Differences in Thermal Stability of Glucosinolates in Five Brassica Vegetables

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Abstract: The thermal stability of individual glucosinolates within five different Brassica vegetables was studied at 100°C for different incubation times up to 120 minutes. Three vegetables that were used in this study were *Brassica oleracea* (red cabbage, broccoli and Brussels sprouts) and two were *Brassica rapa* (pak choi and Chinese cabbage). To rule out the influence of enzymatic breakdown, myrosinase was inactivated prior to the thermal treatments. The stability of three glucosinolates that occurred in all five vegetables (gluconapin, glucobrassicin and 4-methoxyglucobrassicin) varied considerably between the different vegetables. The degradation could be modeled by first order kinetics. The rate constants obtained varied between four to twenty fold between the five vegetables. Brussels sprouts showed the highest degradation rates for all three glucosinolates. The two indole glucosinolates were most stable in red cabbage, while gluconapin was most stable in broccoli. These results indicate the possibilities for plant breeding to select for cultivars in which glucosinolates are more stable during processing.

Keywords: food matrix; phytochemicals; processing; degradation; broccoli; red cabbage; Brussels sprouts; pak choi; Chinese cabbage

INTRODUCTION

Glucosinolates are a group of plant secondary metabolites. In the human diet they are mainly found in *Brassicaceae* vegetables. Over 120 different glucosinolates have been identified to this date. Glucosinolates and their breakdown products are of particular interest in food research because of their proposed anticarcinogenic properties. There are clear indications that they block tumour initiation by modulating the activities of Phase I and Phase II biotransformation enzymes and suppress tumours by apoptosis (Mithen *et al*. 2000; Verkerk *et al*. 2009). Many steps in the food production chain, such as cultivation, storage, processing and preparation of vegetables, may have an impact on levels and thus intake of phytochemicals (De Vos & Blijleven 1988; Dekker *et al*. 2000; Dekker & Verkerk 2003). Domestic treatments of the Brassica vegetables such as chopping, cooking, steaming and microwaving have been shown to affect the glucosinolate content considerably (Verkerk *et al*. 1997, 2001; Verkerk & Dekker 2004; Rungapamestry *et al*. 2006, 2007; Volden *et al*. 2008), while the effects of industrial processes as freesing, fermenting and canning are less studied (Tolonen *et al*. 2002; Dekker & Verkerk 2003; Oerlemans *et al*. 2006). During thermal processing of Brassica vegetables, glucosinolate levels can be reduced because of several mechanisms: enzymatic breakdown, thermal breakdown and leaching into the heating medium. Thermal degradation of glucosinolates in red cabbage has been studied by Oerlemans *et al*. (2006). Differences in stability were observed between the different types of glucosinolates (aliphatic, indoles, aromatic). In this study we investigate the differences in thermal stability of three glucosinolates that occur in five different *Brassica* vegetables upon incubation at 100°C. From the experimental data the kinetic degradation rate constants are estimated. The results indicate the possibilities for plant breeding to select for cultivars in which glucosinolates are more stable during processing.

MATERIAL AND METHODS

Five different Brassica vegetables were purchased from a local supermarket: *Brassica oleracea* (red cabbage, broccoli and Brussels sprouts) and *Brassica rapa* (pak choi and Chinese cabbage). Edible parts were taken and vegetables were microwave treated (300 g, 5 min, 900 W) to inactivate myrosinase. After this the samples were cooled, blended in liquid nitrogen to a fine powder and stored at –20°C until thermal treatments (Oerlemans *et al*. 2006).

The incubation at 100°C was done with samples of 5 g frozen blended vegetable in a heating block. After the incubation time the samples were cooled on ice (Oerlemans *et al*. 2006). Glucosinolate analysis was done by HPLC according to Verkerk *et al*. (2001).

Kinetic parameters were obtained by analysing the breakdown data with a first order degradation model: $C_t/C_0 = e^{-k_d t}$ using Microsoft Excel.

RESULTS AND DISCUSSION

The five different vegetables contain each between seven and nine different glucosinolates. Three glucosinolates occur in all five vegetables: gluconapin, glucobrassicin and 4-methoxyglucobrassicin. These three glucosinolates have been analysed for their thermal stability. In Table 1 the glucosinolate content of the vegetables before the thermal treatment is given.

In Figure 1 the concentration of the glucosinolates is given for all five vegetables during the incubation at 100°C. Large differences in stability between the glucosinolate are observed as was reported by Oerlemans *et al*. (2006).

Surprisingly also large differences are observed in the stability of the same glucosinolate in different vegetables. Brussels sprouts show the lowest stability for all three glucosinolates. The two indole glucosinolates (glucobrassicin and 4-methoxyglucobrassicin) were most stable in red cabbage, while gluconapin was most stable in broccoli.

