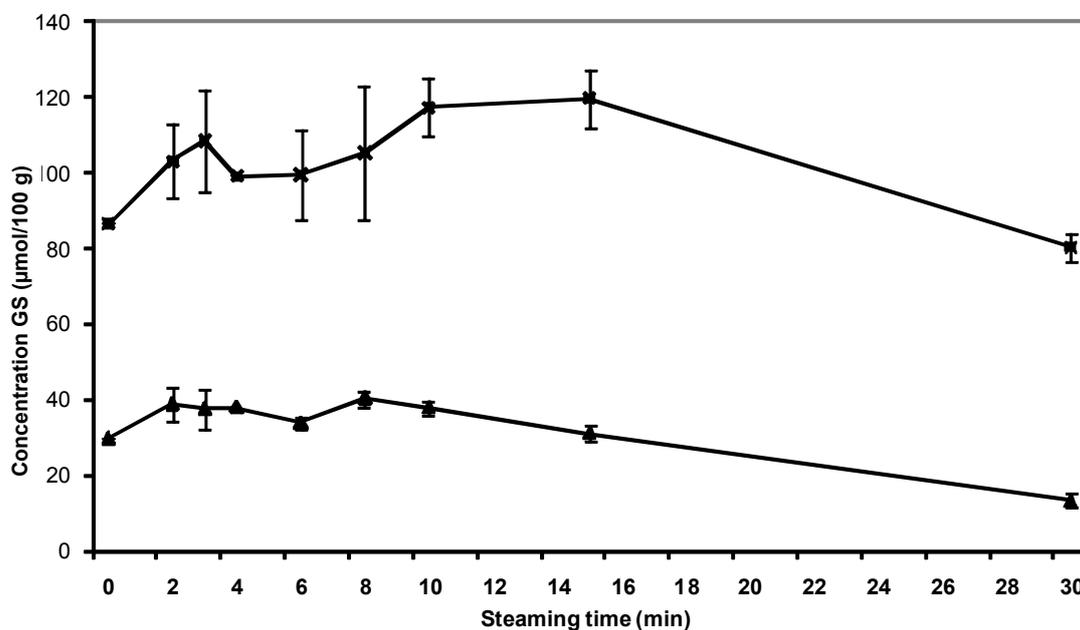


Fig. 1. Effect of steaming on the concentration of total aliphatic (■) and total indolyl glucosinolates (▲) in broccoli.

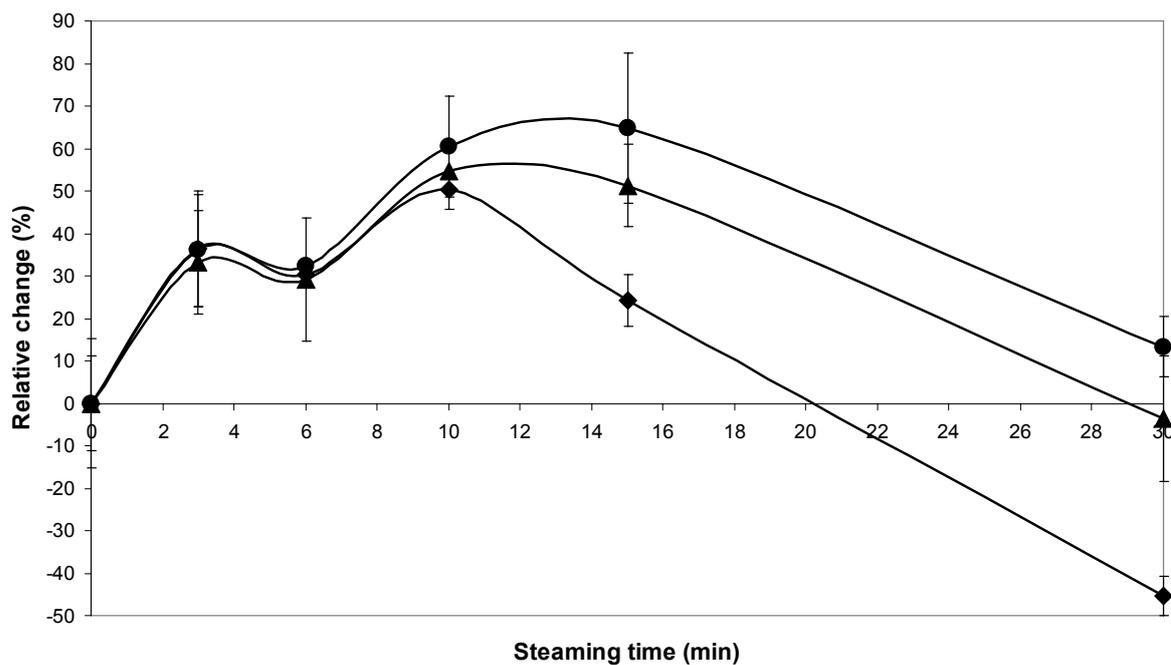


Fig. 2. Relative changes in absolute glucosinolate amount in steamed broccoli compared to the raw material (corrected for the weight gain of the broccoli during steaming); total glucosinolates (▲), aliphatic glucosinolates (●) and indolyl glucosinolates (◆).

