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Review

Glucosinolates in *Brassica* vegetables: The influence of the food supply chain on intake, bioavailability and human health

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Glucosinolates (GLSs) are found in *Brassica* vegetables. Examples of these sources include cabbage, Brussels sprouts, broccoli, cauliflower and various root vegetables (*e.g.* radish and turnip). A number of epidemiological studies have identified an inverse association between consumption of these vegetables and the risk of colon and rectal cancer. Animal studies have shown changes in enzyme activities and DNA damage resulting from consumption of *Brassica* vegetables or isothiocyanates, the breakdown products (BDP) of GLSs in the body. Mechanistic studies have begun to identify the ways in which the compounds may exert their protective action but the relevance of these studies to protective effects in the human alimentary tract is as yet unproven. *In vitro* studies with a number of specific isothiocyanates have suggested mechanisms that might be the basis of their chemoprotective effects. The concentration and composition of the GLSs in different plants, but also within a plant (*e.g.* in the seeds, roots or leaves), can vary greatly and also changes during plant development. Furthermore, the effects of various factors in the supply chain of *Brassica* vegetables including breeding, cultivation, storage and processing on intake and bioavailability of GLSs are extensively discussed in this paper.

Keywords: Bioavailability / Brassica vegetables / Cancer / Glucosinolates / Isothiocyanates

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1 Introduction

In a supply chain of agro-food products, many actors play an important role such as breeders, farmers, distributors, processors, marketers, retailers and consumers. All actors in the supply chain have the task to maintain or create value from raw materials (*e.g.* vegetables or fruit) throughout the entire food supply chain in order to provide consumers with high quality products. However, in the past years the view of product quality has changed drastically. The rising awareness of environmental, nutritional and health concerns have led to changes in consumer behaviour, increasingly demanding only highest quality products [1]. In this respect, food quality is a complex multidimensional con-

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Abbreviations: ACF, aberrant crypt foci; **BDP,** breakdown products; **BITC,** benzyl isothiocyanate; **CA,** controlled atmosphere; **CEB,** 1-cyano-3,4-epithiobutane; **CRC,** colorectal cancer; **DIM,** diindolylmethane; **ESP,** epithiospecifier protein; **GLSs,** glucosinolates; **GSH,** glutathione; **GSTs,** glutathione *S*-transferases; **I3C,** indole-3-carbinol; **MAM,** methylthioalkylmalate; **MAP,** modified atmosphere packaging; **MJ,** methyl jasmonate; **NAs,** nitrosamines; **PEITC,** phenylethylisothiocyanate; **PPF,** photosynthetic photon flux; **QTLs,** quantitative trait loci; **RH,** relative humidity; **SA,** salicylic acid; **SFN,** sulphoraphane

cept which not only depends on the property of the food but also on the consumer and his perception of the food [2].

In order to make quality more tangible for the food scientist, it is suggested that a distinction can be made between intrinsic quality attributes, *i.e.* inherent to the product itself, and extrinsic quality attributes, linked to the production method but not a property of the food itself [3, 4]. Extrinsic factors relate to the way in which the food was produced, like the use of pesticides, the absence of child labour, fair trade regulations, animal-friendliness, the type of packaging material, a specific processing technology or the use of GMOs during the production of ingredients. These extrinsic factors commonly have no direct influence on the characteristics of the product, but they can be of overriding importance in the purchasing policy of some consumers [4].

Intrinsic quality attributes of vegetables are providing the stimuli for consumers and play an important role in the eventual quality perception. Intrinsic quality attributes can be divided, among others, into sensory and health attributes. Sensory attributes refer to the classical aspects of food quality such as flavour, taste, appearance, colour, texture and smell. Taste, for example, is an experience quality that can be evaluated only after purchase and consumption of a product. During the last decades health attributes, such as nutritional and health-promoting values, have become equally (if not more) important as sensorial attributes. However, a health-promoting product property as a choice criterion for consumers is a matter of communication and interpretation of various signals and is not an experienced quality that can be directly evaluated after purchase and consumption of a product. Health attributes like bioactive compounds in horticultural crops (*e.g.* glucosinolates (GLSs), polyphenols and carotenoids) have led to the development of a new image of horticultural product quality such as in governmental campaigns on fruit and vegetables.

There exists a growing amount of evidence for the health benefits of phytochemicals delivered by the wide range of vegetables and fruit we eat.

In this review, we discuss a specific group of phytochemicals called GLS occurring in about 16 botanical families of the order Capparales. For the human diet, representatives of the Brassicaceae are of particular importance as vegetables (*e.g.* cabbage, Brussels sprouts, broccoli, cauliflower), root vegetables (*e.g.* radish, turnip, swede), leaf vegetables (*e.g.* rocket salad), seasonings and relishes (*e.g.* mustard, wasabi) and sources of oil [5]. They are claimed to be the active components responsible for many of the physiological effects proposed for *Brassica* vegetables in different types of studies, including *in vitro*, animal, human and epidemiological studies [6].

We will elaborate all the actors and relevant steps in the food supply chain of *Brassica* vegetables and their influence on intake and bioavailability of GLSs and bioactive breakdown products (BDPs) in relation to human health (Fig. 1).

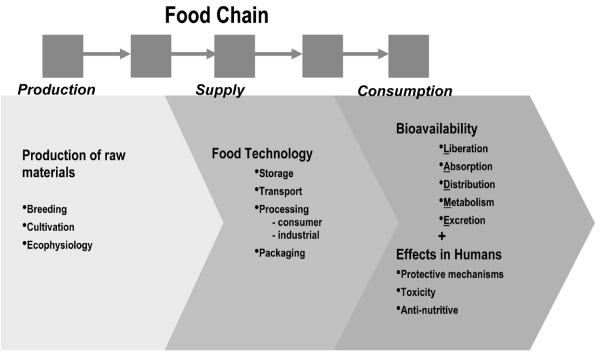


Figure 1. Steps which influence GLS content in the food supply chain.

Furthermore, we will extensively discuss the mechanisms by which *Brassica* vegetables and their components may exert their protective effects. Moreover, the antinutritional effects and the possible toxicity of GLSs will be considered as well as the main food sources in the daily diet. Also, a general strategy for production and supply chain management for optimising GLS intake and improving human health will be proposed.

2 Nature and occurrence

The majority of cultivated plants that contain GLSs belong to the family of Brassicaceae. Mustard seed, used as a seasoning, is derived from *B. nigra*, *B. juncea* (L.) Coss and *B. hirta* species. Vegetable crops include cabbage, cauliflower, broccoli, Brussels sprouts and turnip of the *B. oleracea* L., *B. rapa* L., *B. campestris* L. and *B. napus* L. species. Kale of the *B. oleracea* species is used for forage, pasture and silage. *Brassica* vegetables such as Brussels sprouts, cabbage, broccoli and cauliflower are the major source of GLSs in the human diet. They are frequently consumed by humans from Western and Eastern cultures [7].

GLSs occur in all parts of the plants, but in different profiles and concentrations. Usually, a single plant specie contains up to four different GLSs in significant amounts while, as many as 15 different GLSs can be found in the same plant. Table 1 gives an overview of the GLSs commonly found in these species.

GLSs are β-thioglycoside *N*-hydroxysulphates (also known as (*Z*)-*N*-hydroximinosulphate esters or *S*-glucopyranosyl thiohydroximates) with a side chain R and a sulphur-linked β-D-glucopyranose moiety [8] (Fig. 2). The sulphate group is normally balanced by a (potassium) cation. The side chain R determines whether the GLS is defined as aliphatic, aryl or indole.

In the family Brassicaceae, the plant's genetic background is the major factor determining GLS concentration and composition, although environmental conditions and physiological factors also influence GLS expression and

Table 1. Trivial and chemical names of GLSs commonly found in the family Brassicaceae[a)]

Trivial names	Chemical names of R-groups
Aliphatic GLSs	
Sinigrin	2-Propenyl
Gluconapin	3-Butenyl
Glucobrassicanapin	4-Pentenyl
Progoitrin	2(*R*)-2-Hydroxy-3-butenyl
Epiprogoitrin	2(*S*)-2-Hydroxy-3-butenyl
Gluconapoleiferin	2-Hydroxy-4-pentenyl
Glucoibervirin	3-Methylthiopropyl
Glucoerucin	4-Methylthiobutyl
Dehydroerucin	4-Methylthio-3-butenyl
Glucoiberin	3-Methylsulphinylpropyl
Glucoraphanin	4-Methylsulphinylbutyl
Glucoraphenin	4-Methylsulphinyl-3-butenyl
Glucoalyssin	5-Methylsulphinylpentenyl
Glucoerysolin	3-Methylsulphonylbutyl
	4-Mercaptobutyl
Indole GLSs	
Glucobrassicin	3-Indolylmethyl
4-Hydroxyglucobrassicin	4-Hydroxy-3-indolylmethyl
4-Methoxyglucobrassicin	4-Methoxy-3-indolylmethyl
Neoglucobrassicin	1-Methoxy-3-indolylmethyl
Aromatic GLSs	
Glucotropaeolin	Benzyl
Gluconasturtiin	2-Phenylethyl

a) Modified from ref. [8 – 10].

accumulation. Each species of the family Brassicaceae has a distinct GLS profile characterised by major GLSs as summarised by an actual data set (Table 2). In *Brassica* vegetables, different species of the same genus and different cultivars of the same species have highly variable GLS concentrations [8, 11, 12]. Table 2 shows the GLS concentration ranges of members of the family Brassicaceae.

Previous comprehensive reviews including *Brassica* species (horticultural and agricultural crops) were done by Rosa *et al*. [35] with a data set from the years 1976 to 1991. Fahey *et al*. [8] summarised GLS data from species of the entire order Capparales, but with no quantity indication. Recently McNaughton and Marks [7] developed a food database of total GLSs in fresh but also in frozen, boiled and cooked cruciferous vegetables.

The majority of GLSs are found in every plant organ although the concentration and composition of the GLSs can vary greatly and also change during plant development. For example, radish seedlings showed a five-fold higher GLS concentration in the cotyledones than in roots [36], whereas at harvest, GLSs were present in the root, but only a small amount (<1 mg/100 g fw) was found in the leaves. Moreover, the GLS concentration varies within the plant organs. Comparing seeds and leaves of Ethiopian kale pronounced higher GLS concentration were found in the seeds [37]. In mature flower vegetables, *e.g*. Chinese broccoli and

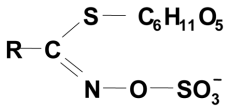


Figure 2. General structure of GLSs.

Choy sum, the generative organs – the flowers – were richest in GLSs [38], while in several *B. oleracea* species, the highest GLS concentration occurred in the roots compared to the shoot [17]. Roots, leaves and flowers of *Eruca* species showed distinct differences in the GLS profiles with high concentrations of 4-mercaptobutylglucosinolate and 4-methylsulphinylbutylglucosinolate in the leaves and flowers, respectively [39]. In addition, the total GLS concentration in pak choi and potherb mustard (*B. juncea*) declined from transplanting to harvest [20] and was higher in young, less developed broccoli heads than in fully developed ones; this effect was mainly caused by a strong decrease of indole GLSs [40]. Furthermore, the highest GLS concentration was observed in the second development stage (42 days after transplanting (DAT)) during poor sulphur fertilisation and in the third stage (49 DAT) during rich sulphur fertilisation, after which GLS concentration decreased until the overmaturity stage [41]. In contrast, in broccoli, the degradation product of the GLS glucoraphanin, sulphoraphane (SFN), increased until the commercial maturity stage [42] and the highest content of glucoraphanin occurred at the mature head stage and then declined as flowering was initiated [43]. Finally, in potherb mustard, sinigrin concentration decreased from seedling to early flowering stage, increased in the late flowering stage and then decreased again during seed maturation [43].

3 Glucosinolates in the food supply chain

3.1 Breeding

The large variation in the content and composition of GLSs in *Brassica* is demonstrated in Table 2. Although this variation is caused by several factors such as environmental factors, including soil, climate and fertilisation, the most important factor determining GLS content is genetic variation. In broccoli, it has been shown that genotype has a significant effect on GLS content in the florets [12, 44]. This provides breeders the opportunity and challenge to produce new varieties of *Brassica* vegetables with adapted a content of various types of GLSs.

Breeders have already altered the levels and types of GLSs in *Brassica* vegetables both indirectly, through selection for flavour and, possibly, by selection for resistance to herbivores, and directly by breeding for health benefits associated with enhanced levels of 4-methylsulphinylbutyl GLS, the precursor of SFN. While the contribution of GLS BDPs to flavour is complex, and, with regard to *Brassica*

Table 2. Major GLSs present in economically important members of the family Brassicaceae

Botanical classification	GLS group	Range (means)	Unit	No.	Ref.
Root vegetables					
Turnip (*B. rapa* ssp. *rapa*)	Total	53.3	mg/100 g fw	1	[11]
		36.0 – 187.0	μmol/g dw	10	[13]
		31.0 – 80.0 (61.9)	μmol/g dw	12	[14]
		50.4 – 81.7	mg/100 g fw	1	[15]
	Aliphatic				
	Progoitrin	0.1 – 20.5	mg/100 g fw	1	[11]
		7.1 – 16.1	mg/100 g fw	1	[15]
	Indole	15.1	mg/100 g fw	1	[11]
		0.4 – 8.2	μmol/g dw	10	[13]
		3.5 – 6.4	mg/100 g fw	1	[15]
	Aromatic	10.2	mg/100 g fw	1	[11]
		23.6 – 35.9	mg/100 g fw	1	[15]
Radish (*Raphanus sativus* var. *sativus*)	Total	87.6 – 332.8 (183.7)	mg/100 g fw	3	[11]
	Aliphatic				
	Dehydroerucin	56.1 – 310.0 (168.5)	mg/100 g fw	3	[11]
		5.9 – 18.2	mg/100 g fw	1	[16]
	Glucoraphenin	1.6 – 15.0 (7.3)	mg/100 g fw	3	[11]
	Indole	3.0 – 8.7 (6.0)	mg/100 g fw	3	[11]
		0.3 – 2.2	mg/100 g fw	1	[16]
Kohlrabi (*Brassica oleracea* var. *gongylodes*)	Total	28.5 – 37.0	mg/100 g fw	1	[11]
	Aliphatic				
	Glucoraphanin	7.8 – 8.7	mg/100 g fw	1	[11]
	Indole	7.2 – 9.6	mg/100 g fw	1	[11]
Leafy vegetables					
White cabbage (*B. oleracea* var. *capitata* f. *alba*)	Total	39.9 – 89.9	mg/100 g fw	1	[11]
		2.5	μmol/g dw	1	[17]
	Aliphatic				
	Sinigrin	20.0 – 22.7	mg/100 g fw	1	[11]
		1.8	μmol/g dw	1	[17]
	Indole	12.0 – 47.2	mg/100 g fw	1	[11]
		0.4	μmol/g dw	1	[17]
Red cabbage (*B. oleracea* var. *capitata* f. *rubra*)	Total	30.1 – 98.3	mg/100 g fw	1	[11]
		17.1 – 29.0	μmol/g dw	6	[18]
		4.6	μmol/g dw	1	[17]
	Aliphatic				
	Glucoraphanin	4.0 – 18.2	mg/100 g fw	1	[11]
	Sinigrin	3.0 – 16.7	mg/100 g fw	1	[11]
		2.1	μmol/g dw	1	[17]
	Glucoiberin	4.0 – 13.6	mg/100 g fw	1	[11]
	Indole	11.7 – 35.5	mg/100 g fw	1	[11]
		0.9	μmol/g dw	1	[17]
Savoy cabbage (*B. oleracea* convar. *capitata* var. *sabauda*)	Total	61.4 – 72.2	mg/100 g fw	1	[11]
		6.9	μmol/g dw	1	[17]
	Aliphatic				
	Glucoiberin	10.4 – 21.2	mg/100 g fw	1	[11]
		1.4	μmol/g dw	1	[17]
	Sinigrin	15.5 – 18.6	mg/100 g fw	1	[11]
		1.5	μmol/g dw	1	[17]
	Indole	18.0 – 43.3	mg/100 g fw	1	[11]
		3.9	μmol/g dw	1	[17]
Brussels sprouts (*B. oleracea* var. *gemmifera*)	Total	16.6 – 36.9 (24.1)	μmol/g dw	4	[12]
		73.0 – 91.4	mg/100 g fw	1	[11]
		15.1 – 35.5 (23.5)	μmol/g dw	2	[19]

Table 2. Continued

Botanical classification	GLS group	Range (means)	Unit	No.	Ref.
	Aliphatic				
	Sinigrin	4.6 – 9.1 (5.5)	μmol/g dw	4	[12]
		22.0 – 25.3	mg/100 g fw	1	[11]
		3.5 – 4.5 (4.0)	μmol/g dw	2	[19]
	Glucoiberin	6.4 – 13.9	mg/100 g fw	1	[11]
		5.2 – 6.6 (5.9)	μmol/g dw	2	[19]
	Indole	4.6 – 7.9 (6.1)	μmol/g dw	4	[12]
		30.7 – 43.5	mg/100 g fw	1	[11]
		5.7 – 21.1 (11.6)	μmol/g dw	2	[19]
Kale (*B. oleracea* convar. *acephala* var. *sabellica*)	Total	12.1 – 18.0 (19.6)	μmol/g dw	2	[12]
		65.4 – 151.1	mg/100 g fw	1	[11]
		3.5	μmol/g dw	1	[17]
		5.5 – 12.7 (8.9)	μmol/g dw	2	[19]
	Aliphatic				
	Sinigrin	7.4 – 13.3 (10.3)	μmol/g dw	2	[12]
		2.2 – 22.7	mg/100 g fw	1	[11]
		53.3	μmol/100 g fw	1	[20]
		0.6 – 1.4 (1.0)	μmol/g dw	2	[19]
	Glucoiberin	13.4 – 16.0	mg/100 g fw	1	[11]
		96.7	μmol/100 g fw	1	[20]
		1.3	μmol/g dw	1	[17]
		1.4 – 3.5 (2.4)	μmol/g dw	2	[19]
	Indole	1.0 – 2.2 (1.6)	μmol/g dw	2	[12]
		48.9 – 109.5	mg/100 g fw	1	[11]
		270.5	μmol/100 g fw	1	[20]
		3.1 – 6.9 (5.1)	μmol/g dw	2	[19]
Chinese cabbage (*Brassica campestris* ssp. *pekinensis*)	Total	9.7 – 33.7 (19.8)	mg/100 g fw	19	[21]
		8.2 – 8.4 (8.3)	μmol/g dw	25	[22]
	Aliphatic				
	Glucobrassicanapin	0.9 – 9.7 (4.3)	mg/100 g fw	19	[21]
	Progoitrin	0.9 – 8.0 (2.7)	mg/100 g fw	19	[21]
	Indole	3.1 – 10.6 (6.3)	mg/100 g fw	19	[21]
	Aromatic	0.8 – 2.6 (1.6)	mg/100 g fw	19	[21]
		4.3 – 5.3 (4.8)	μmol/g dw	25	[22]
Pak choi (*B. rapa* ssp. *chinensis*)	Total	39.0 – 70.4 (53.4)	mg/100 g fw	3	[21]
		84.7 – 290.0 (181.0)	μmol/100 g fw	3	[20]
		5.9 – 12.9 (8.1)	mg/100 g fw	3	[15]
	Aliphatic				
	Gluconapin	24.4 – 157.3 (70.4)	μmol/100 g fw	3	[20]
		2.3 – 7.6 (3.8)	mg/100 g fw	3	[15]
	Glucobrassicanapin	10.5 – 26.6 (18.2)	mg/100 g fw	3	[21]
		13.3 – 38.2 (26.0)	μmol/100 g fw	3	[20]
		0.2 – 2.0 (1.0)	mg/100 g fw	3	[15]
	Progoitrin	2.2 – 39.7 (24.3)	μmol/100 g fw	3	[20]
		0.1 – 1.0 (0.4)	mg/100 g fw	3	[15]
	Indole	2.7 – 4.7 (4.1)	mg/100 g fw	3	[21]
		36.6 – 64.9 (48.7)	μmol/100 g fw	3	[20]
		0.9 – 1.8 (1.3)	mg/100 g fw	3	[15]
	Aromatic	1.1 – 2.3 (1.9)	mg/100 g fw	3	[21]
		8.3 – 15.4 (11.7)	μmol/100 g fw	3	[20]
		0.8 – 2.0 (1.3)	mg/100 g fw	3	[15]
Mustard spinach (*B. rapa* ssp. *nippo-sinica*)	Total	4.7 – 32.2 (18.3)	mg/100 g fw	2	[15]
	Aliphatic				
	Gluconapin	1.4 – 21.7 (7.1)	mg/100 g fw	2	[15]
	Indole	0.9 – 3.6 (1.9)	mg/100 g fw	2	[15]
	Aromatic	0.5 – 2.3 (2.0)	mg/100 g fw	2	[15]

