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Enhancement Of Glucosinolate & Isothiocyanate Profiles In Brassicaceae Crops: Addressing Challenges In Breeding For Cultivation, Storage, and Consumer Related Traits

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ABSTRACT

Glucosinolates (GSLs) and isothiocyanates (ITCs) produced by Brassicaceae plants are popular targets for analysis due to the health benefits associated with them. Breeders aim to increase the concentrations in commercial varieties, however there are few examples of this. The most well known is *Beneforté* broccoli, which has increased glucoraphanin/sulforaphane concentrations compared to conventional varieties. It was developed through traditional breeding methods with considerations for processing, consumption and health made throughout this process. Many studies presented in the literature do not take a holistic approach, and key points about breeding, cultivation methods, postharvest storage, sensory attributes and consumer preferences are not properly taken into account. In this review, we draw together data for multiple species and address how such factors can influence GSL profiles. We encourage researchers and institutions to engage with industry and consumers to produce research that can be utilised in the improvement of Brassicaceae crops.

Keywords: Brassica, Phytochemicals, Plant breeding, Nutrition, Processing, Chemoprotection, Glucoraphanin, Indoles, Broccoli, Cabbage, Mustards

INTRODUCTION

Crops of the Brassicaceae family contain numerous phytochemicals that are known, or are suspected to be, beneficial for human health. These include sulfur-containing glucosinolates (GSLs) [1], which have a range of hydrolysis products that are noted for beneficial effects on human health [2]. GSLs are secondary metabolites that are hydrolysed by myrosinases and modified by specifier proteins into numerous breakdown products [3]; these include isothiocyanates (ITCs), thiocyanates, nitriles, ascorbigens, indoles, oxazolidine-2-thiones and epithioalkanes [4]. This process is part of a complex defense strategy utilised by Brassicaceae plants to protect against herbivory, pests and diseases [5]. These compounds also give the family their distinctive sulphurous, hot, mustard and pepper flavors [6].

Potential health benefits such as anti-carcinogenic and anti-metastatic activity have been linked with these compounds (such as ITCs and indoles) in cell and animal studies [7]. Clinical, epidemiological and pharmacological research in humans has demonstrated beneficial effects *in vivo* on some cancers, on cardiovascular health [8,9], and on neurodegenerative prevention [10]. For these reasons, there is huge interest in enhancing Brassicaceae crop GSL content [11]. Despite initiatives such as the "5-a-day" campaign, fruit and veg consumption remains low in Western countries, and chronic diseases such as cancer and cardiovascular disease are leading to premature deaths [12].

This review will explore prominent species and some underutilised edible Brassicaceae crops with the potential for GSL/ITC profile improvement. The health benefits that have been linked to these compounds and how they can be maximized will also be discussed. We aim to highlight and explore the challenges faced in developing enhanced Brassicaceae varieties in three key areas: plant breeding, agronomic practice, and 'the consumer'. Previous review papers have not directly addressed the discrepancies between scientific research methods and common agricultural and commercial practices, or how plant

51 breeders can use scientific findings to inform their selections. Our goal is not to define the
52 ideal crop for enhancement, but to highlight species that require further study and
53 development. We encourage research groups to consider the entire commercial supply chain,
54 and how this affects plant phytochemistry in a 'real world' context. We also highlight the
55 need for consideration of the sensory preferences and end consumer metabolic genotypes. In
56 this way, commercial breeders/producers can utilise better scientific research to improve crop
57 nutritional density, and make informed decisions about varietal selection and agronomic
58 practice.
59
60 **BRASSICACEAE CROPS & GLUCOSINOLATE PROFILES**
61 **General**
62 Table 1 summarises and compares the GSL profiles of several major, minor and
63 underutilised Brassicaceae crops, and gives examples of typical concentrations that have been
64 reported within the scientific literature. The following section describes these profiles and
65 illustrates how concentrations and profiles vary between species.
66 **Broccoli (*Brassica oleracea* var. *italica*)**
67 Perhaps the most well studied Brassicaceae crop is broccoli [13]. It is a well-known
68 vegetable that is grown and consumed worldwide, and production rates are increasing [14]. The
69 key factor in its popularity from a research perspective is that it contains significant
70 glucoraphanin concentrations in florets and sprouts (Table 1) [11,14–34]. Total reported
71 concentrations in broccoli florets are modest (~7.9 $mg.g^{-1}$ dw, Table 1) compared to other
72 commonly consumed crops such as Brussels sprouts (~13.3 $mg.g^{-1}$ dw). That being said,
73 some varieties have high total concentrations (26.9 $mg.g^{-1}$ dw [16]), well in excess of the
74 average.
75 **Brussels Sprouts (*Brassica oleracea* var. *gemmifera*)**

Although broccoli and kale are most often ascribed with the most potent health benefits associated with GSLs, Brussels sprouts have higher total concentrations than both, on average (~13.3 mg.g^{-1} dw). Although not containing high levels of glucoraphanin, sprouts do have high amounts of glucobrassicin (Table 1) [18,19,25,31,32,34,35].

Cabbage, Red Cabbage, & White Cabbage (*Brassica oleracea* var. *capitata, Brassica oleracea* var. *capitata* f. *rubra,* & *Brassica oleracea* var. *capitata* f. *alba*)

Cabbage is a widely consumed and studied crop, but has modest total GSL concentrations compared to other crops (Table 1) [1,25,31,32,36,37]. White cabbage is similar to the green variety in terms of its overall GSL profile [31,32,38].

Red cabbage contains similar GSLs to white and green cabbages, but differs in the relative amounts present within leaf tissues; e.g. it contains greater concentrations of glucoraphanin and gluconapin, and less sinigrin [1,31,32,39–41]. Overall, average reported concentrations are higher in red cabbages than other types.

Cauliflower (*Brassica oleracea* var. *botrytis*)

Total GSL reports in cauliflower florets range from 0.7 – 11.4 mg.g^{-1} dw, but average ~4.1 mg.g^{-1} dw; much lower than broccoli and Brussels sprouts. The predominant major GSL reported is glucobrassicin (~1.7 mg.g^{-1} dw) [18,19,21,22,25,31,32,34,42].

Chinese Cabbage (*Brassica rapa* var. *chinensis* & *Brassica rapa* var. *pekinensis*)

There are two predominant Chinese cabbage varieties: *B. oleracea* var. *pekinensis* and *B. oleracea* var. *chinensis*. These crops originate and are popular in China and southeast Asia, and have been identified as candidates for GSL accumulation trait improvement through breeding, due to large phenotypic variation [43,44]. Total average GSL contents reported are modest compared to other crops (Table 1). Indolic GSLs make up a large proportion of the overall profile [31,32,36,43–45].