The degradation could be modeled by first order kinetics. The estimated degradation rate constants (k_d) are given in Table 2. The order of stability for the glucosinolates was identical for all five vegetables: gluconapin > glucobrassicin (indole) > 4-methoxyglucobrassicin (indole), this was also reported for red cabbage by Oerlemans *et al*. (2006).

Large differences between the vegetables are also observed for the degradation rate constants. Gluconapin is twenty fold more stable in broccoli compared to Brussels sprouts. Glucobrassicin is six fold more stable in red cabbage compared to Brussels sprouts. 4-Methoxyglucobrassicin is four fold more stable in red cabbage compared to Brussels sprouts.

In this research the stability of the glucosinolates was studied in an environment close to their natural environment (the cellular structure has been disrupted and enzymes have been denatured). Apparently the environment (food matrix) in which

Table 1. Initial content of the three glucosinolate in the five different *Brassica* vegetables

	Initial content (μmol/100 g FW)				
	red cabbage	broccoli	Brussels sprouts	pak choi	Chinese cabbage
Gluconapin	28.7	16.4	37.5	12.1	8.5
Glucobrassicin	13.0	28.3	78.0	2.6	3.5
4-Methoxyglucobrassicin	1.6	3.6	14.6	1.7	11.3

Table 2. First order degradation rates of the three glucosinolate in the five different *Brassica* vegetables at 100°C

	$k_d \times 10^{-2}$ (min^{-1})				
	red cabbage	broccoli	Brussels sprouts	pak choi	Chinese cabbage
Gluconapin	0.2	0.1	2.1	0.3	1.9
Glucobrassicin	0.8	1.5	4.9	2.5	2.7
4-Methoxyglucobrassicin	1.7	5.0	6.8	3.3	2.9

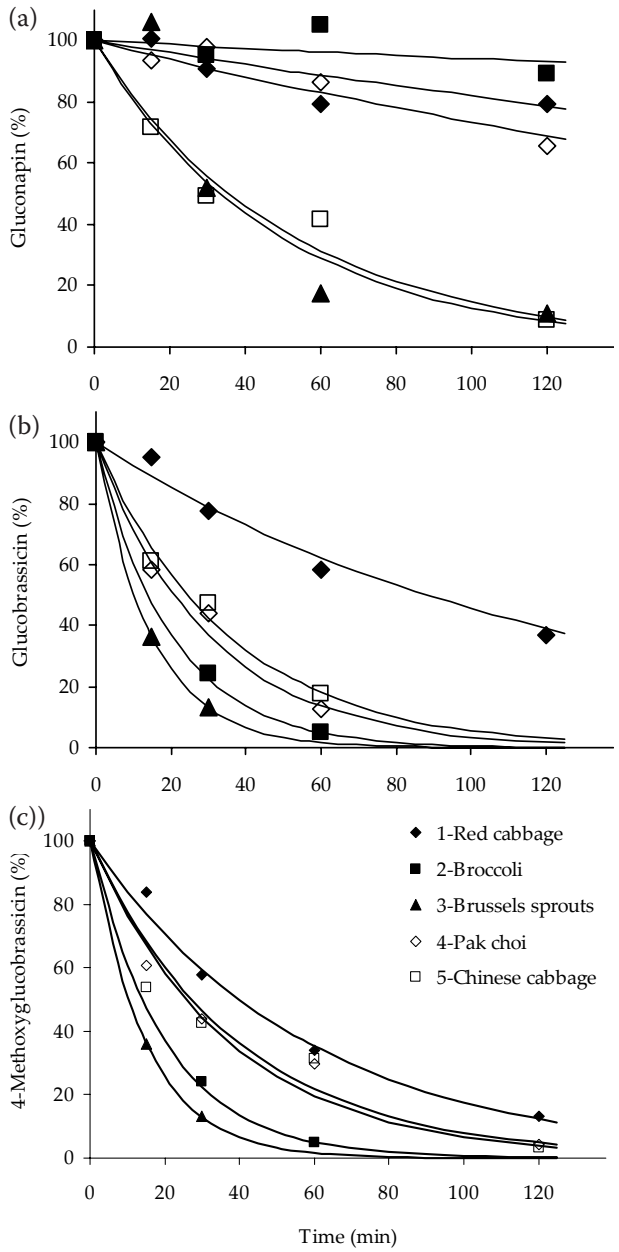

Figure 1. Glucosinolate content during incubation at 100°C for five different Brassica vegetables – (a) gluconapin, (b) glucobrassicin, (c) 4-methoxyglucobrassicin

the glucosinolates are located plays an important role in the rate of degradation. Which factors of the food matrix are responsible for the different degradation rates remains to be investigated. Possible differences in pH and cellular composition will determine the specific glucosinolate degradation rates.

Although differences in cultivation conditions cannot be ruled out it is very likely that differences in the genetic background of the different Brassica vegetables determine to a large extend the observed differences in cellular environment that determine the degradation rates. This would indicate possibilities for plant breeding to select for cultivars in which glucosinolates are more stable during processing. Further research should investigate these possibilities in more detail.

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