Table 2. Continued

Botanical classification	GLS group	Range (means)	Unit	No.	Ref.
Mustard green (*Brassica juncea*)	Total	25.7 – 112.6 (53.3)	mg/100 g fw	2	[15]
		350.0 – 618.5 (482.2)	μmol/100 g fw	3	[20]
	Aliphatic				
	Sinigrin	0.0 – 568.2 (330.3)	μmol/100 g fw	2	[20]
		23.5 – 102.7 (48.2)	mg/100 g fw	2	[15]
	Gluconapin	21.6 – 252.3 (96.8)	μmol/100 g fw	2	[20]
		0.4 – 4.3 (1.7)	mg/100 g fw	2	[15]
	Indole	27.6 – 44.6 (34.1)	μmol/100 g fw	2	[20]
		1.1 – 2.7 (1.8)	mg/100 g fw	2	[15]
	Aromatic	3.2 – 11.0 (6.9)	mg/100 g fw	3	[20]
		0.5 – 2.7 (1.6)	mg/100 g fw	2	[15]
Ethiopian Kale (*Brassica carinata*)	Total	18.0 – 45.4 (31.5)	mg/100 g fw	4	[23]
	Aliphatic				
	Sinigrin	17.6 – 44.8 (30.9)	mg/100 g fw	4	[23]
	Indole	0.4 – 0.8 (0.5)	mg/100 g fw	4	[23]
Rocket (*Eruca sativa*)	Total	8.7 – 12.8	mg/g dw	1	[24]
		11.0	μmol/g dw	1	[25]
	Aliphatic				
	4-Mercaptobutyl	51.6	μmol/g dw	1	[25]
	Glucoraphanin	2.2 – 4.4	mg/g dw	1	[24]
		1.3	μmol/g dw	1	[25]
	Glucoerucin	2.2 – 4.6	mg/g dw	1	[24]
		3.3	μmol/g dw	1	[25]
	Indole	0.1 – 0.3	mg/g dw	1	[24]
		0.5	μmol/g dw	1	[25]
Immature flower vegetables					
Green broccoli (*B. oleracea* var. *italica*)	Total	0.6 – 35.6 (12.8)	μmol/g dw	50	[12]
		15.2 – 59.3	μmol/g dw	11	[26]
		4.6 – 26.9 (15.8)	mmol/g dw	10	[27]
		3.0 – 28.3 (10)	μmol/g dw	14	[28]
		2.5 – 18.6 (10.6)	μmol/g dw	21	[29]
		23.0 – 64.6 (42.7)	mg/100 g fw	3	[30]
		18.9 – 25.2 (21.4)	μmol/g dw	2	[19]
	Aliphatic				
	Glucoraphanin	0.8 – 21.7 (7.1)	μmol/g dw	50	[12]
		4.5 – 28.5	μmol/g dw	11	[26]
		2.4 – 18.4 (15.7)	mmol/g dw	10	[27]
		1.3 – 8.3 (4.0)	μmol/g dw	14	[28]
		0.3 – 12.6 (4.6)	μmol/g dw	21	[29]
		24 – 185 (95)	μmol/100 g fw	32	[31]
		11.6 – 34.0 (22.2)	mg/100 g fw	3	[30]
		4.1 – 14.9 (10.5)	μmol/g dw	2	[19]
		0.37 – 4.7 (2.2)	μmol/g dw	9	[32]
	Indole	0.4 – 6.2 (1.9)	μmol/g dw	50	[12]
		8.6 – 17	μmol/g dw	11	[26]
		1.0 – 4.9 (3.1)	mmol/g dw	10	[27]
		1.8 – 20 (6.4)	μmol/g dw	14	[28]
		0.8 – 5.6 (3.2)	μmol/g dw	21	[29]
		10.5 – 15.2 (13.6)	mg/100 g fw	3	[30]
		6.7 – 14.9 (10.7)	μmol/g dw	2	[19]
Purple broccoli (*B. oleracea* var. *italica*)	Total	26.3	mg/100 g fw	1	[30]
	Aliphatic				
	Glucoraphanin	6.7	mg/100 g fw	1	[30]
	Glucoiberin	3.8	mg/100 g fw	1	[30]
	Indole	14.3	mg/100 g fw	1	[30]
White cauliflower (*B. oleracea* var. *botrytis*)	Total	(15.1)	μmol/g dw	3	[12]
		19.5 – 42.6	mg/100 g fw	1	[11]

Table 2. Continued

Botanical classification	GLS group	Range (means)	Unit	No.	Ref.
		14.1	mg/100 g fw	1	[30]
		9 – 18.2 (13.2)	μmol/g dw	2	[19]
	Aliphatic				
	Sinigrin	5.7 – 12.9 (9.3)	μmol/g dw	3	[12]
		1.4 – 5.9	mg/100 g fw	1	[11]
		3.4	mg/100 g fw	1	[30]
		0.4 – 4.6 (2.0)	μmol g dw	2	[19]
	Glucoiberin	0.5 – 6.6	mg/100 g fw	1	[11]
		2.9	mg/100 g fw	1	[30]
	Glucoibervirin	0.6 – 2.9	mg/100 g fw	1	[11]
		1.0	mg/100 g fw	1	[30]
	Indole	2.7 – 6.1 (4.1)	μmol/g dw	3	[12]
		15.2 – 24.9	mg/100 g fw	1	[11]
		5.6	mg/100 g fw	1	[30]
		5.0 – 14.0 (9.0)	μmol/g dw	2	[19]
Green cauliflower (*B. oleracea* var. *botrytis*)	Total	17.6 – 46.9 (32.2)	mg/100 g fw	2	[30]
	Aliphatic				
	Glucoiberin	1.2 – 27.7 (14.5)	mg/100 g fw	2	[30]
	Glucoibervirin	0.0 – 4.8 (2.4)	mg/100 g fw	2	[30]
	Indole	10.4 – 11.6 (11.0)	mg/100 g fw	2	[30]
Purple cauliflower (*B. oleracea* var. *botrytis*)	Total	35.69	mg/100 g fw	1	[30]
	Aliphatic				
	Glucoraphanin	11.6	mg/100 g fw	1	[30]
	Glucoiberin	4.6	mg/100 g fw	1	[30]
	Indole	17.7	mg/100 g fw	1	[30]
Mature flower vegetables					
Chinese broccoli (*B. rapa* var. *alboglabra*)	Total	149.4	mg/100 g fw	1	[30]
		420.0	μmol/100 g fw	1	[20]
	Aliphatic				
	Gluconapin	76.0	mg/100 g fw	1	[30]
		146.7	μmol/100 g fw	1	[20]
	Glucoraphanin	39.7	mg/100 g fw	1	[30]
		118.9	μmol/100 g fw	1	[20]
	Progoitrin	19.1	mg/100 g fw	1	[30]
	Indole	9.93	mg/100 g fw	1	[30]
		127.0	μmol/100 g fw	1	[20]
Sprouts					
Green broccoli (*B. oleracea* var. *italica*)	Total	24.2 – 56.1	μmol/g dw	1	[33]
		29.2 – 81.7	μmol/g dw	1	[34]
	Aliphatic				
	Glucoraphanin	23.3 – 67.6	μmol/g dw	1	[34]
		11.1 – 28.7	μmol/g dw	1	[33]
		17.4 – 49.5	μmol/g dw	1	[34]
	Glucoiberin	4.7 – 12.5	μmol/g dw	1	[33]
		5.9 – 18.1	μmol/g dw	1	[34]
	Indole	7.7 – 14.0	μmol/g dw	1	[34]

No., number of investigated cultivars; fw, fresh weight; dw, dry weight. Aromatic GLS comprises exclusively gluconasturtiin.

vegetables, largely overstated, there has undoubtedly been a trend towards more mildly tasting *Brassica* vegetables over the last few decades. Thus, while there has been a change in consumer preference for specific types of *Brassica*'s, such as a trend away from cabbage through cauliflowers to heading broccoli, recent cultivars of certain types of *Brassica* vegetables have probably been bred for milder flavour by indirect selection against certain GLSs, namely 2-propenyl and 3-butenyl GLSs occurring in certain cabbages and Brussels sprouts. This has led to either selection for low levels of these GLSs, or the same level of structural different GLSs that do not have a significant effect upon flavour, notably 3-methylsulphinylpropyl and 4-methylsulphinylbutyl GLSs. Likewise, it is conceivable that selection for

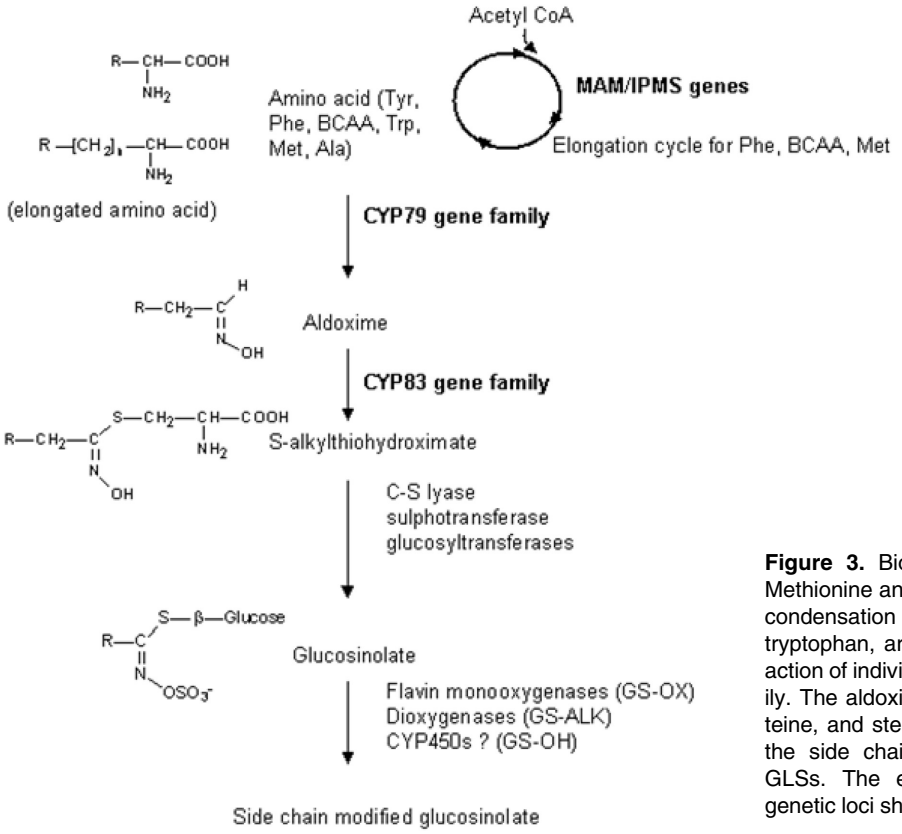


Figure 3. Biochemical model for GLS synthesis. Methionine and phenylalanine are elongated through condensation with acetyl CoA and then, along with tryptophan, are converted to aldoximes through the action of individual members of the CYP79 gene family. The aldoxime undergoes condensation with cysteine, and stepwise converted to GLSs, followed by the side chain modification of methionine-derived GLSs. The enzymes shown above underlie the genetic loci shown in Fig. 4.

resistance to certain herbivores may have led to indirect selection for certain GLS profiles, although there is little documented evidence. In contrast, there has been direct selection for higher levels of 3-methylsulphinylpropyl and 4-methylsulphinylbutyl GLS in broccoli, specifically to explore whether cultivars with higher levels of these GLSs can provide enhanced health benefits, discussed in greater detail below.

3.1.1 Genetic basis for GLS accumulation and diversity

Breeding for altered GLS profiles and content does not specifically require an understanding of the biochemical pathways and relationship between different metabolites – breeders can empirical select for the desired biochemical or flavour profile without necessarily understanding the underlying genetic basis. However, the recent advance in knowledge of the biochemical and molecular genetic basis of GLS biosynthesis has enabled a more systemic approach to breeding, and has also provided an explanation of the diversity of GLS profiles that occur in *Brassica* vegetables.

3.1.2 Molecular genetics and biochemistry of GLS biosynthesis

Major advances in the understanding of GLS biosynthesis over the last decade through the use of the 'model' plant species *Arabidopsis thaliana* has enabled a molecular genetic dissection of the biosynthetic pathway. This has resolved several aspects of the biochemistry of GLSs that had proved intractable *via* a biochemical approach, and has enabled the identification of genes coding for structural enzymes within the biochemical pathway. Knowledge of these genes may enable the design of molecular makers for use in breeding programs. Attention has now turned to identifying various transcription factors that seem likely to regulate the coordinated expression of these genes. The initial step in biosynthesis is the conversion of either a primary amino acid or a chain elongated amino acid (see below) to an aldoxime (Fig. 3), through the activity of gene products of the CYP79 gene family, each of which has substrate specificity for different amino acid precursors. For example, within *Arabidopsis*, the products of CYP79F1 and F2 catalyse the conversion of elongated homologues of methionine to the corresponding aldoximes [45], CYP79B2 and CYP79B3 convert tryptophan to their aldoximes [46] and CYP79A2 convert phenylalanine to its aldoxime [47]. The aldoxime conjugates with cysteine which acts as the sulphur donor, and then cleaved by a C – S lyase [48]. The resultant potential toxic thiohydroximates are 'detoxified' by glycosylation by a soluble UDPG/thiohydroximate glucosyltransferase (S-GT) to produce a desulphoglucosinolate and sulphation by a soluble 3′-phosphoadenosine 5′-phosphosulphate (PAPS): desulphoglucosinolate sulphotransferase. In contrast to the CYP79 enzymes, these latter steps exhibit

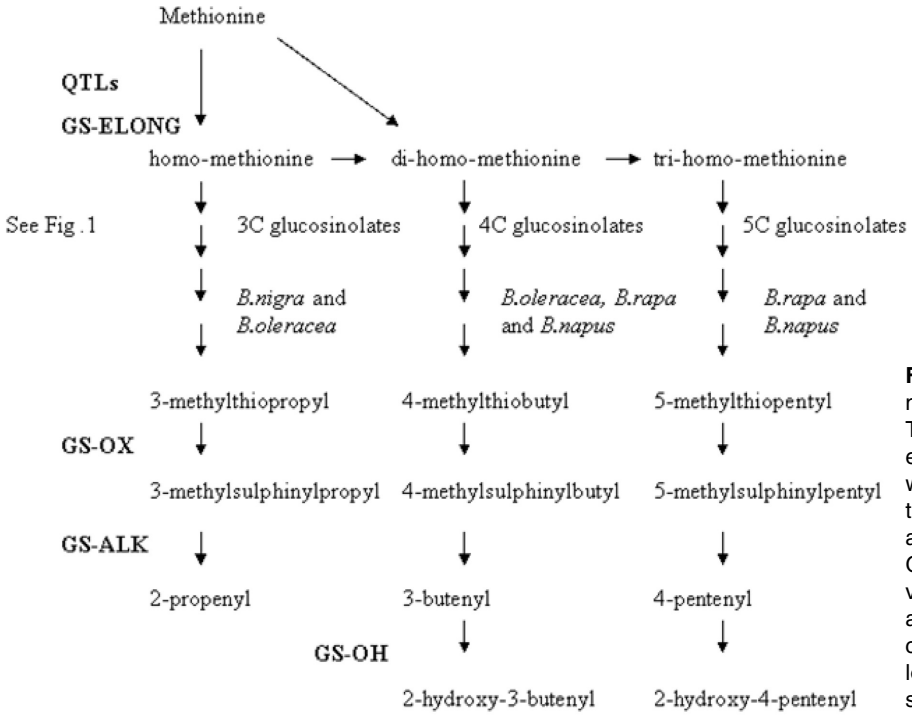


Figure 4. A working genetic model of methionine-derived GLS biosynthesis. The total level of GLSs is determined early in biosynthesis, and is associated with the initial entry of methionine into the pathway catalysed by MAM genes at GLS-ELONG loci. Subsequently, GLS profiles are determined by allelic variation at the GLS-OX, GLS-ALK, and GLS-OH loci. In general, there is considerably more variation at these loci in *B. oleracea* that *B. rapa*, making selection for specific profiles possible.

no specificity towards the nature of the amino acid precursor.

Many of GLSs found within *Brassica* vegetables are derived from chain elongated forms of methionine or phenylalanine (Fig. 3). Biochemical studies, involving the administering of ^{14}C-labelled acetate and ^{14}C-labelled amino acids and subsequent analysis of the labelled GLSs [49], suggests that amino acid elongation is similar to that which occurs in the synthesis of leucine from 2-keto-3-methylbutanoic acid and acetyl CoA. The amino acid is transaminated to produce a α-keto acid, followed by condensation with acetyl CoA, isomerisation involving a shift in the hydroxyl group and oxidative decarboxylation to result in an elongated keto acid which is transaminated to form the elongated amino acid. The elongated keto acid can undergo further condensations with acetyl CoA to result in multiple chain elongations. Studies in *Arabidopsis* have identified genes similar to isopropylmalate synthase as being particularly important in determining the extent of chain elongation of methionine prior to GLS synthesis. These methylthioalkylmalate (MAM) synthases catalyse the condensation of acetyl CoA, as the methyl donor, with a α-keto acid derived by amino acid transanimation. Different members of this family can catalyse different numbers of rounds of elongation [50 – 52], due to different specificities for the length of the α-keto acid.

Following GLS synthesis, the side chains can be modified by hydroxylation, methoxylation, oxidation, desaturation, conjugation with benzoic acid and glycosylation. Some of these modification genes have been characterised as 2-oxogluturate dependent dioxygenases [53, 54] but other types of genes are also involved [55].

3.1.3 Breeding for altered GLS content in *Brassica* vegetables

With regard to methionine-derived GLSs, two processes act independently from each other to determine the GLS content in *Brassica* crops. Firstly, that determining the types of GLSs and secondly, that determining the overall amount of these GLSs. The first of these processes is under strict genetic control. Thus, a particular genotype will express the same ratio of GLS side chains when grown in different environments, although the overall level may vary considerably. In *B. oleacea*, a series of Mendelian genes have been identified and located on linkage maps that determine the length and chemical structure of the side chain [44, 53, 56 – 58]. The underlying genes are likely to correspond directly to cloned genes that have been functionally analysed in *Arabidopsis*, described above. In addition, quantitative trait loci (QTLs) have been identified that determine the overall level of GLSs in both *B. napus* [59, 60] and *B. oleracea* [61, 62]. The genetic regulation of indolyl glucoisnolates derived from tryptophan is far less advanced. This is partly due to the considerable greater effect the environment has on the levels and proportions of the different indolyl GLSs making genetic analyses more complex.

Schematically, GLS biosynthesis can be represented as shown in Fig. 4. This provides a suitable working model for breeding, and which can be integrated with the more biochemical model shown in Fig. 3.

Genetic variation at the different GLS loci enables selection for different profiles, while allelic variation at the QTLs determine overall amount. For methionine-derived GLSs, the overall level is determined either by the initial entry of methionine into the pathway, possibly through the activity of MAM genes at GLS-ELONG loci, which are coincident with QTLs determining levels in both *Arabidopsis* and *Brassica* [50, 62]. Mendelian genes determine the length of the aliphatic side chain, so that in *B. oleracea* there is either one round of methionine elongation leading to 3C GLSs, such as 3-methylsulphinylpropyl and 2-propenyl, or two rounds of elongation leading to 4C GLSs, such as 4-methylsulphinylbutyl or 3-butenyl GLS. In contrast, *B. rapa* and *B. napus* do not manufacture 3C GLSs but accumulate either just 4C GLSs, or both 4C and 5C GLSs. These chain elongated methionine homologues provide the structural basis for side chain modifications after the synthesis of the common glycone moiety. Thus, the methylthioalkyl GLSs are converted by flavin monoxygenases at the GLS-OX loci [55] to methylsulphinylalkyl GLSs, which are in turn modified to alkenyl and hydroxyl-alkenylglucosinolates through 2-oxogluturate dependent dioxygenases at the GLS-ALK [53], and unknown enzymes (but possible CYP450s) at the GLS-OH loci, respectively. One can consider blocks in the pathway, so that specific GLSs accumulates as endpoints in the pathway. For example, methylsulphinylalkyl GLS accumulate in broccoli as this botanical variety lacks functional alleles at the GLS-ALK locus [44]. Other forms of *B. oleracea* with a functional GLS-ALK allele, and all forms of *B. rapa* and *B. napus* synthesis alkenyl GLSs, and, potentially hydroxyl–alkenyl GLSs. Thus, the interaction of alleles at QTLs regulating overall levels interact with those determining side chain length and subsequent chain modifications to determine the overall GLS content.