Chinese Kale (*Brassica oleracea* var. *alboglabra*)

Also known as gai lan, Chinese kale is a popular crop in China and southeast Asia, but not well known in other parts of the world. It is noted for high GSL concentrations (compared with broccoli florets). Total concentrations in mature leaves have been reported to be 14.9 $mg.g^{-1}$ dw [21] (broccoli florets: ~7.9 $mg.g^{-1}$ dw). In sprouts, GSL concentrations have been reported as high as 98.2 $mg.g^{-1}$ dw [46] and as low as 3.7 $mg.g^{-1}$ dw [47].

Collards (*Brassica oleracea* var. *sabellica*)

Collards are an understudied variety of *B. oleracea*, but have high total GSL concentrations (18.2 $mg.g^{-1}$ dw). Sinigrin concentrations (6.5 $mg.g^{-1}$ dw), glucobrassicin (4.6 $mg.g^{-1}$ dw), progoitrin (2.9 $mg.g^{-1}$ dw) and glucoiberin (1.0 $mg.g^{-1}$ dw) make up the typical profile [18,19].

Ethiopian mustard (*Brassica carinata*)

Ethiopian mustard is a traditional leafy crop of Africa and contains modest GSL concentrations. These include minor amounts of glucoalyssin, gluconapin, progoitrin, glucobrassicin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin and gluconasturtiin, with the vast majority composed of sinigrin (Table 1) [48]. The crop is underutilised in terms of breeding and could be developed to a higher quality, both for human consumption and as a potential biofumigant crop [49].

Ezo-wasabi (*Cardamine fauriei*)

Ezo-wasabi is a niche herb crop that originates from Hokkaido, Japan. It is a popular herb in this region and is characterised by a pungent wasabi-like flavor due to very high GSL concentrations. Abe et al. [50] identified three GSL compounds within leaves: glucoiberin, gluconapin and glucobrassicin. Total concentrations were reported to average 63.0 $mg.g^{-1}$ dw.

Kale (*Brassica oleracea* var. *acephala*)

Kale has been reported as having a wide range of health benefits, including those associated with GSLs [51]. Total concentrations are generally modest [18,19,25,37,52], but some

studies report concentrations higher than broccoli. A comprehensive analysis of 153 field-grown cultivars by Cartea et al. [37], found the average concentrations to be higher at 10.7 $mg.g^{-1}$ dw. The profile consists of predominantly aliphatic GSLs: with some aromatic and indole compounds present. The concentrations of the latter are reported as being highest, on average.

Kohlrabi (*Brassica oleracea* var. *gongylodes*)

Kohlrabi stems are low in GSLs with average concentrations amounting to ~2.2 $mg.g^{-1}$ dw. The profile is composed of glucoiberin, glucoraphanin, glucoalyssin, glucoiberverin, glucoerucin, glucobrassicin, gluconasturtiin, and neoglucobrassicin, with some other trace GSLs identified [18,19,31,32].

Leaf rape & Turnip rape (*Brassica napus* var. *pabularia* & *Brassica napus*)

Rapeseed leaves contain modest GSL amounts, but like collards are not widely consumed by the public. The bulk of the leaf rape GSL profile is made up of glucobrassicanapin, progoitrin and gluconapin [53]. Turnip rape by contrast is composed predominantly of gluconapin [36]. Sprouts have relatively high GSL abundances compared to the mature leaf tissue (Table 1) [11].

Maca (*Lepidium meyenii*)

Maca roots are not commonly consumed in western diets, but are prominent in South American cuisine. Three main cultivar forms are consumed (red, purple, and black) and powders are popular as "food supplements" with anecdotal health benefits attributed to them. The species is an ideal candidate for improvement efforts, as it contains a wide variety of traits and compounds with purported health benefits, such as phytosterols [54].

Total GSL concentrations are high relative to other root Brassicaceae with the primary compound being glucotropaeolin, and secondary glucolimnathin. This profile makes

150 the crop somewhat unique among Brassicaceae, with only glucoalyssin and glucosinalbin
151 shared with more common cultivated species [54].

152 **Moringa (*Moringa oleifera*)**

153 Moringa species are non-cruciferous known for the high concentrations of aromatic
154 GSLs found within tissues [55], and the unusual multiglycosylated conformation of their
155 structures. Within leaf tissues 4-α-rhamnopyranosyloxy-benzyl GSL (glucomoringin) is the
156 dominant compound, with lower concentrations of acetyl-4-α-rhamnopyranosyloxy-benzyl,
157 which exists in three isomeric forms (Ac-Isomer-GSLs I, II, III); these latter molecules each
158 have an acetyl group at different positions on the rhamnose moiety. Due to the nature of these
159 structures, standard methods of desulfation extraction are not recommended for moringa as
160 artifacts are formed, which are not reflective of intact GSL analysis. A method for the stable
161 extraction of these compounds has been developed by Förster et al. [56]. For this reason, papers
162 utilising desulfation extraction in moringa should not be taken as representative of GSL
163 profiles *in planta*.

164 The concentrations of GSLs reported for moringa leaves vary greatly (Table 1) [57–60],
165 due to diverse growing environments and cultivar choice. Stems and roots tend to have lower
166 concentrations of glucomoringin and the acetyl isomers, but are noticeably higher than for
167 more commonly consumed crops such as kohlrabi and rutabaga.

168 **Mustard Greens (*Brassica juncea*)**

169 Like collards, mustard greens are high in GSLs (~25.9 $mg.g^{-1}$ dw), but not widely
170 consumed due to their pungent and bitter tastes. Virtually all of the GSL profile is composed
171 of sinigrin [18,19,36]. There are a large diversity of accessions and cultivars of this species, which
172 provides an excellent resource for any breeding programs focused on culinary improvement.

173 **Radish (*Raphanus sativus*)**

Radish encompasses several varieties such as 'common' radish, China Rose [11] radish, and Spanish black radish [61]. GSL concentrations reported from radish sprouts are very high [11] compared to some reports for roots [31,32]. There is special interest in the compound glucoraphasatin (also known as dehydroerucin) contained within radish tissues. It has been postulated that the cell detoxification properties of its ITC (4-methylthio-3-butenyl ITC; MIBITC) are comparable to sulforaphane (SFN) [61].

Rocket (*Eruca sativa, Diplotaxis tenuifolia, Diplotaxis muralis* & *Erucastrum* spp.)