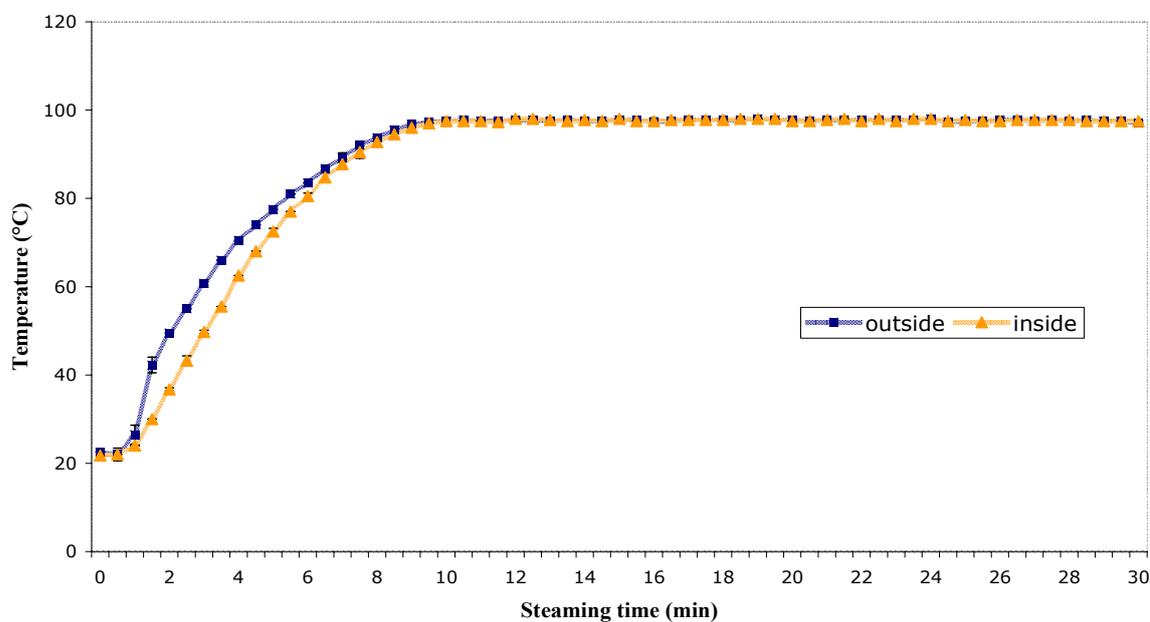


Fig. 3. Temperature profiles of broccoli during steaming: ▲ = measured inside broccoli cylinder; ■ = measured outside broccoli cylinder.

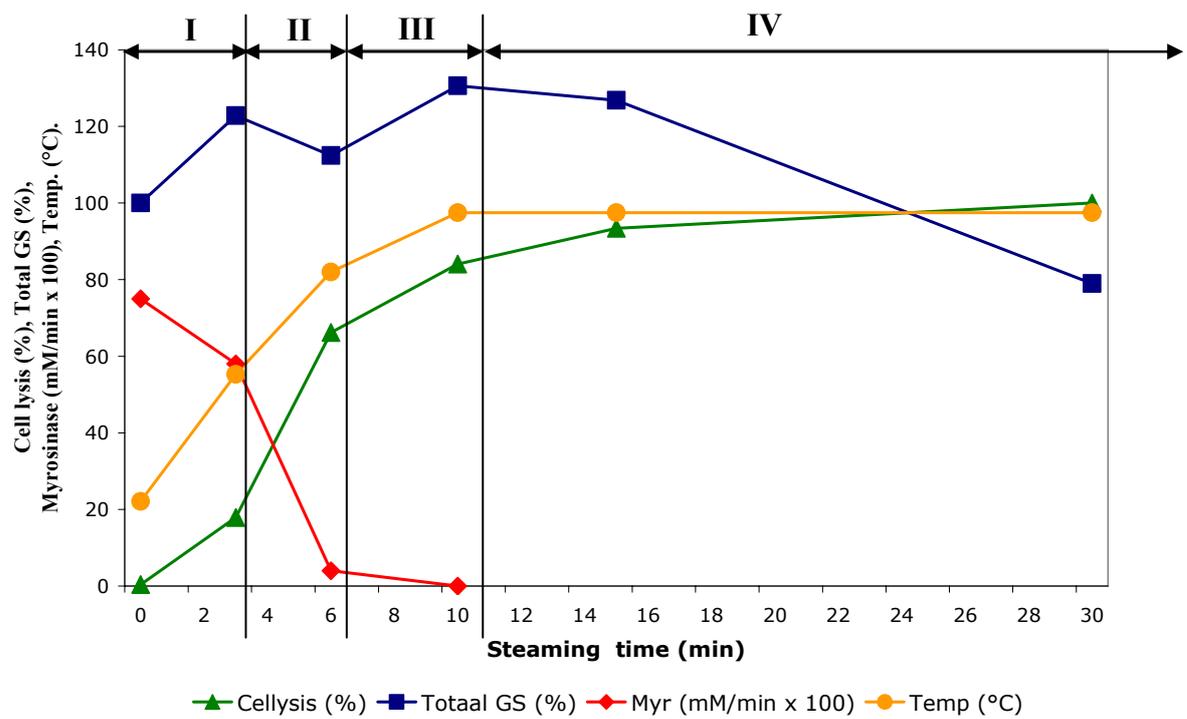


Fig. 4. Changes in glucosinolate concentration, myrosinase activity, cell lysis and temperature during steaming of broccoli florets. See text for explanation of phase I-IV.