3.1.4 Sources of variation

Existing *Brassica* vegetable cultivars provide a valuable source of genetic variation. In general, there is far more variation in both amount and diversity of GLSs in *B. oleracea*, including the related wild $n = 9$ forms [44, 63], as opposed to *B. rapa*, in which all genotypes have at least one functional *ALK* allele resulting in only alkenyl or hydroxyalkenyl GLSs accumulating in tissues. There is also considerable variation in overall amount of GLSs in *B. oleracea*, enabling there to be selection for both higher levels – for example to enhance those with specific health attributes, or lower levels of certain GLSs that may contribute to undesirable flavour attributes.

3.1.5 Breeding for higher levels of GLSs in broccoli

The best example of deliberate breeding for high level of health promoting GLSs is probably selection in broccoli for higher levels of 3-methylsulphinylpropyl and 4-methylsulphinylbutyl GLSs [61–63], the precursors of the isothiocyanates iberin and SFN, respectively. This was achieved by crossing a standard cultivar with *B. villosa*, a wild forms of *B. oleracea* from Sicily, which accumulated high levels of 3-methylthiopropyl GLS in flower buds As expected, the hybrid had high levels of the target GLS, 4-methylsulphinylbutyl, due to the interaction of genes within the two parents, and a series of backcross introgressed two regions of the *B. villosa* genome that contained relevant QTLs for high GLS content into a commercial agronomic heading broccoli background. These high GLS broccoli cultivars have subsequently been used in human intervention trials, and shown to deliver about four times the amount of SFN to the systemic circulation than standard cultivars [64]. It is important to note that the isothiocyanates derived from these GLS contribute little to flavour, so while it is practically possible to enhance their levels, increasing levels of certain other GLSs that result in more pungent iosthiocyanates, such as 2-propenyl or 3-butenyl, may not be desirable.

3.1.6 Future challenges for breeders

Developing breeding lines with specific GLS profiles that are similar to those which occur within the cultivated *Brassica* species and their immediate wild relatives is relatively easy. The genetics basis to altering GLS profiles is relatively simple and it is possible to predict rates of recombination to obtain specific profiles. Likewise, while total levels are determined by a small number of QTLs, they are relatively easy to select for within a breeding programme, if sufficient analytical capacity if available. Molecular markers for both Mendelian genes and QTLs are available. The two major challenges are, firstly, to be able to rapidly recover agronomic characters such as heading times and appearance that are of critical importance within horticultural crops, and, secondly, to obtain GLS profiles that do not occur within the specific biological species. For example, currently, it is not possible to select for methylsulphinylalkyl GLSs within *B. rapa*, as all known genotypes of this species have functional GLS-ALK alleles, resulting in the accumulation of alkenyl GLSs. Likewise, it would be interesting to introduce some of the long chain methionine-derived GLSs, such as 7-methylsulphinylheptyl GLS found in watercress [65] into horticultural forms of *B. oleracea* and *B. rapa*, but the required MAM alleles for methionine elongation to do not seem to be present within these species. Whether it will be possible to derive these profiles through genetic modification or interspecific and intergeneric recombination remains to be seen. Finally, while there have been considerable advances in breeding for methionine-derived GLSs, breeding for specific indolyl GLS derived from tryptophan appears much more problematic.

3.2 Cultivation

In addition to the genetic influence, ecophysiological parameters such as climate factors, *e.g*. irradiation and temperature, as well as nutrition and water supply also affect GLS content. Moreover, application of certain chemical agents can also enhance GLS levels. Thus, all of these factors can influence GLS content and composition, and therefore play a role in determining final GLS levels both at preharvest and harvest [66].

3.2.1 Climate factors

Climate factors such as temperature, radiation, and photoperiod have been reported to affect GLS concentration [67 – 70].

A few studies have even examined and identified an interaction between genotype and climate factors on GLS concentration in broccoli [71] and in Chinese cabbage [22], and conclude that the effect of genotype was greater than that of climate factors. For example, 60% of aliphatic GLS synthesis was reported to be regulated by genotype, whereas 33 and 21% of total indole GLS content can be explained by climate factors alone and genotype × climate factors, respectively [27]. In addition, total and individual GLS levels in 11 broccoli cultivars were generally higher in late (August – January) compared to early (April – July) seasons; however, primary inflorescences harvested in June (early crop) generally contained the highest GLS levels overall [26]. This was proposed to be because of advantageous climate factors, *e.g*. lower temperatures compared to July. Higher GLS levels at lower temperatures in different seasons were also found for broccoli and cauliflower [30] and Asian turnip [15]. Moreover, watercress plants grown under long day conditions and at temperatures of 10 or 15°C had at least 50% higher gluconasturtiin concentrations compared to plants grown at 20 or 25°C [72]. However, higher temperatures (>30°C) induced stress in cabbage during head development and resulted in enhanced GLS levels [18]. Therefore, GLS biosynthesis seems to undergo dynamic changes in response to temperature. In *B. oleracea* leaves, the concentration of total and aliphatic GLSs was 44 and 45% higher at 12°C and 114 and 125% higher at 32°C, respectively, compared to levels recorded at 22°C under constant light conditions. Moreover, these levels are directly correlated with myrosinase activity on a fresh weight basis [74]. Further, Pereira *et al*. [34] also reported that in two cultivars of broccoli sprouts, higher concentrations of total and aliphatic GLSs were present at 11 and 33°C than at intermediate temperatures.

Currently, there are only a few reports that statistically correlate GLS levels with specific climatic factors in mature vegetables using linear and quadratic terms [19, 75, 76]. Greenhouse-grown broccoli was cultivated after head induction at three different daily mean temperatures (in the range from 7.2 to 19.7°C) under two different daily mean radiation levels (in the range from 1.9 to 13.4 mol m^{-2} day^{-1}) [76]. Broccoli grown in temperatures of ≤12°C combined with increasing radiation produced high contents of alkyl GLSs (especially glucoraphanin). In contrast, high contents of the indole GLS glucobrassicin were found under high temperatures (>18°C) and low radiation (<6 mol m^{-2} day^{-1}) conditions. A reason for the different responses among the GLS groups could be because the various enzymes involved in each GLS' synthesis are affected differently by temperature and radiation. For example, the alkyl GLSs are derived from methionine *via* flavin-containing monooxygenases which are light dependent [77]. The conversion of tryptophan to indole GLS is catalysed by peroxidases, which are rather temperature dependent [78]. In five botanical groups of *B. oleracea*, high concentrations of both total and indole GLSs generally corresponded to cultivation at higher temperatures and photosynthetic photon flux (PPF) as well as to longer day length [19]. Total and indole GLS concentrations had negative linear but positive quadratic relationships with temperature and day length, and positive linear but negative quadratic relationships with PPF. Glucoraphanin concentrations were only influenced by PPF and day length.

Besides temperature, PPF, and photoperiod, GLS concentrations can also be increased by exposing growing plants to red light [72] and elevated atmospheric CO_2. Elevated atmospheric CO_2 (685 – 820 ppm) in comparison to ambient CO_2 (430 – 480 ppm) concentration increased the total and aliphatic GLS (glucoraphanin and glucoiberin) levels in broccoli, while indole GLSs decreased [79]. Moreover, changing N content and N/S ratios under different atmospheric CO_2 concentrations as well as alterations in photochemical processes within the plant's photosynthetic system increased C content and could influence the contents of total and individual GLSs. Finally, a decrease in indole GLS levels was also found in cabbage seedlings leaves, while aliphatic GLS content remained unaffected at elevated CO_2 concentrations (720 ppm) [80].

3.2.2 Nutrient supply

Generally, GLS content and composition can be influenced by S, N, and Se supply. While no difference in total GLS content in broccoli grown between 15 and 150 kg/ha S fertilisation conditions was detected [81], S fertilisation between 23 and 92 kg/ha enhanced the glucoraphanin content in broccoli heads [82]. When broccoli plants were fed with a S supply from 0.075 to 1 g/plant, an increasing S supply of up to 0.6 g S *per* plant, led to an increasing overall total GLS content in broccoli [83]. This effect was mainly caused by the alkyl GLS glucoraphanin derived from the S-containing amino acid methionine which needs free inorganic sulphur for its biosynthesis, and less so by the indole GLSs where inorganic sulphur is only needed when tryptophan is converted *via* indolic thiohydroximate formation into indole GLSs.

In contrast for N supply, Krumbein *et al*. [83] observed a 70% reduction of the alkyl GLSs glucoraphanin and glucoiberin in broccoli supplied with 200 kg N/ha in comparison with plants receiving no N fertilisation. GLSs in rocket salad were also influenced by the ammonium-nitrogen to nitrate-nitrogen ratio [24].

Total GLSs and glucobrassicin concentrations in cabbage were maximised at low N (125 kg/ha) and high S (125 kg/ha) supply without N × S supply interaction [84].

Balance between N and S supply also played an important role in the regulation of the GLS synthesis in turnip [85, 86], pakchoi [87], and broccoli [88]. In broccoli grown under controlled experimental conditions, total GLS concentrations were high at insufficient N supply independent of the S level and low at insufficient S supply in combination with optimal N supply, mainly due to the presence of the alkyl GLSs glucoraphanin and glucoiberin [88]. Furthermore, with S supply above 6 g/kg dry matter and an N/S ratio lower than 10:1, GLS concentrations were on average around 0.33 g/kg fresh matter and differed significantly from those plants characterised by higher N/S ratios [88]. In contrast to aliphatic GLSs, indole GLS levels were highest at high N and S supply [85, 87, 88]. Assumingly, this observed effect is because plants assimilate inorganic sulphate into cysteine that is subsequently converted into methionine [89], and this reduction step is regulated by N content [90]. Furthermore, the *de novo* synthesis of indole GLSs from tryptophan is limited by the thiohydroximate sulphur donor (*e.g*. cysteine or methionine) [89].

In the case of Se, increased Se fertilisation was found to decrease GLS production and this was attributed to competitive Se and S uptake by the plant [91, 92].

3.2.3 Water supply

Water stress is known to increase GLS content in watercress [93], Portuguese cabbage [94], and red cabbage [73]. Low rainfall during the vegetation period increased GLS content in most of the *Brassica* vegetables studied to date, *e.g*. cabbage, Brussels sprouts, kale, cauliflower, and kohlrabi [26, 73, 81], but decreased GLS content in red radish roots [11]. In broccoli, SFN content was reported to double as a response to reduced water supply [95]. In contrast, Robbins *et al*. [92] found that water stress reduced total GLS and also glucoraphanin content, the precursor of SFN. These strongly contradicting results suggest that in broccoli, GLS content is also modified by other environmental conditions besides water supply.

Zhang *et al*. [96], for example, found that the influence of water supply on GLS content in turnip roots (*Brassica rapa* ssp. *rapa*) is largely dependent on S content. Changes in (i) S availability in soil [97], (ii) S uptake by roots at different stages of growth [98, 99], and (iii) high-affinity S transporter gene expression that is primarily regulated by S supply [100] as triggered by varied water supply all might contribute to differences in S content, and hence in higher GLS contents under reduced water supply.

3.2.4 Application of chemical agents

Currently, only limited information is available on the effects on GLS content due to preharvest application of chemical agents such as amino acids and signalling molecules.

There are almost no reports on the effect of methionine fertilisation on the GLS content in vegetable crops despite methionine being a precursor in alkyl and alkenyl GLS syntheses [101]. However, methionine application *via* foliar fertilisation [102] or leaf stalk infusion increased the methylsulphinyl GLSs glucoraphanin and glucoiberin in broccoli by up to 16% [103], but methionine foliar spraying had no effect on aliphatic GLS content in radish roots [102]. Thus, it seems that the influence of methionine on aliphatic GLSs may differ between vegetable types, *e.g*. inflorescence versus root vegetable.

Salicylic acid (SA) and methyl jasmonate (MJ) serve as signalling molecules and are induced by pathogen infestation [104] and mechanical wounding [105]. These elicitors trigger signal cascades that activate several defence responses such as the synthesis of phytochemicals, *e.g*. GLSs [106]. In Teltow turnip (*B. rapa* ssp. *rapifera*), treatment with either SA or MJ increased total GLS yields mainly due to increases of aromatic gluconasturtiin and indole GLSs, especially in the secondary roots and exudates [107]. Moreover, Kiddle *et al*. [108] reported that gluconasturtiin biosynthesis was also induced by SA. The increase of gluconasturtiin in all plants parts (leaves, roots, exudates) after SA and MJ application might be explained by elicitor induction of CYP79A2 [109, 110] that converts phenylalanine to aromatic aldoxime and is equally expressed in leaves and roots [110]. Moreover, individual SA and MJ application lead to increased indole GLS content in Teltow turnip with SA influencing glucobrassicin and 4-methoxy-glucobrassicin content more strongly than MJ in both plants and exudates. However, neoglucobrassicin content was most positively influenced by both elicitors in all plant parts.

3.3 Storage and packaging

There are several interpretations of the expression ‘Fresh’ vegetables. Fresh is used for vegetables just picked from the garden, but also for vegetables bought from the shop. Even after some days by the consumer, vegetables are still called ‘fresh’. Obviously, time, temperature, humidity and gas conditions are very important parameters for maintaining quality after harvest. In this respect, transport and storage are very important steps in the logistic chain between harvest and consumer's purchase. However, the conditions during logistics are optimised for visual quality as freshness, colour and appearance but not for high retention of phytochemical levels.

Vegetables belonging to the *Brassica* family have a broad variety of external appearances and associated variation in shelf life. Broccoli is a very perishable vegetable and postharvest senescence results in loss of chlorophyll, deterioration of cellular structure, degradation of macromolecules and mobilisation of nutrients rapidly after harvest [111]. Storage at cooler temperatures delayed the symptoms of senescence at the biochemical and gene expression levels. In general, GLS levels mirror visual quality in broccoli as they usually decrease during postharvest handling. Cabbage and Brussels sprouts have a much longer shelf life.

3.3.1 Storage

Time and temperature during storage of *Brassica* vegetables have been shown to affect the GLS levels in different ways. The aliphatic GLS glucoraphanin and the indole GLS glucobrassicin are the most prominent ones present in broccoli. Rodrigues and Rosa [112] evaluated GLSs levels in the principal and secondary inflorescences of fresh broccoli and after various postharvest treatments. Inflorescences stored for 5 days at 4°C showed a decrease in total GLSs of 16 and 4%, respectively, for the principal and secondary inflorescences. However, a strong decrease was observed when broccoli was left at room temperature (20°C) for 5 days (79% for principal inflorescences and 64% secondary inflorescences). The glucoraphanin content in broccoli florets declined by 82% after 5 days at 20°C, but was lowered only 31% at 4°C [112].

Rangkadilok *et al*. [113] reported approximately 50% decrease of glucoraphanin in broccoli heads after 7 days at 20°C stored in plastic bags as well as in open air, but no decrease was found after 7 days storage at 4°C. On the other hand, Vallejo *et al*. [114] showed a reduction of glucoraphanin for almost 50% in broccoli stored for 7 days at 1°C. Prolonged storage of the broccoli at retail conditions (3 more days at 15°C) lowered the glucoraphanin concentration in total with 65%.

In contrast, indole GLSs increased in concentration during 9 days storage at 10°C in broccoli florets [9]. Similarly, the indoles 4-hydroxyglucobrassicin and 4-methoxyglucobrassicin increased significantly after chopping and 48 h storage of broccoli at ambient temperature, while all other GLSs decreased [115].

A high relative humidity (RH) of 98 – 100% is recommended to maintain postharvest quality in broccoli. However, RH appears to be a critical factor in GLS retention when postharvest temperatures rise above approximately 4°C [116].

3.3.2 Controlled and modified atmosphere

Controlled atmosphere (CA) storage and modified atmosphere packaging (MAP) is very effective in maintaining quality of *Brassica* vegetables in order to extend their marketability [117, 118]. At present, only limited information is available on the postharvest GLS dynamics of highly consumed *Brassica* vegetables stored under CA or packed in modified atmosphere.

Radishes stored in modified atmosphere (8% O_2 + 5% CO_2) and also mini broccoli heads packed at 1% O_2 + 21% CO_2 showed after an initial decrease an accumulation of aliphatic GLSs after 5 and 7 days of storage, respectively, at temperatures between 8 and 10°C [119, 120]. Moreover, the glucoraphanin and glucoiberin contents of mature broccoli heads stored in a CA (0.5% O_2 + 20% CO_2) were reported to increase tendentiously during 7 days of storage [9]. Hansen *et al*. [9] proposed that this increase could be associated with enhanced levels of metabolites (*e.g.* amino acids, amines) being available for a *de novo* GLS biosynthesis that originated from the decomposition of other compounds. It is assumed that the increase in GLS content by a *de novo* biosynthesis in controlled and modified atmospheres is a stress response due to the increased CO_2 and decreased O_2 concentrations. The hypothesis of stress-induced accumulation of GLSs is supported by Bennett and Wallsgrove [121], who detected increased levels of GLSs due to environmental impact. The oxygen dependence of the cytochrome P450-dependent monooxygenases of the CYP 79 family catalysing the formation of aliphatic aldoxime – a key regulatory step in aliphatic GLS biosynthesis [122, 123] – seems not to be a limiting factor in mature broccoli for the *de novo* biosynthesis in postharvest, since an O_2 level of 0.5% enables an increase of aliphatic GLSs. Regarding the decreasing contents of glucoraphanin and glucoiberin of mini broccoli at very low O_2 concentration of 1%, it could be assumed that younger broccoli heads have a more pronounced O_2 sensibility than mature broccoli heads [120]. Kays [124] also stated that susceptibility to low O_2 conditions is related to the product's nature such as the stage of development.

In contrast, Rangkadilok *et al*. [113] found in broccoli stored at 4°C under CA conditions (1.5% O_2 + 6% CO_2) for up to 25 days or stored in MAP (0.2% O_2 + 15% CO_2) for up to 10 days no significant changes in the main aliphatic GLS glucoraphanin. Moreover, Vallejo *et al*. [114] demonstrated a distinct decrease of aliphatic and indole GLSs by 71% in low-density polyethylene film-wrapped broccoli (17% O_2 + 3% CO_2) within 7 days at 1°C. These results indicate that enhanced CO_2 concentrations are necessary for preventing loss in GLSs, even when the storage temperature is very low at 1°C. However, regarding the decreased content of aliphatic GLSs in mini broccoli and mini cauliflower at 1% O_2 + 21% CO_2, strongly enhanced CO_2 concentrations (21%) should be precautionary avoided for preventing degradation of aliphatic GLSs in mini broccoli and mini cauliflower.

Mature broccoli heads stored at 0.5% O_2 + 20% CO_2 [9] and in modified atmosphere packed mini cauliflower (1% O_2 + 21% CO_2) [120] showed a increasing or unchanged contents of indole GLSs, respectively, while mature broccoli in MAP with low CO_2 levels (3%) showed a decrease in

contents for all individual indole GLSs, particularly for neoglucobrasscin [114].

The application of starch coating at untopped radishes inducing internal altered gas atmospheres in the product itself has only limited preserving effects. A degradation of total GLSs mainly due to the alkenyl GLSs could not be avoided, even if starch coating reduced the respiration rate [125].

Degradation of GLSs is caused by GLS hydrolysis catalysed by myrosinase which is activated by tissue damage or loss of cell integrity during product senescence [8, 35]. Chong and Berard [126] have already reported that cold-stored cabbage showed a rapid decline of GLSs at the beginning of product senescence. However, in mini broccoli and mini cauliflower under modified atmosphere no senescence symptoms, *e.g.* colour changes, were visible. Thus, it could be assumed that the decreasing GLS contents should not related to myrosinase activity, but to GLS transport processes. As shown by *A. thaliana*, GLSs could be transported by phloem enabling a GLS exchange between the individual plant organs [127, 128]. It is assumed that during 1-wk-packaging GLSs were transported from the florets to the stalks due to changing source – sink relationship induced by enhanced transpiration at the cut stalk edges.