The rocket (rucola, arugula, roquette) species *Eruca sativa, Diplotaxis tenuifolia,* and *Diplotaxis muralis* are often grouped and classed together due to the similarity in GSL profiles. Other species, known as dogmustards (*Erucastrum* spp.), are also morphologically and phytochemically very similar to rocket.

Rocket species have five major GSL constituents: glucoraphanin, diglucothiobeinin, glucosativin, dimeric-glucosativin (DMB) and glucoerucin (Table 1) [62–65]. By comparison to broccoli, total average GSL concentrations are higher for rocket (*E. sativa*: ~15.3 $mg.g^{-1}$ dw; *D. tenuifolia*: ~11.2 $mg.g^{-1}$ dw), but average glucoraphanin concentrations are lower (*E. sativa*: ~2.0 $mg.g^{-1}$ dw, *D. tenuifolia*: ~1.7 $mg.g^{-1}$ dw).

Dogmustard and annual wall-rocket (*D. muralis*) profiles are somewhat different from 'wild' (*D. tenuifolia*) and 'salad' (*E. sativa*) species, but not as well studied. Dogmustard GSL profiles are low in total concentration, but much of this is glucoraphanin. Annual wall-rocket is high in this GSL too, by comparison to the commercially cultivated species, but few cultivars have been characterised to-date. It is also high in diglucothiobeinin, DMB and glucoerucin, giving a moderate total GSL concentration [65].

The existence of dimeric GSLs in rocket species has proved controversial, with many papers accepting the hypothesis that they are products of extraction, without any supporting experimental evidence. Work by Cataldi et al. [66] a decade ago cast significant doubt on this

assumption, but has largely gone unnoticed within the literature. The addition of tris(2-carboxyethyl)phosphine (TCEP) to rocket extracts is common within the literature, and acts as a reducing agent to break disulfide bonds, such as those that exist in DMB and diglucothiobeinin. This so-called 'prevention of artifact formation' may actually be drastically modifying the GSL profile from its natural configuration. As is seen in *Moringa* spp., multiglycosylated GSLs do occur in nature, and so it is not inconceivable that these compounds are naturally synthesised. Little is known about rocket GSL biosynthesis beyond compounds common to other species (e.g. glucoraphanin and glucoerucin). The pathway for glucosativin, and indeed dimeric GSL, biosynthesis has yet to be elucidated [67], and even less is known about their possible evolutionary and biological functions. In light of these unresolved questions dimeric compounds have been included in Table 1.

Rutabaga (*Brassica oleracea* var. *rapifera*)

Rutabaga (or swede) is consumed as a root crop and undergoes heavy processing and cooking before consumption (i.e. peeling, chopping & boiling) to soften the tissue. Raw GSL concentrations have been reported to range between 3.5 and 5.6 $mg.g^{-1}$ dw, with progoitrin reported as the most abundant GSL overall. The GSL profile is very diverse (Table 1), with concentrations being particularly high in sprouts [31,32,68].

Spider plant (*Cleome gynandra*)

C. gynandra is known by several other common names, including: Shona cabbage, African cabbage, spiderwisp, chinsaga and stinkweed. It is a popular leafy vegetable in African traditional diets, and is routinely consumed for its purported medicinal properties. Despite this popularity, current cultivars perform poorly, making the species an ideal candidate for improvement [69]. Only one GSL is reported for spider plant, which is 3-hydroxypropyl (glucoerysimumhieracifolium; Table 1) [70], and is most concentrated in the stems, siliques and flowers, with low leaf abundance [69].

Watercress (*Nasturtium officinale*)

Watercress is a crop that is gaining popularity in foods such as soups and smoothies, as well as a traditional garnish [71]. Like rocket, watercress cannot be considered domesticated due to a lack of breeding programs, and the tendency for commercial crops to be propagated through clonal cuttings rather than seeds [6]. Its GSL composition is made up almost entirely of gluconasturtiin. Its ITC is phenylethyl-ITC (PEITC) and is known to infer potential health benefits in humans [6].

Small amounts of indolic GSLs are also found within tissues (Table 1) [36,71], but few aliphatic GSLs have been reported. Total concentrations are modest (~5.0 $mg.g^{-1}$ dw) but like rocket species, cooking is not essential before consumption.

White Mustard (*Sinapis alba*)

White mustard leaves are not widely consumed due to their pungent attributes. They are high in GSLs, which is almost entirely made up of the aromatic GSL glucosinalbin [24]. These crops are predominantly used as biofumigants to control soil borne pests, such as nematodes.

PLANT BREEDING

General

To quote Dr. Howard-Yana Shapiro, "It is not so much a question of more food. It is more a question of better food" [72]. This statement encapsulates the ethos of breeding Brassicaceae crops for enhanced GSL content. The trend in many crop breeding programs over the last 60 years has been to increase yield, but this has come at the expense of nutritional value in some instances [73]. It is hoped that by creating new and nutritionally dense varieties, development of chronic diseases such as heart disease, cancer, and dementia can be reduced through elevated concentrations in people's diets.

249 Cereal crops have seen the greatest interest and investment in terms of genomics and
250 breeding improvement over the last 150 years. It has been estimated that plant breeding has
251 accounted for 58% of the increases in maize yields seen between 1930 and 1980 [74], and if the
252 same concerted effort were to be made in Brassicaceae vegetables, it is not inconceivable that
253 compounds related to health-benefits could also be improved.
254 As pointed out by Goldman [75], the irony is that many of the most beneficial health
255 compounds are being bred out of crops because they are also responsible for pungency and
256 sensory traits which consumers dislike. But this could be remedied through breeding by also
257 looking at corresponding ratios with free sugars, some amino acids, and the relative
258 abundances of green-leaf volatiles. These have been shown to infer reductions in the
259 perceptions of such traits, while maintaining GSL concentrations [76].
260 The majority of genomic research for traits related to GSL metabolism has been
261 conducted in species such as *A. thaliana* [77] and *B. oleracea* [78]. *De novo* genome sequencing
262 costs are still high, but falling, and this may entice new exploration of minor Brassicaceae
263 crop genomes in unprecedented fashion. There is however still a lack of understanding within
264 the literature of how new Brassicaceae varieties are developed commercially through plant
265 breeding methods. Such considerations are often absent from many nutritional, biochemical
266 and medical studies [79]. Individuals who are skilled and adept at computational genomics,
267 practical plant breeding, cultivation, analytical chemistry, and molecular biology techniques
268 are scarce, and having a deep knowledge of these fields and how they each interact is
269 challenging. This may be a reason why breeding efforts for phytochemical health traits to-
270 date have lagged behind physiological traits as it requires interdisciplinarity, even when
271 genomic information is available [80]. It is likely that in the private sector molecular breeding is
272 already well established in some Brassicaceae crops, but the degree to which these efforts

273 have focused on GSL improvement are not readily apparent in commercial varieties available
274 for human consumption.