3.4 Industrial and culinary processing

Brassica vegetables are, prior to consumption, subjected to different ways of processing, culinary as well as industrial. Culinary treatments of *Brassica* vegetables as chopping, cooking, steaming, stir-frying and microwaving have received more attention the past years and have been shown to affect the GLS content considerably. Typically, postharvest physical disruption of the plants such as chewing, chopping, blending, juicing, cooking, freezing/thawing, and high temperature leads to cellular disruption and subsequent mixing of GLSs and myrosinase to form isothiocyanates and other BDPs. These processes influence the levels of GLSs, the extent of hydrolysis and the composition, flavour and aroma of the final products.

Processing of *Brassica* vegetables has complex influences on the food matrix affecting the level of GLSs:

(i) enzymatic hydrolysis by myrosinase,
(ii) myrosinase inactivation,
(iii) cell lysis and leaching of GLSs, BDPs and myrosinase in cooking water,
(iv) thermal degradation of GLSs and their BDPs,
(v) increase of the chemical GLS extractability,
(vi) loss of enzymatic cofactors (*e.g.* ascorbic acid, iron).

These different mechanisms are discussed into more detail in Section 7 where an approach of predictive modelling of the mechanisms affecting GLSs during processing has been described. The effects of the various types of processing in relation to these mechanisms have hardly been studied systematically. Moreover, because of the almost infinite variations possible in all the parameters, a systematic approach is needed that is based on modelling techniques.

3.4.1 Chopping/shredding

Chopping of fresh plant tissues creates optimal conditions for myrosinase and a high degree of GLS hydrolysis can be expected. Song and Thornalley [129] showed that fine shredded vegetables (5 mm) markedly declined the GLS content after 6 h at ambient temperature; losses up to 75% of the total GLS content were seen for Brussels sprouts, broccoli and cauliflower and *ca.* 60% for green cabbage. Also, the extent of GLS loss increase with postshredding time. However, the authors stated that when vegetables were shredded into larger pieces, losses of total GLSs remained below 10%. It should be taken into account that water was added to the vegetables after storage (50% w/w) which provokes the autolytic degradation of GLSs.

In contrast to reported findings, Verkerk *et al*. [115] observed elevated levels of all indole and some aliphatic GLSs after chopping and prolonged exposure of *Brassica* vegetables to air. In white cabbage, a 15-fold increase of 4-methoxy and 1-methoxy-3-indolylmethyl GLSs was noted after 48 h stored of chopped cabbage. Chopping and storage of broccoli vegetables resulted in a strong reduction of most GLSs, except for 4-hydroxy- and 4-methoxy-3-indolylmethyl GLSs, which increased 3.5- and 2-fold, respectively. It was hypothesised that chopping triggers a *de novo* synthesis of GLSs, especially indolyl GLSs, by mimicking pest damage as defence mechanism in harvested *Brassica* vegetables [115].

3.4.2 Low temperature

Low-temperature storage processes such as freezing and refrigerating can alter the metabolism of GLSs. Significant loss of GLSs can occur due to freeze-thaw fracture of plant cells and accessibility of myrosinase to GLSs with subsequent enzymatic conversion during thawing. The effect of freezing – thawing without previous inactivation of myrosinase was demonstrated by Song and Thornalley [129] with 33% loss of total GLSs in various *Brassica* vegetables and Quinsac *et al*. [130] with almost complete degradation of GLSs in sprouts of sea kale. Total GLSs presented a high loss rate during cold storage of broccoli, mainly due to decrease of the major GLSs present in broccoli inflorescences namely glucoraphanin (almost 50% decrease), glucobrassicin, and neoglucobrassicin [114].

3.4.3 Fermentation and pickling

The most common fermented *Brassica* product is sauerkraut. Cabbage fermenting probably dates back to the ancient past; it was certainly known in the middle ages. According to documented sources, cabbage was fermented in nearly every household in Germany in the seventeenth

century [131]. Until recently, the effect of cabbage fermentation on the course of GLS hydrolysis and on the content of the products released from GLS was unknown. First data concerning the content of GLS degradation products in fermented cabbage were published in 1980 by Daxenbichler [132]. Yet, due to detection limits of analytical methods at that time Daxenbichler's studies were limited to two compounds determined with GLC method and colorimetric determinations of total content of thiocyanate ions, isothiocyanates and 5-vinyloxazolidine-2-thione. Only in recent years some studies appeared giving a better insight into the GLS fate during cabbage fermentation.

3.4.3.1 Effect of fermentation on direction and rate of GLS hydrolysis

It is not known whether GLS degradation process during cabbage fermentation proceeds in the microbiological, chemical or enzymatic way through the action of native myrosinase, or if perhaps it follows from the interaction of the mentioned possibilities. Neither is it known whether GLS hydrolysis proceeds in plant tissue or if GLS are released from plant tissue along with the juice excreted during the initial fermentation stage and are next hydrolysed elsewhere.

Experimental data seem to indicate that GLS can be hydrolysed already in the initial stage of fermentation [133]. In this stage, lasting 2–3 days, intensive respiration processes of plant tissues proceeded leading to a rapid release of carbon dioxide and environment acidification. In correctly proceeding course of natural fermentation after 4–5 days pH gradually lowers to 3.4–3.7. Conditions favourable for myrosinase action may cause that GLS partly or totally undergo enzymatic degradation. We cannot exclude participation in the initial stage of fermentation of bacterial flora nonspecific for fermenting process in the GLS degradation, either. During spontaneous fermentation, the development/growth of proper bacterial flora takes place in the end of initial stage, when the juice excreted by plant tissues is rich in components, among others sugars, necessary for this development. Intensity of diffusion and plasmolytic processes depends on salt addition and on temperature. Apparently these two physicochemical parameters have the influence on the rate of GLS hydrolysis.

Spontaneous fermentation ends after 7–10 days. If starter bacteria inoculations are used, fermentation can be shortened even to 3 days [134]. Then, GLS degradation might proceed with participation of some strains of milk acid bacteria [135].

The kind of compounds released during GLS hydrolysis depends on a number of factors such as environment pH, presence of Fe^{+3} ions and epitiospecific protein [136] or presence of ascorbic acid [137]. Additionally, in processes undergoing with participation of microorganisms, like fermentation, there should be considered the capability of microorganisms for directed GLS decomposition resulting in release of only one out of several possible products [135].

Cabbage fermented with aliphatic GLS hydrolysis products contained isothiocyanates, nitriles and cyclisation products of some isothiocyanates [133, 134, 138]. In view of literature data, it appears that fermentation favours GLS hydrolysis in the direction of releasing isothiocyanates. Their content in the final product was generally higher than that of respective cyanates [133, 138].

The main product of indole GLS hydrolysis was ascorbigen [133, 139]. This compound is formed in acid environment during the reaction of indole-3-carbinol (I3C) with ascorbic acid [137]. Apart from ascorbigen, fermented cabbage contained also small amounts of ascorbigen dimers and trimers as well as direct products of glucobrassicin hydrolysis – I3C and indole-3-ACN.

The results of previous studies suggest that the kind of GLS hydrolysis products in the final product does not principally depend on the fact if fermentation was spontaneous or controlled through application of various bacteria strains. The factor determining the presence of a given product may rather be the level of native GLS in the raw material. Lack of glucoibervirin or gluconasturtiin derivatives in fermented cabbage obtained by Tolonen *et al*. [134, 138] were due to lack of these compounds in the cabbage used for studies. It should also be mentioned that lack of commercially available standards of GLS degradation products, both aliphatic and indole, causes that not all the compounds, even those coming from decomposition of dominating GLS, may have been analysed by the authors of the quoted studies.

3.4.3.2 Factors determining the amount of GLS decomposition products in fermented cabbage

Fermented cabbage is produced in autumn and consumed throughout the whole winter period. The key issue for consumer is not only the amount of GLS decomposition products after cabbage fermentation but also their stability during storage of fermented cabbage.

It appears obvious that the contents of particular products of GLS hydrolysis in the final product depend on individual GLS contents in the raw material used for fermentation. Yet, the way of conducting fermentation may considerably modify their amount in the final product. Already in the initial fermentation stage intensive excretion of gases may cause losses, especially of volatile products of sinigrin and gluconapin degradation. The losses of these compounds will depend on physicochemical parameters and on the course of fermentation. Application of starter bacteria cultures had a significant effect on the contents of particular compounds. Particularly high differences were found for isothiocyanates from decomposition of sinigrin, glucoiberin and glucoraphanin [134].

As a result, application of various bacteria strains for fermentation resulted in two- to three-fold differences in the total content of decomposition products in sauerkraut [134]. Mean contents of decomposition products in naturally fermented cabbage were lower than mean contents of these compounds in the fermentation initiated by bacteria strains, but the differences were generally statistically nonsignificant [138]. During storage of cabbage for 2 – 17 wk the content of isothiocyanates gradually decreased [133]. The losses of particular compounds were diversified and ranged from 15 to 90%. For cyanates content a different tendency was observed: the content of 1-cyano-3-(methythio)propane increased two-fold between the second and fifth week. In the case of allyl cyanide and 1-cyano-3-(methylsulphinyl)propane, after initial decrease, their contents increased. As a result of various directions of change the total content of aliphatic GLS decomposition products decreased by 30% between the second and seventeenth week. Storing cabbage had no effect on the content of indole compounds from glucobrassicin decomposition [133].

Variety of factors that may influence the content of particular decomposition products causes that relative contents of these compounds expressed as per cent of native GLS content range within a broad bracket. It is worth noticing that there are high relative contents of compounds from glucoraphanin decomposition and relatively high content and stability of ascorbigen which was the main product in stored naturally fermented cabbage [133]. This is especially important since these compounds are ascribed with anticancerogenic properties [140, 141].

The results of previous studies give only fragmentary knowledge on the effect of fermentation on GLS fate. Due to complexity of fermentation process explaining of this problem requires further intensive studies. Perhaps aware obtaining of the final product with high content of compounds desirable from the human health point of view and low content of potentially toxic compounds derived from GLS decomposition will become possible. This will, however, require control over all stages of fermented cabbage production, from selecting raw material, through fermentation process, to producing and storing the final commercial product.

3.4.4 Blanching

Blanching of vegetables is usually carried out to give the vegetables a softer texture, decrease or inactivate enzymatic activity, and increase shelf life. Blanching is mostly applied as pretreatment step prior to further processing such as heat sterilisation, dehydration or freezing. Wennberg *et al*. [142] investigated the effects of blanching of shredded white cabbage. After 5 min of blanching the total GLS levels had been decreased substantially in two tested cultivars by 50 and 74%. The individual GLSs were affected to different degrees. Cieslik *et al*. [143] investigated the effects of blanching in several different vegetable, finding a reduction by 2 – 30% for total GLS levels.

3.4.5 Domestic cooking

Boiling of *Brassica* vegetables in water reduces GLS levels significantly. GLSs and some of their hydrolysis products are water-soluble and on boiling a substantial proportion of these compounds will be leached into the cooking water. The amount of losses depends on the sort of vegetable, cooking time, ratio vegetable/water and also on the type of GLS [144 – 147]. Vallejo *et al*. [145] compared high pressure cooking with conventional cooking and showed significant losses of total GLSs in both treatments (33 and 55%, respectively). They observed higher losses for indole GLSs than aliphatic GLSs.

Song and Thornalley [129] demonstrated a progressive decrease in total GLS content after boiling for 30 min of 58% in Brussels sprouts, 65% in green cabbage, 75% in cauliflower and 77% in broccoli.

Differences in losses by leaching of GLSs in the cooking water could be explained by various reasons. It is expected that the extent of leaching of GLSs will vary between different types of vegetables, *e.g*. the configuration of Brussels sprouts will be prevent leaching of GLSs more than in broccoli. Also, the degree of shredding will cause differences in losses. Furthermore, differences in leaf thickness and waxiness, fibre content and composition could contribute to the variation in losses. Other explanations could be the variation in diffusivity of the GLSs [142].

3.4.6 Steaming

Steaming and stir-frying as culinary treatment of vegetables seems more mild processes since high retention of GLSs appeared to occur. No direct contact of the vegetables with water during steaming prevents leaching and solubilisation of GLSs in the cooking water, only after extended steaming some leaching may occur in the condensation water that is dripping from the product [129, 148].

3.4.7 Microwave

According to Vallejo *et al*. [145] microwave cooking (5 min 1000 W) resulted in substantial loss up to 74% of total GLSs in broccoli. Microwave cooking of cabbage (8 min 850 W) with 10% w/w water produced 8% loss of sinigrin [149]. Verkerk and Dekker [150] measured total and individual GLSs in red cabbage after various microwave treatments varying in time and intensity. Interestingly, they demonstrated high retention of GLSs during the microwave treatments and observed an increase in levels associated with the applied energy input. Moreover, high some time – energy input combinations resulted in levels exceeding the total GLS content in the untreated cabbage. They ascribed these findings to an increased extractability of GLSs by thermal treatment (changes in the vegetable matrix). These findings were in agreement with a study by Song and Thornalley [129] that showed no significant loss of GLSs after microwaving vegetables for 3 min at 900 W.

3.4.8 Stir-fry cooking

Stir-fry cooking is one of the typical cooking methods from Asian countries and it is becoming more popular worldwide. Song and Thornalley [129] stir-fried green cabbage, cauliflower and Brussels sprouts for 3 – 5 min with cooking oil (preheated to 200°C). They stated that the GLS content was not significantly changed by this cooking procedure. It appeared that the temperature upon addition of the vegetables quickly decreased to 120°C and remained stable at that level. They concluded that the stir-fry procedure inhibited the myrosinase activity rapidly resulting in high retention of GLSs.

3.4.9 Industrial processing

During industrial processing of *Brassica* vegetables (*e.g.* canning), the thermal treatment can affect GLS levels considerably. Oerlemans *et al*. [147] described thermal degradation of individual GLSs in red cabbage. Degradation of all the identified GLSs occurred when heated at temperatures above 100°C. The indole GLSs 4-hydroxy-glucobrassicin and 4-methoxyglucobrassicin appeared to be most susceptible to thermal degradation, even at temperatures below 100°C. Canning, the most severe heat treatment, will result in substantial thermal degradation (73%) of the total amount of GLSs.

4 Bioavailability of GLSs and derived products

For any compound to exert a systemic activity it needs to be absorbed by the body and reach the target tissues at appropriate dose levels and in an active form; it needs to become bioavailable to the body. Bioavailability is a term borrowed from pharmaceutical sciences, were absolute bioavailability is used to describe the exact amount of a compound that reaches the systemic circulation. It is calculated as the fraction of the area under the curve (AUC) after oral ingestion compared to the AUC after intravenous administration. In nutrition, however, relative bioavailability, comparing the bioavailability of a compound from different sources, is a commonly applied term.

Numerous endogenous and exogenous parameters affect the liberation from the food matrix, absorption, distribution, metabolism and excretion and thus the bioavailability of bioactive compounds such as GLS-derived isothiocyanates, indols and nitriles, including epithionitriles is highly variable. As direct consequence, the biological response in different populations might vary significantly. Accordingly, a recent randomised, placebo-controlled chemoprevention trial concerning the effect of broccoli sprout hot water infusion on the disposition of aflatoxin and phenanthrene, no significant effects were found when taking the overall study group, but a highly significant correlation between the urinary excretion of isothiocyanate metabolites from broccoli sprouts and aflatoxin as well as phenanthrene detoxification. Based on these findings, the bioavailability of isothiocyanates, *e.g*. SFN is the key to its activity [151].

GLS derived bioactive compounds are recognised by the body as xenobiotics. As such they undergo extensive xenobiotic metabolism, mainly in the liver, small intestine and the corresponding metabolites rather than the parent compounds are likely to reach the target tissues in the body and being the bioavailable and bioactive form. Therefore, results of *in vitro* studies applying phytochemicals, *e.g*. GLS hydrolysis products or plant extracts to organotypic cell cultures or specific tissues need to be carefully interpreted and metabolite *versus* free aglycone availability at tissue level studied.

Knowledge concerning the bioavailability is essential to an understanding of the variable responses to GLS-derived bioactive compounds, to identify population groups that would particularly benefit from a diet rich in *Brassica* vegetables and, eventually, to maximise the health benefits of GLS derived compounds in the general population.

4.1 Liberation, absorption, distribution, metabolism and excretion (LADME)

4.1.1 Liberation

The first major step for any compound to be bioavailable covers the release of the active component and dissolution into a complex matrix of digestive fluids and food.

As emphasised above, hydrolysis products rather than intact GLSs are responsible for observed biological effects. Most GLSs are chemically and thermally stable and therefore hydrolysis is mainly enzymatically, and more specifically, myrosinase driven. Following tissue disruption, myrosinase and GLSs come into contact, causing hydrolysis of the thioglucosidic bond and the formation of a range of bioactive compounds: ITCs, nitriles and elemental sulphur, thiocyanates, epithionitriles, oxazolidine-2-thiones or indolyl compounds. The chemical structure of the resulting product depends on the side chain structure, the reaction conditions and myrosinase activity [152]. Thus, at a pH of 6 – 7, the major hydrolysis products are stable ITCs. Nitriles are the major degradation products under acidic or alkaline conditions and after inactivation of myrosinase. In the presence of the epithiospecifier protein (ESP) and Fe^{2+} ions, myrosinase-catalysed hydrolysis of alkenyl GLSs is directed towards epithionitrile formation [153]. In consequence, different processing and storage conditions of the vegetables, *e.g*. freezing, chopping, conventional cooking, steaming, microwave cooking may result in very different amounts and profiles of GLS BDPs. Even cooking conditions, *e.g*. starting with cold *versus* hot water will affect myrosinase and ESP activity and the formation of isothiocyanates *versus* nitriles. Myrosinase is relatively heat stable and may easily survive blanching or even short term boiling of the plant material while microwave-cooking is extremely

efficient at inactivating myrosinase. ESP is less heat stable and will loose its activity at temperatures around 60°C enhancing the formation of isothiocyanates. During storage low degree cell damage may occur, accompanied by competing processes of hydrolysis and *de novo* biosynthesis of specific GLS.

4.1.1.1 Mastication

In vivo, the maceration in the mouth is the first step that results in further cell rupture, release of GLSs and, depending on previous processing, the formation of bioactive GLS hydrolysis products. As there is no additional enzyme activity in the oral mucosa and saliva, the formation of ITCs *versus* nitriles is likely to follow the mechanisms already described for hydrolysis at neutral to weakly alkaline conditions (pH of the saliva in healthy subjects is around 7.4) and clearly depend on the presence of myrosinase activity released from the plant material. The mechanism of ITC *versus* nitrile formation during food consumption, which was also shown to be species and cultivar depended, and the possible role of a 'nitrile-forming factor' are still unclear.

4.1.1.2 Gastric and small intestinal digestion

Stability tests under acidic conditions, such as present in the empty stomach (pH 2) have shown that most GLSs are relatively stable. Accordingly, Maskell and Smithard [154] have shown that the overall drop in total GLSs was on average 14% after the simulated gastric digestion and 32% when followed by the simulation of a 4 h digestion in the small intestine and that individual GLSs were differently affected with losses ranging from 3 to 23% and from 7 to 28%, respectively [154]. Further losses during digestion can occur as a result of unspecific adsorption and binding to other meal constituents, especially proteins and peptides [155]. In consequence, when incubating intestinal contents with intact GLSs Michaelsen *et al.* observed that the average recovery of the initial doses was only 58% even if myrosinase was inactivated [156]. Furthermore, digestion of the food matrix may cause additional cell lysis enabling extraction and subsequent release of GLSs and myrosinase [154, 156, 157].

Animal and human data support the evidence for (i) a low extend of GLS hydrolysis during gastric and small intestinal digestion, (ii) potential losses due to interactions with the food matrix and digestive products, *e.g.* proteins and peptides, (iii) further GLS and myrosinase release followed by (iv) GLS breakdown as a result of cell rupture [156, 158]. Plant-derived myrosinase seems to contribute significantly to the GLS hydrolysis *in vivo* and thus food processing prior to the ingestion is an important factor to ensure the formation of the desired ITCs from precursor GLSs prior or during digestion. This is particularly true since acidic conditions, such as present in the stomach, favours nitrile formation as shown by Lo *et al.* [159], who did not detect any free ITCs in their samples collected from the intestinal contents and faeces.

The stability of GLS-HP present in the food or formed during maceration and digestion is highly variable. I3C, for example is relatively unstable under the acidic conditions in the stomach forming dimers and different condensation products [160, 161]. ITCs are known to be highly reactive and thus instable. They readily bind to amino acids and proteins forming thiourea derivatives and dithiocarbamate esters. When egg white protein was treated with benzyl-ITC, lysine content and availability was significantly decreased to 60% as well as the bioaccessibility of the ITC [162, 163]. According to Björkmann, addition of radiolabelled ITCs at a level normally present to rapeseed meal resulted in a 36% binding of the ITC to meal components. This binding was independent from the individual ITC structure and reaction time but the reaction rate increased linearly with the amount of ITC added and with increasing pH [155].