275 A minority of people in Western countries consume an adequate amount of vegetables
276 [81], and even fewer are likely to consume the recently reported optimum of ten-per-day [82].
277 Breeders are recognising that getting consumers to eat more vegetables is not a realistic goal
278 [83]. Instead, breeding strategies are concentrating on elevating compounds such as GSLs and
279 ITCs through selection so that the vegetables on offer to the consumer have a higher
280 nutritional density. A large proportion of people could benefit from resultant new varieties
281 without having to modify their diets at all.

282 Much of the reported health effects are attributed to the GSL hydrolysis products of
283 glucoraphanin, glucoerucin and glucobrassicin [84], which could be increased through
284 appropriate breeding selection. The ITC and indole products (SFN, erucin and indole-3-
285 carbinol; I3C, respectively) have shown strong anti-carcinogenic effects in cell and animal
286 studies [85], but as will be discussed, these studies are limited in their applicability to humans
287 and day-to-day consumption. There are many different factors that must be considered when
288 breeding for modified GSL profiles. These will be discussed in the following sections; see
289 Figure 1 for a summary.

290 **Breeding For Increased Glucosinolate Content**

291 As highlighted within Table 1 there is huge scope for individual crop improvement, as
292 evidenced by the diversity of GSLs and concentrations reported [86]. There are very few
293 examples of successful stabilisation of GSL concentrations across environments however [87].
294 In order to develop enhanced varieties, species diversity must be scrutinised on a large
295 number of cultivars/accessions before any breeding or genomics can take place [79].

296 In *Arabidopsis thaliana* quantitative trait loci (QTLs), and the generation of robust
297 single nucleotide polymorphism (SNP) markers have allowed detailed understanding of

298 numerous genotypes [88]. In order to develop such comparable resources for specific
299 Brassicaceae crops, breeders and researchers must have a comprehensive and extensive
300 knowledge of the cultivar breeding history, as well as a detailed knowledge of the GSL/ITC
301 types produced across environments [89]. Due to the complexity of the *Brassica* genome and
302 comparatively long life cycles of commercial crops, generating such genetic resources can
303 take decades.

304 The GSL pathway itself in *Brassica* and *Arabidopsis* is now well elucidated [90] and it
305 is possible to identify orthologous genes for biosynthesis, transcriptional regulation and
306 environmental response in other species [87]. MYB transcription factors control the complete
307 GSL biosynthetic pathway, and also influence primary and sulfate metabolic pathways.
308 Differing transcript levels associated with MYB genes has been shown to affect indole GSL
309 accumulation and the related metabolism products when plants are under pathogen stress [91].

310 Aliphatic GSLs are synthesised from the amino acid methionine, and indolic GSLs
311 from tryptophan [92]. The gene *BoGSL-PRO* in *B. oleracea* converts methionine into
312 dihomomethionine and a chain-elongation process begins. This is further regulated by other
313 genes such as *BoGSL-ELONG*, and determines the carbon side-chain length (e.g. propyl,
314 butyl, pentyl, etc.). Other genes, such as *BoGSL-ALK,* further modify the R-group later in the
315 synthesis pathway, and determine its final configuration [77].

316 GSL biosynthesis levels are regulated by plant defense signaling compounds, such as
317 salicylic acid (SA), ethylene and jasmonic acid (JA). The synergistic or antagonistic crosstalk
318 between these three compounds determines the relative gene expression. Genes such as
319 *CYP79B2, CYP79B3, CYP79F1* and *CYP79F2* regulate the GSL biosynthesis pathway and
320 determine the overall GSL tissue profile, influencing the ratios between aliphatic and indolic
321 GSLs [93]. The level to which these and other biosynthetic genes are expressed depends on the
322 stimuli that initiate transcription, which can be both biotic and abiotic in nature. The

relationship with primary sulfur metabolism is also important for GSL production, as two to three sulfur atoms are required per aliphatic GSL molecule [94].

The difficulty comes in generating breeding populations and having resources large enough to develop such detailed knowledge in non-model species. Some papers have advocated plant selection based on highest total GSL concentrations [37,44], however this is an unsophisticated approach, as not all GSLs produce breakdown products which are beneficial for health, or positive for consumer acceptability. It also does not account for the potentially harmful effects of specific GSLs when ingested in large quantities.

The most comprehensive and thoroughly tested example of a crop bred for enhanced GSL content is *Beneforté* broccoli. This variety is an F_1 hybrid derived from an original cross between *B. oleracea* var. *italica* and *Brassica villosa* – a wild relative. The resultant variety is able to assimilate sulfur at an enhanced rate, but also allocate greater amounts to methionine-derived GSL production, rather than partitioned into the form of S-methylcysteine sulfoxide (SMCSO). SMCSO levels are reduced by an average of ~7% in plants containing the introgressed *B. villosa Myb28* allele, which in turn corresponds to a reciprocal increase in glucoraphanin [23]. Sarikamis et al. [20] also introgressed markers from *B. villosa* into broccoli which are associated with genes controlling the ratios between glucoraphanin and glucoiberin. Selection for such genes could influence the downstream health beneficial effects to the consumer.

Another area that could be targeted through breeding is hydrolysis product pathway modification. It is known for example that a gene in *A. thaliana* called *epithiospecifier modifier 1* (*ESM1*) encodes a protein that inhibits epithiospecifier protein (ESP) function, preventing it from converting GSLs into nitriles. Identifying, selecting and breeding for such genes in Brassicaceae crops would be instrumental for improving the predictability of hydrolysis product formation. Nitriles are much less bioactive than ITCs, and it would be

348 favourable to decrease production of them [95]. This would lead to increases in ITC abundance
349 and enhance potential health benefits. Selecting for GSL accumulations alone is therefore not
350 sufficient to produce enhanced varieties; ITC abundance ratios must also be considered, as
351 these vary between species, varieties and genotypes [96].

352 The variability of GSL concentrations in crops is due to genetic responses which are
353 influenced by environmental interactions [17]. The specific mechanisms responsible for such
354 large variations seen in varieties are complex [97], and are not well understood in the
355 commercial supply chain context. Few research papers have replicated the food system to
356 determine the effects on GSL and hydrolysis product concentrations from a plant breeding
357 perspective [98], and so it is difficult to make informed selections.

358 If products like *Beneforté* are to be developed for other species, it will require
359 screening a large number of germplasm accessions in multiple environments, and
360 phytochemical analysis throughout the commercial food chain [86]. Gene bank accessions are
361 an underutilised resource for breeding enhanced GSL accumulation traits. Screening these
362 large collections for enhanced traits is challenging, but wild genotypes with enhanced
363 glucoraphanin, glucoerucin, glucoraphenin, glucoraphasatin, glucoiberin, sinigrin and indole
364 GSLs may be found [37]. Blueprint breeding schemes for this method of introgression already
365 exist [20] and so it is feasible that other crops could be developed with enough time and
366 resources.

367 Developing the genomic tools to improve varieties will also be necessary in future.
368 Despite detailed knowledge of the *Arabidopsis* and *Brassica* genomes there are few other
369 related crops that have been sequenced. Developing analogous genetic markers, linkage and
370 QTL maps using these species will serve for a time to screen for common GSL traits;
371 however, species such as rocket, radish and watercress have very different GSL profiles to *B.*
372 *oleracea* and *Arabidopsis*. As such, the time will come when full genome sequences will be

373 required for these crops, to develop and enhance GSLs/ITCs with a high level of precision [80].
374 Having species specific SNPs associated with GSL/ITC QTLs, genes, transcription factors,
375 and other plant defense and senescence pathways will be a powerful tool for enhancing crops,
376 and significantly reduce the generation number required to develop new breeding lines and
377 varieties [89].

378 **Breeding For Decreased Glucosinolate Content**

379 From the late 1960s to the mid-1990s, much of the focus on GSLs and the associated
380 hydrolysis products was in relation to adverse health effects. There was concern surrounding
381 goitrogenic compounds, which are produced from the GSLs epiprogoitrin and progoitrin. The
382 oxazolidine-2-thiones and thiocyanate compounds produced by the hydrolysis of these GSLs
383 interfere with thyroid metabolism and induce a condition known as goiter. In the presence of
384 nitrate they also undergo nitrosation reactions, which is thought to have negative health
385 consequences [99]. High doses of GSL-derived nitriles have also been shown to be toxic [100] but
386 reports are conflicting [101]. This has led to arguments for decreasing certain GSL compounds
387 in Brassicaceae crops through selective breeding. Progoitrin, sinigrin, gluconapin and indole
388 GSLs have all been cited as contributors to bitterness [87], and a reduction is thought to
389 improve consumer acceptance [102].

390 Sinigrin is common (in low concentrations) in important crops, such as cabbage, kale,
391 broccoli and Brussels sprouts (Table 1). The relative abundances in these are minor compared
392 to those found in mustard greens (~16.6 $mg.g^{-1}$ dw), Chinese kale sprouts (~8.4 $mg.g^{-1}$ dw)
393 and collards (6.5 $mg.g^{-1}$ dw) [18,19]. The reduced bitter compound concentrations in commercial
394 crops have led some to speculate if this is partly the reason why pesticides have to be used so
395 intensely, as these varieties may be more prone to disease and herbivory [102].

396 There are opposing opinions relating to sinigrin concentrations within Brassicaceae
397 foods. Sensory analysts advocate its reduction, as it is "*regarded not as a health benefit but*

398 *as a major sensory defect*" [102]. Other studies by contrast have argued that sinigrin should be
399 increased due to the associated health benefits of allyl-ITC (AITC) [37]. Opinions expressed in
400 sensory quality reviews perhaps do not appreciate how difficult 'removal' is from a breeding
401 perspective, or what the effects are from a pest and disease management standpoint. These
402 compounds do not exist simply for the pleasure or displeasure of the human species. It
403 perhaps demonstrates a misunderstanding of the endogenous function of such compounds
404 within plants, and ignores any health benefits they have.

405 Progoitrin has been found to be prevalent in Chinese kale sprouts (~14.8 $mg.g^{-1}$ dw),
406 collards (2.9 $mg.g^{-1}$ dw [18,19]), and leaf rape (2.2 $mg.g^{-1}$ dw [53]; Table 1). Arguments have been
407 made for progoitrin reduction in commercial crops because of the association between its
408 degradation products and goiter [87]. Double recessive alleles of GSL biosynthesis genes have
409 been identified and utilized in reducing concentrations in rapeseed to improve livestock feed
410 [90]. Similar efforts to reduce harmful GSLs in other Brassicaceae is a realistic goal, but must
411 be targeted so that beneficial GSL accumulation is not affected.

412 Most arguments for the goitrogenic effects of GSLs are outdated and unsupported in
413 humans, however. Not all GSLs have goitrogenic breakdown products, and so are unlikely to
414 adversely affect otherwise healthy humans [103]. Most cited evidence stems from studies in
415 herbivores, such as rabbits and cows, which can ingest large amounts of seed meal and leaves
416 a day [104,105]. Assuming humans who eat Brassicaceae vegetables don't have a severe pre-
417 existing thyroid condition, and are not suffering iodine deficiency, there is little evidence of
418 healthy people developing goiter through ingestion of leaves, sprouts, roots, or indeed the
419 milk of animals that consume large GSL quantities [103]. At low-moderate levels the
420 compounds are beneficial to humans and enhance cellular defenses against cancer and other
421 diseases [106].
422

CULTIVATION, POSTHAVEST PROCESSING & STORAGE

General

Improved genetics and phytochemical content through breeding must be synergistic with improvements in Brassicaceae agronomy and cultivation methods. Important aspects to be considered when attempting to enhance GSL concentrations through breeding include: appropriate varietal selection, responses to fertilizer application, water availability, harvest time/growth stage, light levels, and local temperature [107–112]. These factors and many more can have a significant impact on the quantities of GSLs produced by plants (see Table 2). It has been reported that GSLs can be enhanced through better and more informed cultivation methods by up to ten times in the case of broccoli and cauliflower, and doubled in radish [86].

Varietal Selection

It is well documented that GSLs and the respective breakdown products vary between species, within species, and even within individual cultivars [86]. The data collated in Table 1 gives examples of this variability, with large concentration ranges reported for species according to different growing environments (e.g. field or glasshouse).

It has been reported that a high degree of variation in GSL concentrations can exist between plants of the same variety (e.g. in *Marathon* broccoli heads) [113]. This poses a significant challenge, especially if varieties are uniform hybrids for morphological traits; and indicates just how great an impact environment has upon GSL accumulation. In experimental terms, it has been suggested that replicates be increased or samples pooled to create a 'representative' picture [113]. This is perhaps a neater approach statistically, but obscures the inherent variation present between plants of the same variety, giving a false sense of uniformity. If plants have not been selected for GSL profile modification, it is unsurprising that such high variations exist [96]; therefore the development of uniform breeding lines and varieties will mitigate this by considering individual plant chemotypes and sensotypes.