4.1.1.3 Colonic fermentation

A substantial proportion of intact GLSs from food may not be absorbed in the small intestine and, based on data of pig ileal digesta, Maskell and Smithard [154] suggested that about 60% of most intact GLSs reach the colon unmodified. In the colon, this proportion can be hydrolysed by the colonic microflora but the precise role of microbial myrosinase activity is controversial [164 – 168]. Incubating human faeces for 2 h with cooked watercress juice resulted in 18% hydrolysis of total GLSs and the formation of ITCs [169]. The corresponding nitrile was not detected, but based on studies in sheep rumen, these could have been formed and immediately further metabolised [170]. Compared to the levels of allyl GLS found in the colon (10 μmol), the level of the corresponding ITCs (100 nmol) was low, indicating a fast absorption of the ITCs formed or the preferential formation of products other than ITCs. Combourieu *et al.* [171] confirmed the latter and showed *in vitro* that allyl and benzyl GLS were transformed quantitatively by human colonic microflora into allylamine and benzylamine, respectively and not into the corresponding ITCs.

In contrast, in human it was shown that reducing the bowel microflora by mechanical cleansing and antibiotics, lead to a significant decrease of urinary ITC metabolite (dithiocarbamate) excretion from 47% to a negligible amount [158]. Based on these human data, there seems to be no doubt about the importance of the gut microflora in the intestinal ITC formation but interindividual differences in the appearance of bacterial strains exhibiting myrosinase activity may result in very different hydrolytic activities as shown by the apparent discrepancies [156].

In summary, dissolution, gastric and intestinal digestion as well as GLS degradation by the colonic microflora determines the stability of bioactive GLS-HP but most of all their formation from the parent GLSs.

4.1.2 Absorption and first pass metabolism at gut level

The most important parameters affecting the early phases of the plasma concentration time curve are the rate and extent of absorption and presystemic metabolism. Small intestinal absorption of intact GLSs has been proposed in a number of studies [159, 164, 165, 172 – 174]. Good evidence has been provided by Michaelsen *et al*. [156] who studied the transport of GLSs from the mucosal to the serosal side of rodent everted gut sacs. They observed a transport rate that was structure and side chain dependent (0.39 and 0.18 μmol h^{-1} g^{-1} for benzyl and allyl GLS, respectively) [156]. When studying the transport rate it was shown that GLS are likely to be absorbed by passive diffusion or facilitated transport while active transport was excluded [156, 172, 175]. The relevance of a possible absorption of intact GLS in humans could so far not be confirmed [176].

Unlike the relative polar and, at neutral pH ionised GLSs, their degradation products show log *P* values in the range of 0.2 – 4.4 and a lower molecular weight. This implies a high potential for membrane partitioning, enabling efficient absorption by passive diffusion. Indeed, numerous studies describing the absorption of structurally different GLS-HP in animal models [172, 173, 175, 177 – 179] and in humans [180, 181], showing the fast absorption as measured indirectly by urinary excretion. Ye *et al*. [182] applied the cyclo-condensation method to the detection of dithiocarbamates in blood. Following a single dose of 200 μmol of ITCs (mostly SFN), they found a rapid absorption reaching a peak plasma concentration of 0.94 ± 2.27 μmol/L after 1 h.

As the 'gold standard' for studying effective intestinal permeability and first pass metabolism an intestinal perfusion technique (Loc-I-Gut) was developed and applied in drug studies [183, 184] but also used to investigate SFN absorption and metabolism [185]. Because of its lipophilicity (log *P* (octanol/water) = 0.72 [186]) and a low molecular weight of 177, SFN rapidly diffused into the cells of the intestinal lining [185] were rapid conjugation with glutathione (GSH) is likely to be the driving force of this diffusion. As a result millimolar concentrations of intracellular SFN – GSH (several hundred fold over the extracellular concentration) have been determined in cell culture [187]. Initial uptake rates were closely correlated with the nonenzymatic second-order rate constants of GSH conjugation and with cellular GSH levels [188, 189]. The accumulation kinetics, the maximum levels of accumulation, and their excretion out of the cell was shown to depend on the structure of the individual ITC [189, 190]. Furthermore, Kolm *et al*. [188] have shown that GSTs M1-1 and P1-1 were the most efficient enzymes and that ITCs are among the most rapidly conjugated substrates of GST.

While cysteine conjugates of ITCs can be absorbed intact [177, 191], GSH conjugates were shown to require release of free ITC prior to absorption [187].

Diindolylmethane (DIM) and 2,3-BII, the main hydrolysis products of indole-GLS derived products were detected by De Kruif *et al*. [160] in the liver of rats, suggesting that they are absorbed by the small intestine. To our knowledge, the extent and mechanism of absorption of indole GLS derived products has not been investigated in detail.

There is also limited knowledge on the absorption of GLS derived nitriles, but the chemical structure and nature implies a fast absorption in the small intestine. Thus 3,4-epithiobutanenitrile, one of the most prevalent GLS derived nitriles was shown to be rapidly taken up by experimental animals. Peak values of radioactivity appeared 1 h after application of the radiolabelled compound [192].

4.1.3 Distribution

Distribution involves the movement of a compound between the intravascular space (blood) and the extravascular space (body tissues). Proteins in plasma, which bind very strongly, *e.g*. to ITCs, include albumin and glycoproteins but only free compounds exert diffusion pressure across most membrane. Based on their chemical structure and properties, it is unlikely that intact GLSs reach human tissues as such, whereas their BDPs, especially ITCs or rather the metabolites thereof are distributed throughout the body and accumulate in different tissues.

A whole body autoradiographic study in rats suggested that apart from the gastrointestinal tract, liver and kidneys, only the blood contained relatively higher concentrations of ITC metabolites (Franklin, E. R., unpublished, cited in [177]). Following application of ^{14}C labelled ITCs to rats, high concentrations of ^{14}C appeared rapidly in stomach, small intestine, ceacum, and colon, intermediate concentrations in pancreas and spleen, and very low concentrations in heart and brain. For the alimentary tract, the time of peak ^{14}C concentration was shown to depend on the rate of intestinal passage and was different for the two ITCs tested. After a rapid absorption into the blood, peak levels of ^{14}C occurred 4 – 8 h in the heart, liver and lungs and were nearly constant in the kidney over a time period of 8 h [193].

The basis for the distribution of ITCs throughout the body is the reversibility of their binding to amino- and thiol-groups and especially the high degree of binding to cysteine and GSH [162, 163, 181, 194]. The resulting thiol-disulphide exchange give rise to an enormous range of possible intermediates and transport forms of ITCs, *e.g*. mixed disulphides and protein disulphides.

With low millimolar concentration in the blood, serum albumin is the major binding target and carrier of ITCs. Distribution into individual tissues involves the permeation of membranes following the general principles described for small intestinal absorption. Accordingly, ITCs can only passively diffuse into the cells as unbound compounds or as L-cysteine derivatives, where GSH conjugation is driving passive diffusion. Intracellular accumulated GSH conjugates have been shown to be rapidly excreted where both,

the multidrug resistance associated protein-1 (MRP-1) and P-glycoprotein (Pgp-1) are likely to be involved [195].

Due to analytical limitations there are only few studies approaching the distribution of GLS, GLS-HP and their metabolites in humans. The development of a sensitive and reliable method for measuring ITCs and metabolites in plasma and tissues has enabled pharmacokinetic studies in human and scientific progress in this field. Based on this method, Ye *et al*. have shown a rapid absorption and appearance of ITCs and their metabolites in the blood (0.94 ± 2.27 μmol/L). This level subsequent declined following first-order kinetics with a $t_{1/2}$ of 1.77 ± 0.13 h, indicating a fast distribution and/or metabolism [182].

There is only little data on the distribution of nitriles. Following the fate of radioactivity after gavage of ^{14}C and ^{35}S labelled 3,4-epithionitrile a rapid and almost linear decrease of radioactivity was observed for 13 h in the liver, kidney, stomach, and small intestine, respectively. Three days after administration, the highest levels were found in the blood suggesting an efficient binding to blood constituents. A high reactivity towards tissue macromolecules was shown and might affect cellular functions, cause mutagenicity and carcinogenicity of the nitriles at high doses.

Dashwood *et al.* studied the disposition of radiolabelled I3C in rainbow trout for 72 h. 75% of the initial 3H-dose was detected in the stomach 0.5 – 12 h post-treatment. Radioactivity accumulation was observed in the liver, reaching 1 – 1.5% of the original dose between 48 and 72 h [196].

Human data on the disposition of hydrolysis product of indole GLS are not available. As the hydrolysis products of the indole GLS are strongly implicated in several health issues and are already available as dietary supplements, this gap in knowledge needs to be addressed.

4.1.4 Metabolism and excretion

Bioavailability is often limited by rapid and extensive metabolism. As an absorption of intact GLSs has not been confirmed in humans, the metabolites found in urine and faeces are most likely derived from GLS-HPs, rather than of the parent GLSs [197].

The liver is the second and major metabolic barrier for xenobiotic bioavailability. It contains high concentrations of GSH and shows the highest GST activity in the organism. As described for small intestinal ITC metabolism, enzymatic and nonenzymatic conjugation with GSH is the major route of metabolism [177, 198]. At high concentration of ITCs this may lead to a temporary GSH depletion and an increased binding to cellular macromolecules [187]. The structure of the ITC determines the extent of this binding, but also the rates of nonenzymatic and enzymatic conjugation with GSH [198] and might explain very different excretion curves and thus bioavailability of individual ITCs [158, 199]. Accordingly, allyl ITC and SFN showed similar urinary metabolite excretion (44 and 47% for allyl ITC and SFN, respectively) but very different half-lives of urinary excretion, 2 h for allyl ITC and 12 h for SFN [199]. *In vitro* studies have shown that SFN is a poorer substrate for individual GSTs and this may be the reason for the differences in half-life and finally bioavailability [198].

The kidney is the major organ involved in the conversion of GSH conjugates into the corresponding *N*-acetyl-S-cysteine conjugates (Fig. 3) [200]. Because of high GSH concentrations in the liver, and high NAT activities in liver and kidney, the mercapturic acid derivatives can be formed prior to excretion in the kidney, or presystemically and undergo enterohepatic cycling as shown in rats [201]. In humans, Shapiro *et al.* [199] observed a biphasic excretion curve after the ingestion of horseradish with a second maximum after 6 h, indicate that enterohepatic recycling is relevant in humans too.

Structure depending, individual ITCs are subject to extensive phase I metabolism resulting in a broad range of conjugates and explaining different urinary recoveries of their structural corresponding mercapturate [201]. Accordingly, when SFN was administered to rats it gave three different mercapturates in the urine, where the mercapturate of erucin ITC, the sulphide analogue of SFN, accounted for 12% of the initial dose. Surprisingly, rats that were gavaged with erucin ITC excreted 67% of the applied dose as SFN mercapturate, indicating a bioconversion of the ITC, with favourable oxidation of the sulphide. The appearance of an unsaturated GSH conjugate in bile and urine indicates that SFN also undergoes dehydrogenation [201]. If this biotransformation can be confirmed in humans, it would demonstrate that the consumption of *Brassica* vegetables rich in glucoerucin (*e.g*. rocket salad) may give rise to the same active components *in vivo* as glucoraphanin containing broccoli species. Today, ITC bioavailability is mostly determined by the directly derived mercapturic acid derivative in the urine, not considering biotransformation *via* phase I metabolism. As phase I metabolism may contribute considerably to the biotransformation of ITCs and their metabolites, bioavailability and efficacy studies need to include a comprehensive analysis of metabolite profiles in blood, urine and faeces.

Organic nitriles in general are detoxified by sulphur transferases into the less toxic thiocyanate (SCN^-) and the corresponding aldehyde. Accordingly, Lange *et al*. detected thiocyanate as one of the major metabolites (23%) after phenylacetonitrile administration to rats. A further 20% of the administered dose was excreted as glycine conjugate, but 57% phenylacetonitrile could not be accounted for [164]. Multiple, competing pathways for the metabolism of nitriles have been postulated, and are defined by the structure of the organonitrile. Thus, Wallig *et al*. [202] observed significant differences in the metabolism and toxicity of 1-cyano-3,4-epithiobutane (CEB) and n-valeronitrile were CEB administration caused only small increases in urinary SCN^- (4.5-fold) and in hepatic and pancreatic nonprotein thiol concentrations (1.5- to 2.4-fold), while animals treated

with *n*-valeronitrile showed a 95- to 170-fold increase in urinary SCN^- and only minimal effects on tissue nonprotein thiol concentrations. Enhanced tissue nonprotein thiol concentrations after CEB treatment indicated increased tissue GSH levels and suggests the involvement of GSH in its metabolism and detoxification [202]. Analysis of CEB-derived urinary metabolites revealed a single predominant urinary metabolite that was identified as the corresponding mercapturate: *N*-acetyl-S-(4-cyano-2-thio-1-butyl)-cysteine [203]. Brocker *et al*. [192] showed that the same applies to the n-1 homologue of CEB and concluded that nucleophilic opening of the epithio group is the underlying mechanism.

Considering that the nitriles are major products of GLS hydrolysis, and that nitrile metabolism determines the nature of their effects, it is surprising how little conclusive knowledge exists on this matter and on their elimination from the body.

I3C metabolism was only studied *in vitro*, where different intermediate products and metabolites were identified: indole-3-carboxaldehyde, 5-hydroxyindolecarboxaldehyde and the corresponding carboxylic acid. Both, the mixed function oxidase and alcohol dehydrogenase systems, appear to be involved [204, 205]. In MCF-7 cells, enzymatic and nonenzymatic reaction with cellular thiols such as cysteine and GSH were identified as major metabolic routes of I3C biotransformation [206]. Apart from the carboxyaldehyde and carboxylic acid metabolite, substantial amounts of DIM accumulated in the nucleus with major implications for the biological activitiy. When 3-methylindole, a structurally very similar compound to I3C was administered to experimental animals, the corresponding mercapturate, 3-[(*N*-acetylcystein-*S*-yl)-methyl]indole, was identified in the urine as excretory forms of the 3-methylindole – GSH adduct [207].

GSH conjugation, followed by *N*-acetylation, and subsequent excretion as the corresponding mercapturate appears to be the major and a common metabolic pathway of structurally different GLS hydrolysis products. Since identical metabolic pathways imply competition for substrates (*e.g.* GSH and proteins) and enzymes (*e.g.* GST) involved in their metabolism, GLS hydrolysis products are likely to interact and interfere with each other's metabolism but this has not been investigated.

4.2 Effect of genetic polymorphisms on GLS metabolism and interindividual variation

A combination of genetic and environmental factors is responsible for large interindividual variations, which are also referred to as 'pharmacological individuality'. Polymorphisms of genes coding for phases I and II metabolising enzymes and transcriptional modulation of these genes by xenobiotics and environmental factors can result in significant differences concerning bioavailability and efficacy of GLS hydrolysis products. Accordingly, when Shapiro *et al*. [199] compared the excretion of dithiocarbamates after administration of intact GLSs and their corresponding ITCs, they observed a tendency for 'high or low dithiocarbamate excretors', regardless of whether they received intact GLS or ITCs. Getahun confirmed the latter, showing dithiocarbamate excretion ranging from 17 to 78% of the ingested GLSs for uncooked watercress and from 1 to 7% for cooked watercress [169]. In contrast, very low inter- and intraindividual variations were apparent (CV 9%) when studying the extent of excretion of ITC-derived dithiocarbamates among ten volunteers given horseradish. In addition, the authors determined a linear dose dependency over an eight-fold dose range when applying the same vegetable. As the subject numbers for both studies were low, results on interindividual variations of dithiocarbamate excretion are non conclusive, but very likely.

The extent and duration of bioefficacy depends on extend and duration of bioavailability and thus upon the rate at which the GLS hydrolysis product is metabolised and excreted. As described above, GSTs in the liver or intestinal mucosa play an important role in the metabolism of GLS hydrolysis products. The two GST isoforms GST M1 and GST T1 are subjects to genetic polymorphisms, and geographic as well as ethnic variations in genotype frequencies are known for both genes. Indeed, GSTμ polymorphism has been implicated as a variable, which determines the protective effect of broccoli against the development of precancerous adenomatous polyps in human populations [208]. In a recent epidemiological study, Lin *et al*. [209] observed a protective effect of broccoli consumption against adenomatous polyps only in subjects with the GSTM1 null genotype because ITCs are conjugated and excreted more slowly in subjects who do not express GSTμ, so that exposure of target tissues to the protective compound and/or metabolite is higher and prolonged. In contrast to these results, a study by Seow *et al*. [210] on different GST genotypes (M1/T1/P1) failed to show a difference in urinary dithiocarbamate excretion between GSTM1-null and GSTM1-positive subjects ($p = 0.61$) and between subjects with different GSTP1 genotypes ($p = 0.77$). However, urinary excretion of ITC conjugates was significantly higher among GSTT1-positive subjects relative to GSTT1-null subjects ($p = 0.006$). The strength of the association between GSTT1 genotype and urinary dithiocarbamate excretion was shown to depend highly on the level of cruciferous vegetable consumption (or dietary ITC levels), which might be due to saturation of other metabolising enzymes at higher dose levels [210].

Human *N*-acetyltransferases, NAT1 and NAT2, are major enzymes involved in the final step of metabolism of GLS hydrolysis products, such as ITCs and are polymorphic [211]. For drugs it is well described that different acetylation capacities cause significant differences in drug efficacy and in the susceptibility to certain types of diseases. Therefore, it is surprising that polymorphisms in NATs in

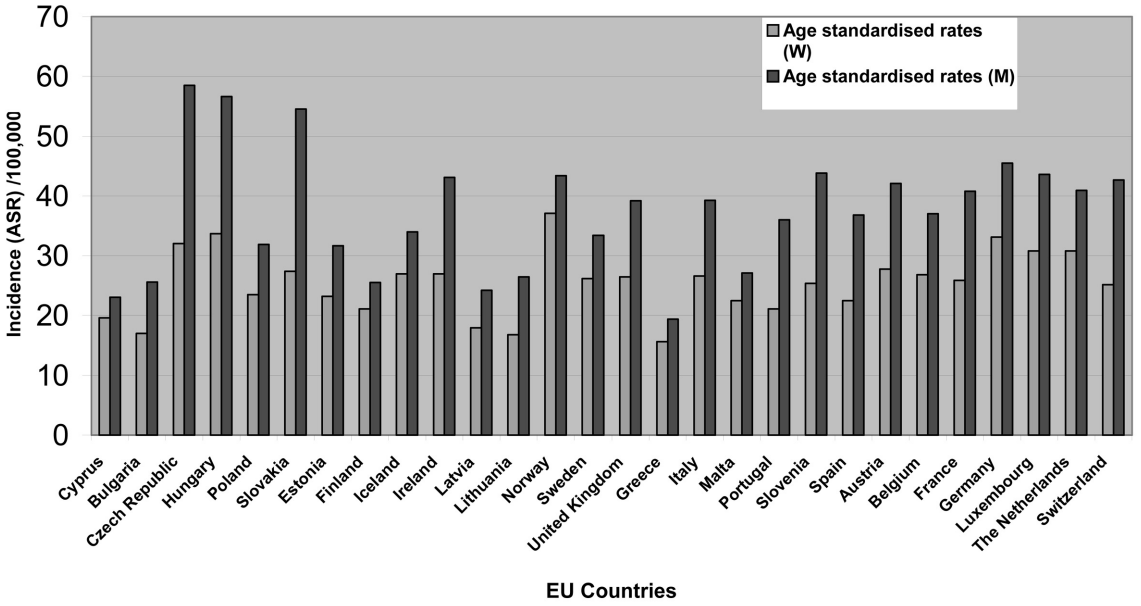


Figure 5. Estimated rates of CRC incidence (ASR/100 000) for men and women in EU countries (GLOBOCAN, 2002 figures) [275].

relation to the metabolism of GLS hydrolysis products have not been studied closely.