448 **Light Intensity**

449 It has been demonstrated in *A. thaliana* that UV-B radiation can induce gene
450 expression that promotes GSL accumulation [114]. In crops such as broccoli and cauliflower it
451 has also been observed that increased light levels can increase glucoraphanin and glucoiberin
452 concentrations within florets [86,115]. In an excellent recent paper by Moreira-Rodríguez et al.
453 [116] it was demonstrated that 24 hours after exposure to high UVB treatment, broccoli spouts
454 showed large increases in GSL concentrations. This included a 73% increase in
455 glucoraphanin, 78% increase in glucobrassicin, and a 170% increase in 4-
456 methoxyglucobrassicin. The authors indicated that UVB radiation triggers signal transduction
457 pathways, leading to up-regulation of GSL biosynthesis genes as part of a UV protection
458 mechanism. Within a segregating population of plants, it is theoretically possible to select for
459 plant with genes predisposing them for such higher accumulations. With more advanced
460 genetic analysis of such genes, it should also be possible to identify polymorphisms
461 underlying the propensity for increased glucoraphanin and indole GSL biosynthesis. As the
462 authors discuss, it may be theoretically possible to 'tailor' GSL profiles to a degree, by
463 exposing sprouts to differing combinations of UVA and UVB light intensities. As with most
464 studies of this kind, only a single variety of broccoli was used, and so it is not possible to
465 determine how much these responses vary according to genotype. It was also not determined
466 how these respective increases affected ITC/nitrile/indole production. Other studies have
467 noted that GSL profiles are not necessarily indicative of myrosinase activity or hydrolysis
468 product profiles [110]. Nevertheless, the results indicate that this is an area for future study, and
469 it would be intriguing to determine how such responses vary within segregating populations
470 of broccoli and other Brassicaceae.

471 GSL accumulation is generally much higher when plants are exposed to longer
472 periods of light. A study by Kim et al. [117] showed that GSL concentrations of Chinese

473 cabbage seedlings were up to 6.9 times higher in plants exposed to light ten days after
474 sowing. This suggests that raising seedlings in the dark for several days may increase the
475 potential accumulations within the plants at later developmental stages.

476 GSL concentrations also fluctuate according to diurnal rhythms imposed by exposure
477 to light and dark. Huseby et al. [118] demonstrated that relative expression of genes associated
478 with GSL biosynthesis in *A. thaliana* were significantly increased in plants grown in dark
479 conditions before being exposed to light, compared with those which were only exposed to a
480 normal diurnal cycle. This implies not only that GSL biosynthesis can be influenced by light,
481 but also that GSL concentrations can be enhanced through controlled exposure. Huseby et al.
482 also saw GSL concentrations peak eight hours after light exposure was initiated in a diurnal
483 cycle, with concentrations then subsequently declining. This has large implications for
484 commercial operations that may harvest at specific times during the day. More research is
485 needed to understand how these mechanisms function in commercial crops, but it is likely
486 that recommendations for optimum harvest times could be generated in order to maximise
487 GSLs.

488 Different light wavelengths that are applied to Brassicaceae crops also cause differing
489 effects on GSL concentrations. Blue light has been shown to increase total GSLs in ezo-
490 wasabi leaves [50] and turnip roots [119] (Table 2) via possible activation of GSL biosynthesis
491 enzymes. This mechanism has been postulated but not verified, and is thought to impact
492 aliphatic and aromatic GSLs, not indolic, as there is no corresponding increase for these
493 compounds under blue light [120]. This phenomenon could be exploited in controlled
494 environment cultivation or vertical farming methods, to improve the nutritive value of niche
495 microleaf and baby leaf crops. In contrast, increased levels of red and far-red light have
496 resulted in elevations of gluconasturtiin in watercress. It has also been reported that red light

497 (640 nm) applied to kale sprouts increases the production of specific GSLs, such as sinigrin;
498 but other wavelengths have no significant effect [107].

499 **Environmental Temperature**

500 Unlike light intensity, increasing temperature does not have a reciprocal effect on
501 GSL concentrations. Myrosinase activity is known to increase with higher daily mean
502 temperature, and it is hypothesised that this leads to increased GSL degradation upon harvest
503 [86]. It has been noted that high summer field temperatures have a detrimental effect on specific
504 GSL concentrations at the point of commercial harvest in 'salad' rocket, but this is not
505 indicative of postharvest concentrations, which have been observed to increase during shelf
506 life storage [98].

507 There are reports of increasing GSL concentrations with warmer weather in kale and
508 red cabbage [1], but these come from spring and autumn comparisons where differences in light
509 levels may contribute more to the elevations observed than the relative increase/decrease in
510 temperature. Steindal et al. [52] found a specific increase of sinigrin in kale at low growing
511 temperatures. Schonhof et al. [121] analysed broccoli at different growth temperatures and
512 found that low temperatures increased aliphatic GSLs, and high temperatures increased
513 indolic GSLs. This trend was not observed by Steindal et al. [52] in kale, where both high and
514 low temperatures (32°C & 12°C) increased aliphatic GSLs. The authors suggested that cold
515 temperature stress is beneficial for GSL accumulation, but is dependent on the organs and
516 species in question.

517 **Water Availability**

518 In broccoli plants it has been observed that a reduction in water availability causes
519 large increases in GSL concentrations [86]. This may be due to a concentration effect within the
520 plant tissues, but it is also possible that this is a defensive response in times of vulnerability
521 and stress. Various reasons have been hypothesised for such increases when plants are

experiencing drought, including increased synthesis of sugars, amino acids, and sulfur availability [107].

As with other abiotic factors influencing GSL concentrations, there are conflicting reports. Some studies suggest that increased rainfall in the spring (coupled with increasing temperatures) increases GSLs [1]; but these interacting factors, combined with lengthening days and stronger light might be the primary cause. The timing of irrigation before harvest also impacts the abundance of GSLs, and is another factor for consideration [107].

Sulfur Application

Fertilizer application to Brassicaceae crops is common practice in the commercial setting but can lead to changes in GSL composition. High sulfur doses applied to crops can facilitate sizeable increases in GSLs with known health benefits (Table 2) such as glucoraphanin [23]. Application to broccoli plants (600 mg S $plant^{-1}$ [86]) has been shown to increase concentrations. Combined with a reduction in watering, this can also boost the concentration, but at the sacrifice of yield [86]. Fertilizer cost may be a limiting factor for many growers, however. So while sulfur application to enhance GSLs may be effective, farmers will not be likely to adopt it unless yields can be maintained.