4.3 Biomarkers for GLS exposure

Linking knowledge on the bioavailability of GLS derived bioactive compounds to their effects is a key step in the exploitation of the beneficial potential of these compounds. Biomarkers are developed to draw this link, relating the consumption of specific compounds in food to the biological outcome, and hence, are essential for understanding the association between diet and health. GLS-derived hydrolysis products occur at low doses and are relatively weakly biologically active in the short term. Each compound has very specific activities, multiple targets, producing potentially both, beneficial and, at supra-nutritional doses, potentially adverse effects. This poses particular problems in determining the net effect, especially since plant foods contain a complex mixture of GLS related active compounds as well as other phytochemicals and bioactive micronutrients with a wide range of physiological effects. On this basis, one of the first questions relates to how much contribute GLS hydrolysis product, to a specific physiological outcome and secondly, to what extent does this affect the health status *versus* disease outcome.

Several authors proposed measurement of the dithiocarbamates excreted with the urine as biomarkers of exposure to ITCs. However, since mercapturic acid derivatives only account for a proportion of ITCs and the metabolic pathway is still only partly understood, more work needs to be conducted to validate the link between tissue bioavailability and urinary dithiocarbamates as marker of exposure.

As described above, high inter- and intraindividual variations in the bioavailability of, and response to, GLSs and GLS hydrolysis products have been observed. In particular, the genetic variability, and especially the overall impact of GST and NAT polymorphism on the bioavailability has the potential to serve as a biomarker of susceptibility and should be further explored.

Modulation of biochemical endpoints (biomarkers of effect), such as phases 1 and 2 biotransformation enzymes by ITCs, are very early events in the development of chronic diseases, and may give information concerning the mechanism of action of GLS hydrolysis products, GLS containing extracts, or whole diets [212]. To obtain conclusive results on the disease outcomes linked to these early events, surrogate markers, such as late-stage precancerous lesions, recurrence of lesions, micronuclei, cell proliferation, need to be measured on an intermediate term level and finally linked to specific endpoints.

A combination of the three biomarkers (exposure, susceptibility, and effect) should be encouraged to be applied in future intervention studies in order to understand the health effects of GLS derived compounds in different groups of the population showing different susceptibility.

5 *Brassica* vegetables and cancer

5.1 Epidemiology

Colorectal cancer (CRC) is ranked as the fourth most common cancer worldwide with approximately 944 000 cases being diagnosed in 2000, accounting for 9.2% of all new cancer cases [213]. It is the second most common cause of

Table 3. Epidemiological studies of cruciferous vegetables and colon cancer risk

Study type	Population size	Place	Measurement method	Outcome	Ref.
Case – control	353 cases, 618 controls	Wisconsin	Diet history	Significant protection in both proximal and distal colon	[276]
Case – control	286 cases, 295 controls	Majorca	Food frequency questionnaire	Significant protection in both colon and rectum	[277]
Case – control	746 cases, 746 controls	Los Angeles	Food frequency questionnaire	No effect	[278]
Case – control	248 cases, 699 controls	North-East Italy	Food frequency questionnaire	Significant protection	[279]
Case – control	1150 cases, 5746 controls	America-taken from cancer prevention study II	Food frequency questionnaire	Significant protection	[280]
Case – control	203 cases, 425 controls	Singapore	Food frequency questionnaire	Significant protection	[281]
Case – control	488 cases, 488 controls	California	Food frequency questionnaire	Inverse association between cruciferae and polyps	[282]
Cohort	659 colon cases, 375 rectum cases	Netherlands	Food frequency questionnaire	Inverse association for *Brassica*	[225]
Case – control	213 cases, 1194 controls	Singapore	Food frequency questionnaire	Significant 57% reduction in high V low ITC intake	[241]
Case – control	115 cases, 230 controls	Japan	Food frequency questionnaire	Inverse association for broccoli	[283]

death from malignant neoplasms in the EU, with 190 000 new cases *per* year. The cancer occurs almost equally in men and women, as demonstrated in westernised countries, where CRC represents 12.6% of all incident cancers in men and 14.1% in women [214] (Fig. 5).

The majority of epidemiological studies evaluating the association between fruit and vegetable consumption and colon cancer risk have reported inverse associations [215 – 219] although some recent studies have reported conflicting results [220 – 222].

The association between vegetables and colon cancer appears to be stronger for the dark green vegetables [223, 224] and among the subgroups of these vegetables, *Brassica* vegetables have shown strong negative associations between consumption and colon cancer risk in both sexes [225]. Examples of these studies are summarised in Table 3.

The epidemiological evidence indicating a protective role of *Brassica* vegetables in CRC is supplemented by extensive investigations in human volunteers, animal models, and cell culture systems which are discussed below. These studies have not only provided strong support for the epidemiological associations, but also valuable insights into the possible mechanisms behind these effects and the potential phytochemicals present in *Brassica* vegetables. Numerous constituents found in *Brassica* vegetables, including dietary fibre, micronutrients and various other phytochemicals, might contribute to the ability of these foods to reduce cancer risk [226], although the main focus of studies investigating the protective effects of *Brassica* vegetables on CRC has been on GLSs and ITCs.

5.2 Human dietary intervention studies

The difficulties of assessing the anticancer effects of dietary regimens and food constituents in humans have been discussed by Gill and Rowland [227]. As cancer is an impractical endpoint due to ethical considerations, cost and duration, studies have focussed on intermediate endpoints. Three main endpoints have been utilised during these studies: phase I and phase II enzyme activities, carcinogen excretion and antioxidant effects including decreased oxidative DNA damage.

5.2.1 Phase I and phase II enzyme activities and carcinogen excretion in humans

The potential of *Brassica* vegetables to induce both phase I and phase II enzymes during carcinogen metabolism in humans has been demonstrated in many studies [228 – 232]. Induction of the phase I enzyme CYP1A2, known to mediate metabolism of certain carcinogens such as 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), which are found in cooked meat and fish, has been demonstrated in response to *Brassica* vegetables consumption. Although induction of the CYP1A2 enzyme is responsible for carcinogen activation, studies have demonstrated that even following induction of this enzyme, a protective effect of *Brassica* vegetables supplementation on urinary mutagenicity (determined after consumption of a fried meat meal) has been reported [229].

Glutathione S-transferases (GSTs) are a large multigene family responsible for the elimination of activated carcino-

gens from the body by producing highly polar molecules that are readily excreted [233]. GSTs catalyse this detoxification *via* the conjugation of carcinogens with GSH. Low GST activity has been correlated with a higher tumour incidence in the colonic mucosa [234]. Studies in humans have demonstrated that *Brassica* vegetables consumption can increase GST levels and activity in addition to GSH levels in lymphocytes. *Brassica* vegetables (radish, cauliflower, broccoli and cabbage) have been demonstrated to induce GSTμ activity in peripheral lymphocytes [230]. In addition, in response to Brussels sprouts, levels of GSTa and GSTp were increased in plasma and in rectal cells, respectively [235, 236].

Those individuals with an increased risk of adenoma development can be identified by their genotype, *i.e.* the GST polymorphic gene/phenotype. The GSTM1 gene dele-

Table 4. Human intervention studies on antioxidant effects of cruciferous vegetable supplementation

Constituent	Latin name	Dosage	Time period	Subject	End point	Observation	Protective/ adverse effect	Comment	Ref.
Spinach	*Spinacea oleracea*	150 g/day, 75 μM	3 wk	9 females	H_2O_2 (*ex vivo*) induced oxidative DNA damage in lymphocytes	DNA damage ↓, therefore ↑ resistence of lymphocytes to oxidative damage	+ *	↓ Oxidative DNA damage by Spinach may be due to range of constituents, *i.e.* flavones, lutein, folates, and vit c	[243]
Mixture of 3-day-old sprouts of		113 g/day	14 days	10 males 10 females	H_2O_2 (ex vivo) induced oxidative DNA damage in lymphocytes	Reduction in H_2O_2-induced DNA damage	+ *	Consistent with in vitro antigenotoxic effect of crude extract of same vegetable mixture against H_2O_2 oxidative DNA damage in human colon cells	[244]
Broccoli Radish	*B. oleracea* *R. sativus*					No significant change			
Alfalfa	*Medicago sativa*				Antioxidant status				
Clover	*Trifolium pratense*				Plasma antioxidants	No significant change			
Brussels Sprouts	var. *gemmifera* DC.	300 g/day	3 wk	10 males	Urinary 8-oxodG levels	Within the sprouts group levels of 8-oxodG ↓ *28%*	+ NS		[284]
Fruit and Veg + Broccoli (heated)	*B. oleracea*	10 servings/day	15 days	9 males (young) 9 females (young)	Plasma antioxidant capacity	Plasma ORAC values ↑ than baseline values in both old and young in response to fruit and veg	+ * +	Plasma antioxidant capacity was significantly induced in response to fruit and veg consumption but there was no additional effect with broccoli consumption	[285]
		102.4 g/day	2 days	9 males (old) 9 females (old)	(ORAC)	Plasma ORAC levels ↑ in old subjects but not in young in response to broccoli addition	+ * + *		
Spinach	*S. oleracea*	294 g/day	1 day	8 females	Serum and urinary antioxidant Capacity (ORAC)	Urinary ORAC values ↑ 27.5% Serum ORAC – 25%	+ * + *	↑ Serum and urinary antioxidant capacity indicates direct absorption of antioxidants	[285]
Watercress (raw) + H_2O_2 (ex vivo challenge)	*Rorippa nasturtium-aquaticum*	85 g/day	2 months	30 males 30 females 50% cigarette smokers	Oxidative DNA damage in lymphocytes Antioxidant status Plasma antioxidants	Reduction in baseline and H_2O_2-induced DNA damage No change B-Carotene ↑ 33% Lutein ↑ 100%	+ * (greater effect in smokers) +*	Consistent with *in vitro* anti-genotoxic effect of crude extract of same plant against H_2O_2 oxidative DNA damage in colon cells	[286]

+, Protective effect; *, statistically significant.

Table 5. Effects of cruciferous vegetable Consumption in animal models

Constituent	Latin name	Dosage	Time period	Animal	End point	Observation	Protective/ adverse effect	Comment	Ref.
Brussels sprouts extract	var. *gemmifera DC*	7 g/day (G)	5 days	Wistar rats (M)	CYP, QR, and GST activity	No effect noted on CYP1A2, 2B1 2B2 and 2E1 levels GST ↑ 1.3-fold QR ↑ 2.6-fold ↑	+ NS	Consumption CV vegetables ↑ phase II enzymes	[287]
			3 and 7 days		8-OxodG levels in DNA	1.3- and 1.2-fold ↑ for days 3 and 7, respectively	– *	↑ in oxidised DNA damage raises question whether increasing consumption of CV is beneficial	
Broccoli Freeze-dried	*B. oleracea*	20% v/v (D)	5 days	F344 rats (M)	QR Activity	9.1-fold ↑	+*	Differing levels of QR induction probably due to effects of processing methods on myrosinase activity in broccoli	[288]
Dehydrated Hydrolysed						10.5-fold ↑ ↑ QR activity but to a lesser degree of significance	+* +*		
Broccoli tablets	*B. oleracea*	1 g/kg BW (G)	Single dose	ICR (Ha) mice (F)	Colon GST activity GST isoenzyme expression	GST μ activity ↑ 3.5-fold compared to control ↑ GST μ and p expression on day 1	+ *	Demonstrates ability of commercial broccoli supplements to ↑ GST expression in murine colon	[289]
Lyophilised cabbage or Broccoli	var. *sabauda* L. subvar. *cymosa Lam*	10 – 40% (D)	14 days	Sprague – Dawley rats (M)	Colonic and duodenal mucosal GSH levels	Colonic and mucosal GSH levels ↑ in dose-dependent manner	+ NS	GSH levels in colon and mucosa enhanced by cabbage and broccoli	[290]

D, Supplemented in diet; G, gavage; +, protective effect; –, adverse effect; *, statistically significant; NS, not significant; BW, body weight.

tion is the best-studied polymorphism and occurs in up to 50% of populations depending on their ethnic background [237]. *Brassica* vegetables have been shown to exert protective effects on colon cancer risk in individuals with a specific genotype; however, these effects are dependent on a variety of other variables such as smoking and age [238, 239]. The findings of these studies have suggested the positive genotypes are desirable due to an increase in detoxification of carcinogens in these individuals [233], however, in those individuals with the null genotypes, ITCs may act longer due to slower excretion, and exert their effects in a way other than detoxification [188, 209, 240, 241].

In addition to effects on dietary carcinogens, *Brassica* vegetables have also been demonstrated to alter the metabolism of cigarette smoke carcinogens, such as the nitrosamine (NA), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in humans. After the consumption of watercress, a significant increase in urinary levels of the NNK metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and NNAL-gluc was observed following 2 days of consumption of watercress [242] suggesting that watercress induces UDP-glucuronosyltransferase activity in humans.

5.2.2 Antioxidant activity of *Brassica* vegetables

Measuring oxidative DNA damage in human lymphocytes, in addition to antioxidant status in the blood cells, gives an idea of the integrated rate of DNA damage in the body and is suggested to be a potential biomarker for cancer risk (Table 4).

Consumption of Brussels sprouts, spinach, watercress, or a sprouting vegetable mixture (containing broccoli, radish, alfalfa and clover) significantly reduced DNA damage in lymphocytes, following treatment (*ex vivo*) with H_2O_2, and as measured *via* 8-oxodG excretion [243, 244]. A good correlation has been observed between DNA damage occurring in colonocytes and the levels observed in lymphocytes of subjects participating in supplementation studies [245]. Therefore effects observed in peripheral lymphocytes should be consistent with site-specific effects, such as those seen in the colon.

Table 6. Effects of cruciferous vegetable consumption *in vivo*

Constituent	Latin name	Dosage	Time period	Animal	End point	Observation	Protective/adverse effect	Comment	Ref.
Garden cress juice	*Lepidium sativum* L.	0.8 mL (G)	3 days	F344 rats (M)	DNA damage	IQ-induced DNA damage was reduced almost completely by all constituents	+ *	Garden cress juice and its constituents attenuate genotoxic effects of IQ.	[291]
Glucotropaeolin		150 mg/kg BW (G)	3 days		GST and P4501A2 activities	No effect was observed	No effect	UDPGT-2 induction may be responsible for anti-genotoxic effects and ACF ↓	
BITC + IQ		70 mg/kg BW (G)	3 days		UDPGT-2 activity	Hepatic UDPGT-2 ↑	+ *	Garden cress juice significantly reduced no. of IQ-induced ACF	
		90 mg/kg BW (G)	4 h						
Garden cress juice + IQ		5% v/v (D)	15 days		ACF inhibition	↓ in IQ-induced ACF	+ *		
		100 mg/kg BW (G)	10 alternate days						
Red Cabbage	var. *capitata* subvar. *Rubra*	5% v/v (D)	25 days	F344 rats (M)	ACF inhibition	↓ in IQ-induced ACF	+ NS		[292]
Brussels sprouts + IQ	var. *gemmifera DC*	100 mg/kg BW (G)	10 alternate days			↓ in IQ-induced ACF	+ *		
Brussels Sprouts + IQ	var. *gemmifera DC*	5% v/v (D)	10 alternating days	F344 rats (M)	ACF inhibition	↓ frequency ACF by 41 – 52%	+ *	Marked ↓ in ACF numbers in all areas of colon	[293]
		100 mg/BW (G)							
Brussels Sprouts (raw and blanched) + DMH	var. *gemmifera DC*	20 g/day (D)	28 days	Wistar rats (M)	ACF inhibition	Raw sprouts ↓ DMH-induced ACF but not significant	+ NS	As GLS was given after DMH, antineoplastic effect brought about by suppressing lesion, not mitotic block	[294]
		30 mg/kg BW (SC)				No effect on ACF in response to blanched sprout tissue	No effect		

D, Supplemented in diet; SC, injected; G, gavaged; +, protective effect; *, statistically significant; NS, not significant; BW, body weight.

5.3 Animal studies

The main biological effects observed in animals after exposure to *Brassica* vegetables or purified ITCs are changes in enzyme activities, decreased levels of DNA damage and reductions in colonic aberrant crypt foci (ACF) formation (Tables 5 and 6).

ACF, thought to be the earliest morphological changes to occur during colonic mucosal neoplasia have been observed in the human colon and in rats and mice treated with carcinogens [246], and have been used as a surrogate marker for colon cancer for assessing activity of chemoprotective agents.

From the studies in Table 6 it is suggested that although feeding rodents with *Brassica* vegetables extracts during and after carcinogen exposure reduced ACF formation, this reduction appears to be significantly higher in animals fed the extract prior to and/or during carcinogen treatment. These findings support the role of *Brassica* vegetables at both the initiation and the postinitiation stages of carcinogenesis.

Studies have been carried out to investigate the ability of phenylethylisothiocyanate (PEITC) to protect against ACF formation and DNA adducts, induced by a range of carcinogens as summarised in Table 7. A significant reduction in ACF numbers brought about by AOM and DNA adducts as a result of PhIP was observed. PEITC has also been shown to induce GST activity and GSH content in the colon [247] which could at least in part be responsible for the chemoprotective effects exerted in the digestive tract of rats.

A significant reduction in levels of PhIP- and IQ-induced DNA adducts in response to preinitiation, postinitiation and continuous exposure of I3C has been observed (Table 8). In addition, I3C has also been shown to reduce ACF formation and tumour induction at both the initiation and postinitiation stages of IQ and AOM-induced carcinogenesis [248 – 250]. SFN, benzyl isothiocyanate (BITC) and Sinigrin

Table 7. Anticarcinogenic effects of PEITC in animal models

Constituent	Dosage	Time period	Animal	End point	Observation	Protective/adverse effect	Comment	Ref.
PEITC and AOM	(D) 15 mg/kg BW (SC)	5 wk 2 wk (1 dose/wk)	Sprague – Dawley rats (M)	ACF inhibition	Number of foci induced by AOM not significantly ↓	No effect	Further validation of chemicals for chemoprevention required	[295]
PEITC + AOM	5 μmol (G)	8 wk	F334 rats (M)	ACF inhibition	Total no. ACF ↓ from 153 to 115	+ *	As ITC conjugates are less toxic than parent compounds, doses ↑ four times – yet still no effect	[296]
	15 mg/ kg BW (SC)	2 wk						
PEITC-NAC + AOM	20 μmol (G)	8 wk			No reduction observed for conjugate	No effect		
	15 mg/ kg BW (SC)	2 wk						
PEITC + PhIP	570 or 210 mg/kg BW (G)	2 h	Swiss Albino mice (M)	DNA adduct levels	No ↓ of DNA adducts in colon or livers	No effect	No protective effects of PEITC on PhIP-induced DNA damage noted	[297]
	175 mg/kg BW (G)	Further 2 h						
PEITC + PhIP	816 mg/kg BW (D) 4 μg/g BW (G)	15 days	F334 rats (M)	DNA adduct levels	1.2 – 1.7-fold ↓ DNA adducts in colon	+ *	Significant ↓ DNA adduct levels Useful in cancer initiation prevention	[298]
PEITC	0.045% (w/w) (D)	2 wk	Wistar rats (M)	Colonic GST activity	GST activity ↑ 1.2-fold	+ *	PEITC exerts chemopreventive effects by ↑ GST and GSH	[247]
				Colonic GSH content	GSH content – 1.6-fold	+ *		

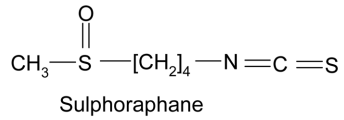
D, Supplemented in diet; SC, injected; G, gavage; +, protective effect; *, statistically significant; BW, body weight.

exerted protective effects against AOM, PhIP and DMH-induced colonic DNA adducts and ACF formation (Table 9).

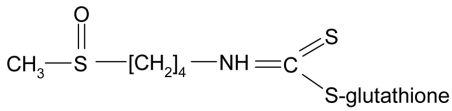
5.4 Anticancer effects of *Brassica* vegetables and components in vitro

In vitro studies have focussed on a number of end points, DNA damage and modulation of phase I and phase II enzymes together with proliferation and apoptosis.

In general, a decrease in genotoxin-induced DNA damage by plant extracts and an increase in protective enzymes have been observed. In addition in one study with watercress extract, a significant decrease in cell invasion through Matrigel (a model for metastasis) was seen [251] (Table 10). Tables 11 – 13 summarise the effects of a range of ITCs and indoles *in vitro*. Overall the studies indicate beneficial effects – induction in apoptosis and inhibition of cell proliferation *via* cell cycle arrest. In addition to the apoptosis-inducing ability of ITCs and indoles, CYP-dependent enzyme activities such as 7-ethoxyresorufin *O*-deethylase were significantly induced by DIM, a BDP of I3C. SFN has been demonstrated to induce both phase II enzyme activity and to inhibit benzo(*a*)pyrene (B(*a*)P) and H_2O_2-induced DNA damage in colonic LS-174 cells.