In radish, a lower amount of sulfur has been reported to be efficacious in increasing glucoraphasatin concentrations (150 mg S $plant^{-1}$) [86], meaning that application on specific crops could be more preferable and affordable from a commercial perspective. Increases in total GSLs, sinigrin, glucobrassicanapin, gluconapin and progoitrin have also been reported with increased sulfur [107]. For an excellent review of sulfur assimilation, its relationship with GSL biosynthesis, and the underlying genetic mechanisms responsible in *Brassica* species, see Borpatragohain et al. [122].

Nitrogen Application

546 With decreasing nitrogen application GSLs have been observed to increase [86]. In
547 combination with sulfur fertilization (60 kg.ha^{-1}), increasing nitrogen (80 – 320 kg.ha^{-1}) has
548 been shown to be ineffective at increasing total GSL concentrations in turnip, but can shift
549 the ratio towards greater indolic GSL production. This is in contrast with sulfur applications
550 at a low level (10 – 20 kg.ha^{-1}) and increasing nitrogen, where aromatic and aliphatic GSLs
551 decrease [123].

552 Experiments by Schonhof et al. [124] in broccoli found that inadequate nitrogen
553 increased GSLs, and inadequate sulfur decreased them. Hirai et al. [125] found that under
554 nitrogen and/or sulfur limited growth conditions in *A. thaliana*, the genes encoding
555 myrosinase enzymes were down-regulated in order to facilitate GSL storage in leaf tissues.
556 The strategy for fertilizing commercial Brassicaceae crops should therefore take these factors
557 into account if enhanced health properties are to be produced.

558 **Methionine Application**

559 Another means of increasing GSL concentration in crops is amino acid application
560 (Table 2). As aliphatic GSLs (such as glucoraphanin) are derived from methionine,
561 application to crops could enhance production in species such as broccoli [86]. It has been
562 applied to broccoli sprouts and rutabaga with encouraging results. In these crops, total GSLs
563 were increased by 19% and 85%, respectively [11]. The effects on glucoraphanin and
564 glucoiberin in the broccoli sprouts were modest, with a 7% increase. By contrast, indolic
565 GSLs 4-hydroxyglucobrassicin, glucobrassicin and 4-methoxyglucobrassicin increased by
566 28%. In the rutabaga the large total increase was due to elevations in both aliphatic and
567 indolic GSLs.

568 Baenas et al. [11] have suggested that the effects are strongest at lower concentrations (5
569 – 10 mM applications) which result in total GSL increases of 21 – 23% in sprouts. Other
570 studies have applied up to 200 mM of methionine and still seen increases of up to 28% [126],

571 though the application method was different. The effects on specific GSLs in sprouts related
572 to health benefits such as glucoraphanin, glucoraphenin and glucoraphasatin seem not to be
573 affected by methionine application according to Baenas et al. [11], but this may be related to the
574 immature growth stage at which plants were tested.

575 **Selenium Application**

576 Selenium is an essential micronutrient for humans. There is a significant relationship
577 between the amount of selenium within the diet and the risk of developing conditions such as
578 cancer, heart-disease and immune system diseases [127]. It has been estimated that 33% of
579 children (age 11-18), 39% of adults (age 19-64), and 44% of older adults (age 65+) consume
580 less selenium than the recommended Lower Reference Nutrient Intake (LRNI)
581 recommendation [128].

582 Research has been conducted to apply selenium to crops (such as broccoli) to enhance
583 nutritional properties [129]. Studies have shown that excess selenium application can reduce
584 GSL content by 90% [30]. By contrast, selenium application to radish plants has been shown to
585 increase glucoraphanin concentrations within roots [129]. With more moderate application, SFN
586 concentrations can be increased in broccoli [130], but other studies have reported no change in
587 sprouts, indicating the optimum benefits of application depend on growth stage [127].

588 **Plant-Bacterial Interaction**

589 In a 2009 paper, Schreiner et al. [36] demonstrated that an auxin-producing bacterial
590 strain (*Enterobacter radicicitans* DSM 16656) could influence and utilise GSL
591 concentrations in several Brassicaceae species. The bacterial strain colonized the plant
592 phyllosphere, and it was hypothesised that the response could be two-fold: 1) that GSL
593 concentrations increased due to defense mechanism activation, and 2) that the bacterial auxin
594 supply to leaves could induce GSL synthesis by metabolism of indole-3-acetaldoxime. The
595 species with the greatest bacterial growth of *E. radicicitans in vitro* had high aliphatic GSL

596 concentrations (*B. rapa* & *B. rapa* var. *chinensis*), whereas aromatic GSL-containing species
597 showed little increase (*N. officinale*).

598 Very few papers have linked bacterial colonisation of leaves with GSL accumulation,
599 but Bell et al. 2017 [98] found strong correlations between GSL concentration and bacterial
600 load of rocket within the commercial supply chain after processing. This could be suggestive
601 of defensive responses due to damage incurred through processing, but also that bacteria
602 influence the GSL profile in some way during shelf life. This is an area of research that
603 requires much more thorough exploration.

604 **Developmental Stage (Ontogeny)**

605 The developmental stage (ontogeny) at which plants are harvested is a significant
606 determining factor in the GSL concentrations that will be ingested by consumers [37]. Crop
607 maturity from a culinary perspective does not always coincide with peak GSL accumulation,
608 as this can vary over life cycle. In broccoli heads, the highest glucoraphanin concentrations
609 have been observed at 180 days after sowing, with a subsequent decline at the onset of
610 flowering [14]. In contrast, Chinese kale GSL concentrations are reported to peak at the sprout
611 growth stage [47].