Sulphoraphane



GST-SF conjugate

Figure 6. ITC conjugation with GSH *via* GST.

5.5 Protective mechanism for CRC

In humans, CRC risk appears to be especially elevated in individuals with a higher exposure to dietary carcinogens which is coupled with a strong capacity to activate metabolically, such carcinogens, leading to increases in DNA adduct levels [229, 252 – 254].

Results of *in vitro* studies, animal model and human intervention studies suggest that ITCs can alter the metabolism of dietary carcinogens such as NAs, polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines

Table 8. Anticarcinogenic effects of I3C in animal models

Constituent	Dosage	Time period	Animal	End point	Observation	Protective/adverse effect	Comment	Ref.
I3C + PhIP	0.1% 10 and 50 mg/kg BW	42 days	F344 rats (F)	DNA adduct inhibition	Range of inhibition from 22.6 to 86.6%	+ *	I3C protects against both carcinogens but it protects against higher concs of IQ than PhIP-may be related to strain	[299]
I3C + IQ	0.02% 0.01%	42 days	Sprague – Dawley rats (F)	DNA adduct inhibition	Range of inhibition from 32.2 to 89.6%	+ *		
I3C + PhIP	0.1%	23 days	F344 rats (F)	DNA adduct inhibition	0.1% ↓ DNA adduct formation in all organs from 68.4 to 95.3%	+ *	I3C inhibits PhIP – DNA adduct formation and accelerates PhIP metabolism, possibly through induction of cytochromes CYP1A1 and CYP1A2	[300]
I3C	0.02%	23 days	F344 rats (M)	CYP1A1 and CYP1A2 levels	↓ adduct levels in colon by 85.6% with 0.1%	+ *		
	1 mg/kg BW (I)	3 days			↓ adducts in colon (60.4%) with 0.02% I3C	+ *		
	0.1 and 0.02% (D)				CYP1A1 ↑ in colon. Both CYP1A1 and 1A2 ↑ in liver	+		
	100 and 200 mg/kg BW							
I3C + PhIP	0.1% (D)	16 wk	F344 rats (M)	ACF inhibition	91% inhibition of ACF	+ *	Support protective role for I3C against PhIP-induced colon carcinogenesis	[301]
	50 mg/kg BW (G)	Weeks 3 and 4 (alternating days)			Complete inhibition of ACF during initiation stage			
I3C + IQ	0.1% (D)	8 wk	F344 rats (M)	ACF inhibition	↓ mean number of ACF	+ *	Potent ↓ of PhIP-induced ACF	[302]
	50 mg/kg BW (G)	Weeks 3 and 4 (alternate days)						
I3C + IQ +DMH	0.001 – 0. 1% (D)	Beginning week 6 up to 1 year	F344 rats (M)	Tumour induction	0.1% I3C resulted in complete absence of IQ-induced colon tumours	+ *	As no effect was observed against DMH, it is suggested that I3C is protective against HAs only, in the postinitiation stage	[248]
	0.03% (D)	5 wk			No effect on DMH-induced tumours			
	20 mg/kg BW (SC)	5 wk						
I3C + AOM	40/80% max tolerated dose (D)	5 wk	F344 rats (M)	ACF	D ↓ ACF formation	+ *	Modulator of phase II enzyme activity also demonstrated to↓ ACF in rats	[249]
	15 mg/kg BW (SC)	2 doses						
I3C + AOM	100 ppm (D)	32 wk	C57BL/6J mice (M)	ACF	Total number of ACF ↓ in comparison to those not fed I3C	+ *	Suggest I3C may be potential chemopreventive agent for CC	[250]
	300 ppm (D) 5 mg/kg BW (SC)	4 wk (1/wk)						
I3C	56 mg/kg BW (G)	1 wk	F344 rats (M)	CYP1A1, GST, QR and GSH levels	9.4-fold ↑ CYP1A1	+*	Greater ↑ in detoxification enzymes by mixture due to I3C and Crambene	[303]
PEITC	0.1 mg/kg BW (G)				1.4-fold ↑ GST	+*	↑ CYP1A1 only by I3C and mixture, therefore, I3C responsible for bifunctional induction	

Table 8. Continued

Constituent	Dosage	Time period	Animal	End point	Observation	Protective/adverse effect	Comment	Ref.
					1.9-fold ↑ QR	+*		
					1.6-fold ↑ GSH	+*		
Crambene	50 mg/kg BW (G)				1.4-fold ↑ GST	+*		
					2.5-fold ↑ QR	+*		
					1.8-fold ↑ GSH	+*		
Mixture	As above				11-fold ↑ CYP1A1	+*		
					2.5-fold ↑ GST	+*		
					6.2-fold ↑ QR	+*		
					Two-fold ↑ GSH	+*		
I3C	50 mg/kg BW (D)	12 months	Sprague – Dawley rats (M and F)	Colonic CYP1A1 and CYP1B1 levels	CYP1A1 band densities ↑ ten- and eight-fold in males and females No effect on CYP1B1	+	Direct toxicity not observed CYP1A1 ↑ may shift PhIP towards detoxification	[304]
DIM (low)	6.6 mg/kg BW (D)	12 months			CYP1A1 band densities ↑ eight- and three-fold in males and females No effect on CYP1B1	+	I3C more potent inducer, therefore DIM suggested as a more preferable chemoprotective agent	
DIM (high)	66 mg/kg BW (D)	12 months						

D, Supplemented in diet; SC, injected; G, gavage; +, protective effect; *, statistically significant; NS, not Significant; BW, body weight.

(HAs). Biochemical investigations demonstrate that the effects of the ITCs against these carcinogens are firstly due to inhibition of phase I enzymes, responsible for their bioactivation. Secondly, they induce the activity of phase II enzymes, such as GST, which play a key role in the detoxification of the activated carcinogen [255] by producing highly polar molecules that are readily excreted. These enzyme modulating activities are a consistent property of a variety of ITCs and it has been observed in animals that ITC accumulation levels are closely related to their potencies in inducing phase II enzymes [187].

The ITCs present in watercress and other *Brassica* vegetables, namely SFN, BITC and PEITC have all been shown to act as substrates for four GSTs, *i.e.* A1-1, M1-1, M4-4 and P1-1 [188]. Detoxification can occur during carcinogenesis either by reaction of the electrophilic carcinogen with an endogenous antioxidant, *i.e.* GSH, or by conversion to stable metabolites that can be readily excreted. GSH is a cellular antioxidant 'buffer' and depletion therefore sensitises cells to radiation, oxidative stress and various chemicals. Figure 6 demonstrates conjugation of SFN with GSH *via* GST.

The ultimate chemopreventive effects of *Brassica* vegetables probably involve complex interactions as it is well established that ITCs can inhibit cancer development *via* a range of mechanisms. In addition to their effects on metabolic enzymes, ITCs have been demonstrated to inhibit cell proliferation by either inducing apoptosis or cell cycle arrest of colon cancer cells *in vitro* (Tables 11 and 12) This has also been observed during animal studies where ITCs were administered after chemical induction had occurred; therefore demonstrating that modulation was occurring after initiation of carcinogenesis occurred [256]. They also appear to be more toxic towards transformed/malignant cells than normal cells of the colon, suggesting ITCs to be promising new agents in cancer therapy through their selective effects on cancer cells [257 – 259].

5.6 Conclusions

Results of human, animal and *in vitro* studies have provided considerable evidence that *Brassica* vegetables and their constituents have the potential to reduce colon cancer risk partly by modulating detoxification enzymes, resulting in prevention of initiation/DNA damage, and partly by modulating postinitiation events in particular inhibition of proliferation and induction of apoptosis. It has been demonstrated from these studies that the protective effects of these vegetables do in part come from their GLS, ITC and indole content, although it is likely that other components, especially antioxidants such as carotenoids and vitamin C, also play a role.

A question still arises over the concentration of ITCs required to exhibit their anticarcinogenic effects without themselves becoming genotoxic. Therefore, it is questionable whether increasing the consumption of ITCs to a high

Table 9. Anticarcinogenic effects of ITCs in animal models

Constituent	Dosage	Time period	Animal	End point	Observation	Protective/adverse effect	Comment	Ref.
SFN + AOM	5 μmol daily (D)	Post = 8 wk Pre = 3 days	F344 rats (M)	ACF inhibition	↓ Total no. ACF from 153 to 109	+ *	As ITC conjugates are less toxic than parent compounds, doses ↑ four times – yet still no effect	[296]
	15 mg/kg BW (SC)	2 doses						
SFN-NAC + AOM	20 μmol daily (D)	Post = 8 wk Pre = 3 - days	F344 rats (M)	ACF inhibition	No effect observed with conjugate	No effect		
	15 mg/kg BW (SC)	2 doses						
SFN + NDMA	0–300 μM (D)	2 wk	Sprague–Dawley rats (M)	CYP2E1 activity	Inhibition constant of 37.0 ± 4.5 μM	+ NS	Suggests ↓ of CYP2E1 by SFN *via* competitive inhibition	[305]
	50–500 μM (D)							
BITC + PhIP	75 mg/kg BW (G)	3 days	F344 rats (M)	DNA adduct levels	66% ↓ in PhIP–DNA adduct levels	+ *	BITC most effective protectant against PhIP–DNA adduct formation in colon	[306]
	50 mg/kg BW (G)							
GSH + PhIP	30 μmol	Co-administered			↓ DNA-adduct formation by approx. 30%	+ NS		
	50 mg/kg BW (G)							
Sinigrin + DMH	400 μg/g (D)	22 h post-DMH	Wistar rats (M)	Apoptosis	No. of ACF ↓ by approx half	+	Although reduction in ACF occurred, difference between those consuming sinigrin diet and those not was not significant	[307]
	30 mg/kg BW			ACF inhibition				

D, Supplemented in diet; SC, injected; G, gavage; +, protective effect; *, statistically significant; NS, not significant; BW, body weight.

level, *e.g. via* supplements, is beneficial. Clearly more human supplementation studies need to be carried out to determine this level, *via* the use of specific colon cancer end points.

6 Toxicity and antinutritional effects of GLSs

6.1 Effects in animals

In the field of animal production, it is well known that feeding rapeseed meal that contains high levels of GLSs may result in a variety of toxic effects which are often manifested as goitre as well as malfunction of liver and kidneys. Although reported dietary tolerance levels vary, ruminants are generally considered to be less susceptible to GLSs as compared with monogastrics. In their review paper, Tripathi and Mishra [260] concluded that total GLS content in diets for lambs, pigs, rabbits, poultry and fish should not exceed 1.5–4.22, 0.78, 7.0, 5.4 and 3.6 mmol/kg diet, respectively. Thus, with pigs considerable care is required in prolonged feeding of high-GLS meals. Reduced feed intake and growth have been reported. Piglets may show enlarged thyroids and poor survival rates when maternal diets include high levels of rapeseed meal. Although GLS intake is less threatening for ruminant animals, there is still some evidence of reduced feed intake and minor liver damage in younger animals, as well as adverse effects on rumen fermentation as shown by decreased production of SCFAs. However, there appears to be no reason for not using rapeseed meals as the major if not the sole source of supplemental protein in diets for adult ruminants.

GLSs themselves are not responsible for adverse health effects, but their degradation products are. Thus the toxic effects have been generally attributed to the formation of isothiocyanates, organic thiocyanates, nitriles and 5-vinyloxazolidine-2-thione (goitrin). This process is brought about by the action of thioglucosidase known as myrosinase. A certain measure of control of the goitrogenic activity of meals is achieved by ensuring destruction of myrosinase during the pretreatment of the seed before extraction. However, such control is only partial, since bacterial thioglucosidases produced in the gut may hydrolyse residual GLSs in the meal as well.

Table 10. Effects of cruciferous vegetable extracts in colon cells *in vitro*

Vegetable extract	Latin name	Cell line	Dosage	End point	Observation	Protective/adverse effect	Comment	Ref.
Mixture of Broccoli	*B. oleracea*	HT29	100 – 200 μL/mL	DNA damage induced by 75 μM H_2O_2	24 h incubation caused ↓ genotoxicity by 30 – 50%	+ *		[244]
Radish	*R. sativus*							
Alfalfa	*M. sativa*							
Clover	*T. pratense*							
Watercress	*Rorippa nasyurtium-Aquaticum*	HT29	0 – 50 μL/mL	DNA damage induced by 75 mM H_2O_2	Significantly decreased DNA damage	+ *		[251]
		HT115		Invasion through matrigel	Invasion significantly inhibited	+ *		
Watercress	*Rorippa nasyurtium-Aquaticum*	HCT 116	0.02 – 1.0 mg/mL	GST activity QR activity	↑ GST and QR activities	+ *	Demonstrates that watercress and broccoli juices result in strong enzyme induction, possibly due to their ITC composition	[308]
Broccoli	*B. oleracea*	HCT 116		GST activity QR activity	↑ GST and QR activities	+ *		

+, protective effect; *, statistically significant.

Table 11. Antiproliferative effects of ITCs and indoles *in vitro*

Constituent	Dosage	Cell line	End point	Observation	Protective/adverse effect	Comment	Ref.
AITC	12 μM	HT29	Cell cycle arrest and apoptosis	Cell cycle arrest occurred in metaphase, common in compounds interfering with microtubule formation	+ *	AITC inhibited proliferation by causing mitotic block associated with a-tubulin disruption	[309]
PEITC	5 – 50 μM	HT29	Apoptosis	Condensed and fragmented nuclei ↑ with ↑ concs PEITC	+	PEITC-induced apoptosis as observed by morphological features	[310]
				DNA fragmentation ↑ in DD manner	+		
PEITC + Z-VAD-FMK + AC-LEHD-CHO + AC-DEVD-CHO	40 μmol/L	HCT 116 and HT29	Apoptosis	Time-dependent ↑ caspase-3 like activity	+* (from 1 to 24 h)	Caspase-3 activity significant ↑ by PEITC.	[308]
	10 – 80 μmol/L			DD ↑ caspase-3 like activity	+* (up to 40 μmol/L)	Role of caspases in PEITC-mediated apoptosis supported	
	75 μmol/L			Pharmacological caspase inhibitors ↓ DNA fragmentation	+*		
	50 μmol/L						
	50 μmol/L						
PEITC, SFN and BITC + DIM	5 mM	LS-174	Apoptosis enzyme induction	Apoptosis -	+	All 3 ITCs and DIM initiated apoptosis	[311]
	300 μM			CYP ↑ by DIM	– *	Indoles are bifunctional inducers and possibly hazardous *via* carcinogen activation	
				Dihydriol dehydrogenase and NQO ↑ by ITCs	+	ITCs are monofunctional inducers	
PEITC, SFN and BITC + DIM	5 mM	CaCo-2	Apoptosis enzyme induction	Apoptosis ↑	+		
	300 μM			CYP ↑ by DIM	– *		
				Dihydriol dehydrogenase ↑ by ITCs	+		

+, Protective effect; –, adverse effect; *, statistically significant.

Table 12. Antiproliferative effects of ITCs and indoles *in vitro*

Constituent	Dosage	Cell line	End point	Observation	Protective/ adverse effect	Comment	Ref.
BITC	10 μmol/L	CaCo-2	Proliferation	↑ doubling times from 32 to 220 h	+ *	Antiproliferative effect of both BITC and PEITC in CaCo-2 cells, due at least in part to activation of G2/M DNA damage checkpoint.	[312]
	5.1 μmol/L			50% ↓ DNA synthesis	+ *	Sustained G2/M phase cell cycle arrest may be due to up-regulation of p21	
PEITC	10 μmol/L			↑ doubling times from 32 to 120 h	+ *		
	2.4 μmol/L			50% ↓ DNA synthesis	+ *		
BITC and PEITC	10 μmol/L			↑ cells in G2/M phase	+ *		
				↑ DNA strand breakage	+ *		
				phosphorylation of G2/M checkpoint enforcer Chk2	+ *		
				↑ p21 expression	+ NS		
PEITC	10 μM	40 – 16 ($P53^{+/+}$) (derived from HCT 116) and 379.2 ($P53^{-/-}$) (derived from 40 to 16) 40 – 16 ($P53^{+/+}$) and 379.2 ($P53^{-/-}$)	Apoptosis	Strong ↑ PARP cleavage at 24 and 48 h in 40 – 16 but weaker in 379.2 at 48 h	+ NS	Apoptosis induction occurring in response to all compounds independently of p53.	[313]
SFN	15 μM		Proliferation	↑ PARP cleavage at 24 h in 40 – 16 and at 48 h in 379.2	+ NS	Differing apoptotic effects between ITCs and indoles consistent with differing antiproliferative profiles	
I3C and DIM	10 μM			Weaker apoptotic effect for both indoles in 40 – 16 cells compared to ITCs	+ NS		
All 4 compounds	0.4 – 50 μM			DD ↓ proliferation PEITC and SFN cytotoxic ↑ 12.5 μM	+* (above 3.1 μM)		
NI3C	0 – 100 μM	DLD-1 + HCT-116	Proliferation and apoptosis	Both compounds caused DD ↓ in both cell lines	+ *	Even at 250 μM I3C did not ↑ apoptosis	[314]
I3C	0 – 450 μM			In HCT 116 cells, NI3C - apoptosis at 30 μM		NI3C is a more potent inhibitor of proliferation than I3C	
I3C	0.1 – 0.7 mM	HT29	Proliferation	↓ proliferation >0.1mM	+ *	I3C has the ability to inhibit cell proliferation of colon cancer cells at concentrations of >0.1mM	[315]

+, Protective effect; *, statistically significant; NS, not significant.

Nowadays, mainly rapeseed varieties that are very low in GLS content such as canola or 'double zero' rapeseed are grown for animal feeding. Still, it must be borne in mind that in some instances even reduced GLS levels in the meal may exert harmful effects such as depressed foetal development and reduced feed intake and consequently poor growth in early weaned pigs.

In summary, from experiences with animals we know that GLSs have at least the potential to induce antinutritional and toxic effects.

6.2 Mode of action of GLSs in creating harmful health effects

Basically the biological activity of GLSs originates from their hydrolysis products which are often isothiocyanates, thiocyanates, epithionitriles, oxazolidine-2-thiones and indolyl compounds. Obviously, the chemical nature of the BDPs depends on the initial structure of the GLSs. This implies that GLSs differ in their potentiality to exert deleterious health effects as observed in animals consuming diets with high concentrations of these compounds. Moreover, amounts of hydrolysis products are determined by the myrosinase activity in the plant cells and in the gut. Consequently, it is not surprising that the biological effects of GLSs vary. High intake may exert toxic effects as shown in animals, while low intake has either no effects or may in some cases even result in health promoting consequences such as anticarcinogenicity, depending upon the hydrolysis products that are formed. For example, high consumption of 2-hydroxy-3-butenyl GLS or progoitrin has been considered toxic in animals and therefore this compound has been

Table 13. Anticarcinogenic effects of SFN *in vitro*

Constituent	Dosage	Cell line	End point	Observation	Protective/adverse effect	Comment	Ref.
SFN and ICZ + BaP + H_2O_2	5 and 1 μM	LS-174	DNA Damage	SFN↓ DNA damage greater than ICZ. Greater effect observed when co-administered	+ *	Demonstrated protective effects of combined SFN and ICZ against BaP-induced and H_2O_2.DNA damage	[311]
	25 μM 100 μM	LS-174	DNA Damage	↓ only observed when co-administered	+ *		
SFN	100 μM	HT29	Proliferation	80% ↓ cell viability in 24 h IC50 reached at 15 μM irreversible	+ *	Strong cytotoxic effect observed with SFN in HT29 cells	[258]
SFN	0 – 50 μM	CaCo-2	Proliferation	No effect on cell viability noted until 30 μM			
				At 50 μM cell viability ↓ 70%	+ *	Demonstrates specificity of SFN	
SFN	0 – 30 μM	HT29	Apoptosis	15 μM caused 75% cell death in 24 h	+ NS	10 – 30 μM clearly induces cell arrest and apoptotic death in dose-dependent manner	[259]
				Almost total cell death observed in 96 h			
				No change noted in p53 expression			
				15 μM displayed condensed chromatin and fragmented nuclei			
SFN	0.01 – 0.1 mmol	HT29	Proliferation	≥0.02 mmol SFN caused inhibition of cell proliferation to be significantly reduced after 72 h	+*	Results may help explain protective effects of vegetables against CRC	[316]
SFN + Roscovitine	15 μM	HT29	Apoptosis	Around 25% apoptotic cells after 24 h	+ NS	SFN causes apoptosis to occur in HT29 cells. Possibly due to activation of cdc2 kinase	[317]
				Around 32% after 48 h	– NS		
	20 μM	HT29	Apoptosis	Reduction in apoptosis to 6.5% with 24 h incubation			

+, Protective effect; –, adverse effect; *, statistically significant; NS, not significant.

largely removed from rapeseed by appropriate breeding. In contrast, SFN which is a metabolite from 4-methylsulphinyl-butyl GLS is considered a putative anticarcinogen [261] and attempts are being made to increase its content in human foods.

The biological mechanisms responsible for the harmful activity of GLS-derived compounds are only partly elucidated. From animal studies it is known that isothiocyanates and thiocyanates behave different in causing antithyroid effects. Certain isothiocyanates interfere with the synthesis of thyroid hormones, while thiocyanates compete with iodine and inhibit iodine uptake by the thyroid gland. In addition to the thyroid gland, main target organs are the liver, kidney and pancreas, showing altered weight and malfunction. The mechanisms for these phenomena are greatly unknown, although carcinogenic processes have been reported. GLS dose–response relationships have hardly been investigated. In rats, toxic effects were observed with daily isothiocyanate doses higher than 10 – 50 mg/kg body weight. At such high concentration, certain isothiocyanates and nitriles may initiate mutagenic, cytotoxic and carcinogenic processes [5].

In some cases, there is not much difference between the deleterious and the beneficial GLS dose. Consequently, the health promoting effects from GLSs are not necessarily more pronounced at higher doses, quite the contrary. This seems to be true for I3C, a hydrolysis product from indole-GLSs. This compound is considered responsible for the modulation of estrogen receptor activity resulting in agonistic and antagonistic effects depending on the dose [262]. Moreover, while I3C may delay mammary tumour formation and may inhibit development of ACF in the colon, it appears to promote carcinogenesis in the liver [263]. Such findings will certainly complicate future proposals for recommended daily GLS intake levels in relation with positive health effects in humans.

In case isothiocyanates act as anticarcinogenic agents, their effects are increasingly explained by their contribution

to the antioxidative potential of cells. This means that isothiocyanates are able to affect the redox status of cells by modulating phase II enzyme expression [5].

6.3 Toxic and antinutritional effects from GLSs in humans?

As mentioned above, particularly monogastric animals are sensitive to GLSs, the negative health effects being dependent on the ingested dose and age. As a consequence, humans should have reasons to take care of their GLS consumption. Based on current knowledge from animal studies, it seems risky to give humans large quantities of GLSs or their degradation products such as isothiocyanates because dose–effect relationships are not known. For example, I3C potentially both inhibits and promotes carcinogenesis. Stoner [263] concluded that this compound is not an appropriate chemoprotective agent for human use in spite of its potential effects on breast and colon cancer. Also, benzyl and allyl isothiocyanates have been shown to act as anticancer agents, but they have also genotoxic and carcinogenic potential [264]. Certainly, depending on the ingested dose and bioavailability, some hydrolysis products from GLSs have chemopreventive and carcinogenic properties. Nevertheless, especially intake of supplements warrants attention as the optimal dose may be exceeded giving rise to negative health effects.

Can any harmful effects on human health be expected from intake of GLSs present in vegetables? GLS content in *Brassica* plants is around 1% of dry matter. Estimated daily consumption of GLSs varies between 12 and 300 mg [146, 265]. To date, there are no reports on deleterious health effects from GLSs in humans consuming normal amounts of *Brassica* vegetables, watercress, rocket salad and radish. In contrast, beneficial effects may not be excluded.

On the other hand, there is no scientific information available regarding allowable dietary levels for various GLSs. This should be investigated, first of all because supplements are becoming available on the market. Moreover, attempts are being made to selectively increase GLS concentrations in human foods in order to generate beneficial health effects. In this context, 4-methylsulphinyl-butyl GLSs as precursors of the putative anticarcinogen SFN appear promising.

7 Modelling variability of GLSs in the food supply chain

Mathematical modelling in the area of phytochemicals is of interest for various applications. Simulation and optimisation of processes in the supply chain is an obvious application [266]. Prediction of the effect of variability in the supply chain on the health benefits of phytochemicals in the human diet is another challenging approach. These two approaches will be discussed in this section. Linking these two approaches together can be used to effectively improve the effect of phytochemicals on human health. Modelling consists of describing a part of the reality in terms of the main mechanisms that are assumed to be occurring. Modelling therefore is always neglecting mechanisms that are assumed to be less important for the variables of interest.

7.1 Dealing with variability in the supply chain

Epidemiological studies on the relation between fruit and vegetable intake and chronic diseases show variable results. In many studies, a small protective effect is found for fruit and vegetable intake and the risk for cardiovascular diseases and cancers. In other studies, these effects could not be found in a statistically significant way. This is clearly illustrated in a review by Steinmetz and Potter [267].

For *Brassica* vegetables, the protective effects is often stronger when compared with vegetable intake in general, but also for *Brassica* vegetables the epidemiological studies show variable results. Experimental animal and mechanistic studies with cell lines or in humans show often clear protective effects of many phytochemicals including GLSs. The effect of variability in the food production chain on the results of epidemiological studies can be predicted by probabilistic simulation of the effects of steps in the supply chain on the level of GLS in the consumed products. Even if a very strong health protective effect of a phytochemical is assumed, this variability will lead to only very small, non-significant, protective effects to be found in epidemiological studies [267, 268].

The two published studies dealing with variability in the supply chain use Monte Carlo simulation of the variability in the supply chain [268, 269]. The main sources of variation in the supply chain were identified as being: cultivars, industrial processing and consumer preparation. Each of these steps can cause at least a ten-fold variation in the starting level of GLS in the vegetable and in the retention of the GLS during processing and preparation. By testing different relations between the intake level of GLS and the health protecting effect the simulation could be calibrated to the typical outcomes of published epidemiological cohort studies. With this calibrated relation the effect of different scenarios to improve human health has been investigated. Increasing the *Brassica* vegetable consumption with 50% will produce far less benefits when compared with a scenario of increasing the level of phytochemicals in consumed products three-fold and reducing the variability in its content three-fold [269]. These increased levels are realistic from a practical point of view given the variation in cultivars, processes and preparation methods. Collaboration within the entire supply chain is however required to deliver these products to the consumer in a reliable way.

7.2 Modelling the effect of processing of GLSs

For GLSs mathematical models have been made to describe the effect of thermal processing in an aqueous environment like cooking, canning, blanching, *etc*. Modelling the consequences of thermal processing in water on the loss of GLS from vegetables has to take into account different mechanism that all affect the level of GLS during the process:

(i) Heating up of the processing water.
(ii) Heat transfer from the processing water into the vegetable.
(iii) Thermal lysis of vegetable cells.
(iv) Increase in extractability of GLS.
(v) Diffusion and Leaching of GLS from the vegetable matrix.
(vi) Diffusion and Leaching of myrosinase from the vegetable matrix.
(vii) Diffusion and Leaching of enzyme cofactors from the vegetable matrix.
(viii) Enzymatic degradation of GLS upon contact between myrosinase and GLS.
(ix) Thermal denaturation of myrosinase in the vegetable.
(x) Thermal denaturation of myrosinase in the processing water.
(xi) Thermal degradation of GLS in the vegetable.
(xii) Thermal degradation of GLS in the processing water.

All these individual mechanisms can be described by mathematical equations that will have parameters that have to be estimated as well as there temperature dependency. The amount of parameters will not allow to estimate them all accurately from an experimental data set, unless many experimental data for many different conditions are available. Therefore it is necessary to simplify the model approach by neglecting certain mechanisms that are expected to have only little effect or only an effect in the initial stages of the processing process.

The following assumptions were made for the processing of fresh-cut cabbage:

Given the size of fresh-cut cabbage (1 – 2 mm × 2 – 5 cm × leaf thickness) it is assumed that the temperature of the vegetable equals that of the processing water.

Increase in extractability (defined as the recovery of GLS from the vegetable matrix by the analytical procedure) has been observed in previous studies to occur during the first minutes of processing. The mechanism by which this occurs is not clear. In the simulations presented here it is neglected.

Describing the diffusion of GLS in the vegetable matrix is possible and may be rate limiting in relatively large vegetable structures like brussel sprouts or broccoli. For fresh-cut cabbage it was negelected.

The model will thus be limited to a description of the observed profiles of GLS both in the vegetable and in the processing water for processing times of 6 min and more. Mechanisms that are included in the model are:

(i) Heat up of the processing water.
(ii) Thermal lysis of vegetable cells.
(iii) Leaching of GLS from the vegetable matrix.
(iv) Enzymatic breakdown of GLS in contact with myrosinase.
(v) Myrosinase denaturation.
(vi) Thermal degradation of GLS in the vegetable.
(vii) Thermal degradation of GLS in the processing water.

The mathematical description is based on differential equations and mass balances describing the mechanism as a function of time and the dynamic temperature profile. A detailed description of this mathematical model based on these assumptions will be published (Dekker *et al.*, 2008, in preparation). In this review, some main applications of the model are presented.

Cell lysis is described by a first order kinetics as this was also observed for the lysis of red cabbage cells by Verkerk [270]. A mass balance is used to relate the fraction lysed cells to the fraction intact cells.

The result of cell lysis is that the lysed part of the mass of the vegetable is in direct contact with the processing water, this means that the volume of the 'free' water phase (processing water plus lysed cell contents) is in fact increasing as more cell are lysed.

According to this model the leaching of GLS is the direct consequence of the cell lysis. The GLS content of the lysed cells is added to the free water phase. No differences in leaching behaviour of individual GLSs are expected according to this mechanism. To describe this mathematically one has to take into account the amount of GLS transferred from the lysing cells, but also the diluting effect caused by the increase of the mass of free water caused by this lysing.

Thermal breakdown is described by first order kinetics, similar as in previous studies [147].

Myrosinase can hydrolyse GLSs in the free water (either outside or within the lysed cell matrix), the active myrosinase concentration can be predicted in the vegetable and in the free water. It is depending on lysis, leaching and first order denaturation. The activity of myrosinase follows Michaelis Menten kinetics. The formation of BDP is equal to the enzymatic degradation of GLS. These BDP will also be susceptible to thermal breakdown.

To be able to predict concentrations in the vegetable, the concentration that corresponds with that should be calculated by taking into account both, the part of the vegetable that is still intact and the part that is already lysed (the lysed part will have the same concentration as the rest of the processing water). All rate constants in the model are temperature dependent following the Arrhenius equation.

For the parameter estimations and the processing simulations, the software programme Athena Visual Workbench (www.athenavisual.com) was used.

In Figs. 7a – d, the simulation results of the model are shown for four typical industrial or domestic processes:

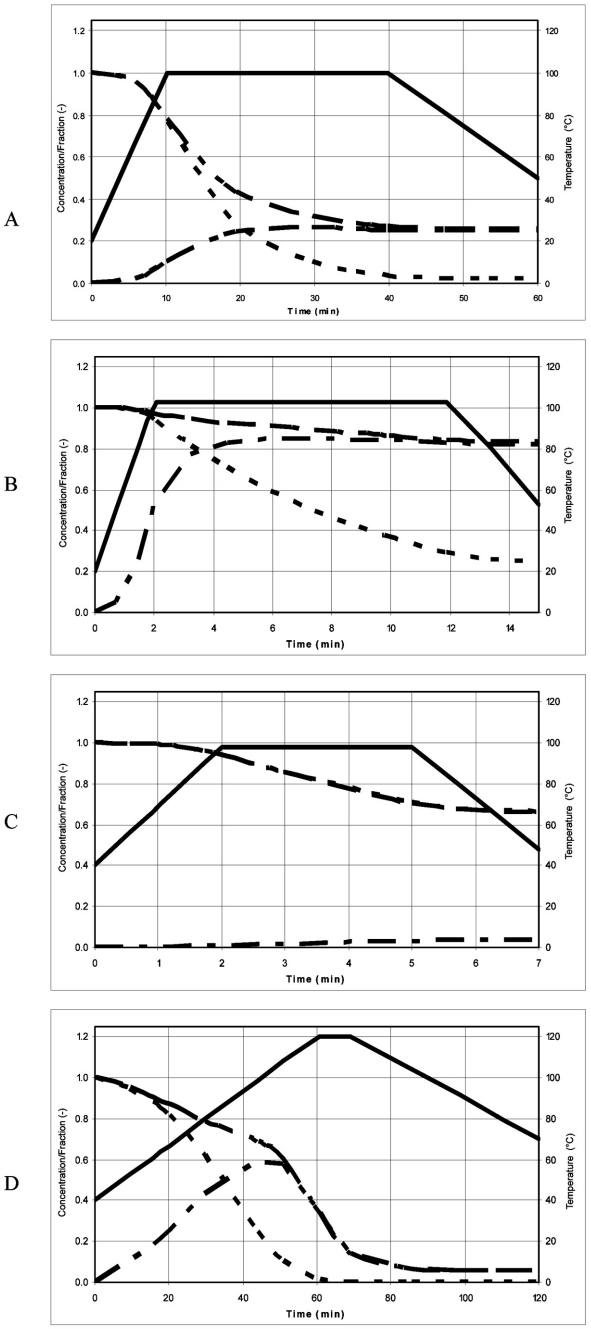


Figure 7. Simulations of a process model for thermal treatment of red cabbage in water (–, temperature; —, fraction intact cells; - · –, concentration GLS in vegetable; · – ·, concentration GLS in water). (A) Cooking (ratio vegetable/water = 1:2); (B) microwaving (ratio vegetable/water = 20:1); (C) blanching (ratio vegetable/water = 1:9); (D) canning (ratio vegetable/water = 1.75:1).

cooking in water, microwaving, blanching and canning. The parameters included in these simulations are based upon experimental data on the GLS glucobrassicin in red cabbage [147, 270]. The simulations shows that leaching of the GLS to the processing water is the main loss during cooking and blanching of red cabbage. These processes resulted in a loss of 75 and 35%, respectively. Thermal breakdown is the most pronounced mechanism for the losses of GLS during canning. The total loss was more than 90%. Microwaving the cabbage with very little water shows the smallest loss of only 18%, mainly due to thermal degradation.

With the model for processing it is very easy to simulate the effect of changing conditions like: Vegetable/water ratio for one particular process, heat-up time, processing temperature/time, *etc*. In this way, the model can be used to optimise the conditions with optimal retention of GLS can be determined. Of course other quality and safety attributes of the product will be important constraints for this optimisation.

8 Supply chain management strategies

8.1 Best practices/recommendations

The delivery of GLSs to consumers by final products is determined by the entire supply chain. The levels of GLSs in current products of *Brassica* vegetables varies over 100-fold [268]. As described in Section 7, increasing the level of GLSs and reducing its variability can have a big impact on the health benefits for the population. In order to achieve controlled levels of GLSs in the most efficient way, the steps in the supply chain with the biggest effect on the final level of GLSs should be identified. A way to do this is the so-called Quality Analysis/Assurance Critical Control Point approach [271]. This method is an alternative to Hazard Analysis Critical Control Point (HACCP) that is only dealing with food safety aspects. QACCP is dealing with final quality of products, which includes health aspects like the level of GLSs of products containing *Brassica* vegetables. By analysing various steps of the supply chain of *Brassica* vegetables Verkerk [270] identified four critical points that have the biggest impact on the level of GLSs in the final products:

(i) Cultivar selection.
(ii) Storage and packaging.
(iii) Industrial processing.
(iv) Consumer preparation.

To develop products with optimal, controlled levels of GLSs various actors should collaborate to design a food supply chain that can guarantee and market products with additional health benefits and value.

8.1.1 Cultivar selection

Plant breeding companies do not screen all their cultivars and breeding lines for the content of phytochemicals like GLSs as it is not part of the standard product specification. As interest in the presence of these compounds is clearly increasing screening the commercial cultivars for GLS levels and market selected varieties for their GLS level and

profile will become more common. Innovative breeding companies already have done such screenings (Verkerk, R., Tebbenhoff, S., Dekker, M., Variation and distribution of GLSs in 42 cultivars of *Brassica oleracea* vegetable crops, *Acta Hortic*. Submitted). On the longer term new cultivars can be developed with even further optimised GLS levels and profiles.

This information of cultivars will be a good starting point for other chain actors like growers, processors and retailers to collaboratively develop and market final products with additional health benefits.

8.1.2 Storage and packaging

Logistics in the supply chain is essential for retention of overall quality of the vegetables as well as prevention of losses of GLSs during storage and transport of the vegetables. In this respect, a continuous cooling chain and altered atmosphere conditions appear to be effective for reducing respiration, while a uniform RH is helpful in reducing transpiration.

8.1.3 Industrial processing

An important starting point for industrial processing is the selection of raw materials as shown in the previous paragraph. Industrial processing can have a large effect on the level of GLSs as described in Section 3.4. By using process models the level of GLSs can be optimised by changing the processing conditions, like time–temperature profile, or the ratio of water to vegetables during thermal treatments. Also novel processing techniques like high pressure sterilisation with lower temperatures and shorter times can reduce the breakdown of GLSs during the production of sterilised vegetables.

8.1.4 Consumer preparation

Selection of products with optimal level and profile of GLSs is the starting point for an optimal intake by consumers. Products containing *Brassica* vegetables could therefore by marketed for their health benefits or their content of GLSs.

During preparation of these products minimal amounts of GLSs should be lost by choosing the best preparation methods. As described in the Section 3.4 methods with low water to vegetable ratio and short preparation times are preferred. Cooking with minimal water level, steaming and microwaving for short times are considered the best ways of preparation to retain GLSs. In addition to reducing losses of GLSs also retention of activity of the enzyme myrosinase is of importance for the formation and bioavailability of the bioactive BDPs. Although these BDPs can be formed by the gut flora, their formation and bioavailability is substantially higher when the GLSs are consumed together with active myrosinase.

By the combined action of these three steps in the supply chain it will be possible to enhance the level of health promoting GLSs substantially. These higher intake have the potential to have a major impact on cancer prevention in the population.

8.2 Best sources and intake levels

Clearly, for producers and consumers looking for the best sources of GLS containing food products in relation to health several considerations are important. In order to have the optimal beneficial effect of the GLS derived BDPs it is important to make the proper choices with respect to: selection of vegetable, selection of cultivar, conditions during industrial processing, conditions during storage and packaging, conditions during preparation and combination with other foods.

8.2.1 GLS profile

Evidence for the potential health promoting effect indicates that not all GLSs can be treated similar. Most reported health effects are based on the aliphatic GLS glucoraphanin and its isothiocyanate BDP SFN. Isothiocyanates of several other aliphatic GLSs have been reported to have a similar but smaller effect on detoxifying enzyme induction. In addition, the aromatic GLSs gluconasturtiin and glucotropaeolin are both also considered as strong anticarcinogens. Care should be taken with indole GLSs, since they have been found to exert also an inducing effect on phase I enzyme systems, which are known to activate some procarcinogens.

Based on the current knowledge it seems safest to select raw materials that contain predominantly aliphatic GLSs, like glucoraphanin, sinigrin and glucoiberin as well as aromatic GLSs.

Chinese broccoli and (green, purple) broccoli, broccoli sprouts (white, green, purple), cauliflower (white, red, savoy), cabbage, mustard green and Ethopian kale are good sources of several of the desired aliphatic GLSs, whereas radish, turnip and watercress are rich in aromatic gluconasturtiin.

8.2.2 Myrosinase activity

It has been shown that the bioavailability of the bioactive isothiocyanates is significantly higher when active myrosinase is present in the food product consumed. The significance of myrosinase-mediated conversion is emphasised by bioavailability studies carried out by Conaway *et al*. [181]. They showed that the bioavailability of isothiocyanates from fresh broccoli is approximately three times higher than that from steamed broccoli, in which myrosinase is inactivated. The microbial gut flora is also capable of forming isothiocyanates but the efficiency is lower compared to the action of plant myrosinase [272]. Food products containing active myrosinase, like sprouting *Brassica* vegetables and shortly cooked *Brassica* vegetables are preferred. Addition of a small amount of raw *Brassica* vegetables to a meal of cooked ones to add active myrosinase is expected to increase the bioavailability of isothiocyanates.

Thus, the efficiency and the type of BDPs formed depend on one hand on the residual GLS concentration and on the other hand on the action of plant myrosinase or the gut flora. However, the digestive fate of GLSs and uptake of BDPs also depends on other factors such as the extent of chewing, food matrix, gastrointestinal transit time, meal composition and individual genotype [272 – 274].

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