612 Sprouts are often the subjects of environmental, elicitation and postharvest studies to
613 increase GSL accumulation [47]. This is because of the fast turnaround times in which crops of
614 such age can be sown and harvested, and because it has been reported that GSLs are of higher
615 concentration at this point. This is thought to be due to a concentration effect as leaves are
616 not fully expanded, and therefore not diluted by growth and expansion [11]. Broccoli,
617 cauliflower and cabbage studies have shown that total aliphatic GSL concentrations decline
618 during a seven day sprouting period, but that indolic GSLs increased [107]. This is a very short
619 space of time compared to the entire plant life cycle, and not representative of peak
620 accumulation. Baenas et al. [11] specified that eight-day-old sprouts were optimum for

621 enhancing GSL concentrations, broccoli, turnip, rutabaga, and radish all much higher than
622 their average reported mature values. They reported broccoli glucoraphanin concentrations of
623 18.3 $mg.g^{-1}$ dw. China Rose radish sprouts are especially rich in glucoraphenin and
624 glucoraphasatin, and rutabaga high in progoitrin. Qian et al. [46] reported total concentrations
625 as high as 98.2 $mg.g^{-1}$ dw in Chinese kale (grown hydroponically). It may be that sprout
626 concentrations vary between species and varieties, and this needs to be addressed by
627 analysing multiple commercial varieties and wild cultivars of each species. Sprout
628 consumption is an uncommon practice for the consumer at the present time, so research in the
629 mature crop may be of more relevance for enhancing GSL intake. That being said there is
630 little consensus on what the best harvest point is to maximise GSL concentrations for
631 individual crops, or even commercial varieties. As pointed out by Bell et al. [62], some studies
632 analysing the GSL composition of mature rocket leaves are often long after a commercially
633 relevant time point, and so this needs to be addressed with consideration for common
634 commercial practices.
635 An excellent paper published recently by Hanschen & Schreiner [110] explored the
636 effects of ontogeny upon GSL and ITC concentrations in broccoli, cauliflower, cabbage,
637 savoy cabbage, and red cabbage sprouts and heads. Importantly, they also tested multiple
638 varieties for each crop, highlighting how important this is as a consideration for enhancing
639 health-promoting compounds. It was observed that both the types and concentrations of GSLs
640 and hydrolysis products differed between sprouts and heads, with up to ten times more
641 present in the former than the latter. It was also apparent that for the tested varieties nitriles
642 were the predominant hydrolysis product, indicating that this is an area for potential
643 improvement through selection of genes related to ITC-nitrile ratios. The authors also pointed
644 out that ‘mini heads’ contained the greatest concentrations of ITCs (such as sulforaphane),
645 and are perhaps a better alternative to fully mature heads in terms of maximizing ITC

646 consumption. The only drawback of the study was that the reported concentrations were for
647 raw plant material, not cooked. As discussed in the following 'Consumer' section, this may
648 have drastic effects upon myrosinases and ESP proteins, and determining the amounts and
649 types of hydrolysis products present at the point of ingestion.

650 In watercress, a crop which does not require cooking, an ontogenic study by
651 Palaniswamy et al. [131] showed that leaves harvested at 40 days of growth after transplantation
652 contained 150% higher PEITC than leaves at 0 days. This was a linear increase with no
653 significant changes at 50 and 60 days. In species such as watercress where establishment of
654 new breeding programs and varieties is difficult (due to the commercial preference of
655 vegetative propagation), the selection of an optimum harvest date may be the most effective
656 way in the short-term to promote maximum ITC formation in commercial crops.

657 **Postharvest Commercial Processing & Storage**

658 It is well known that GSL profiles change during postharvest processing and storage.
659 Processing can alter food matrix composition, which increases the accessibility and
660 bioavailability of compounds [34] such as ITCs. The atmosphere in which produce is stored
661 also affects GSL concentrations [13].

662 In rocket species simulated shelf life storage has revealed that individual GSLs such
663 as diglucothiobeinin increase [63]. After harvest and commercial processing significant
664 increases in glucosativin and SFN have been observed. This indicates that postharvest
665 industrial practices induce GSL synthesis and may boost the health beneficial effects for the
666 consumer [98]. Glucoraphanin has likewise been shown to increase [63] or remain stable [98]
667 throughout cold storage conditions, and the increases in ITCs over nitriles during storage has
668 also been documented [96]. These results are encouraging, as it was previously assumed that
669 concentrations would be detrimentally affected by rigorous harvest and washing procedures.

These trends have also been reported in broccoli, where total GSLs have been shown to increase by up to 42%, but at high storage temperature (10°C) [132]. It has been suggested that increases in glucoraphanin are due to the vegetative state of the broccoli heads [14]. At cold-chain temperatures (0-4°C) results are more conflicting; Rybarczyk-Plonska et al. [14] reported no changes in GSL concentrations, Fernández-León et al. [133] reported increases in aliphatic GSLs and decreases in indole, and Rodrigues & Rosa [134] saw stable indole GSLs, but a 31% reduction in glucoraphanin.

When combined with the addition of low postharvest light (13-25 μmol m^{-2} s^{-1}) at 10°C and 4°C, aliphatic GSL concentrations have been observed to increase by up to 130%, with 4-methoxyglucobrassicin also increasing [14]. It is unclear if the shift to warmer temperature during storage has any implication for tissue degradation or increased microbial load. These increases are arguably the result of stress responses due to the shifts in temperature from 0°C [14], with the relative increases seen are dependent upon dose, frequency, and duration of UV-B exposure [135]. Increases have been reported for 4-hydroxyglucobrassicin at 18°C with 25 μmol m^{-2} s^{-1} light [14], but it is difficult to see how these recommendations can be applied to commercial produce.

THE CONSUMER

General

Some consumers are becoming more health conscious, and while not always the primary decision in purchasing and eating food, nutritional content is an aspect which is more evident in the decision-making process [86]. They are looking for products that are "healthy" and "natural", and scrutinizing the nutritional value of Brassicaceae crops [136,137]. This is especially the case for young consumers, who are open to trying new foods [138]. That being said, the average contribution to the "five-a-day" that Brassicaceae account for is between 0.2

695 – 0.5 servings [137], and even further from the optimum "ten-a-day" [82]. This section will
696 explore the processes relating directly to the consumer after purchase, such as cooking,
697 sensory perceptions and preferences, and human health and metabolic aspects.

698 Previous reviews have addressed the mechanisms involved in processing and the
699 changes initiated in GSL and ITC profiles [2,139]. Few however have done so with the purpose
700 of using such data to inform plant-breeding selections and improving the varieties
701 themselves, rather than the methods used to process them. The effects of cooking on ITC
702 formation in one variety of cabbage may not be the same as another, for example. The taste
703 of one rocket variety may be preferred over another because of underlying phytochemical
704 interactions with ITCs. The relative stability of myrosinases between broccoli varieties may
705 determine the formation of ITCs over nitriles. All of these are quantifiable traits that can be
706 used to inform breeding selections, and can be linked to the biochemistry and physiology of
707 plants, which are ultimately determined at the genetic level.

708 **Cooking Methods**

709 The means by which produce is prepared by the consumer influences the amounts of
710 beneficial compounds that are ingested [140]. This includes all aspects relating to peeling,
711 chopping and cooking. Depending on the species, this affects GSL concentrations and the
712 production of hydrolysis products that are responsible for health benefits.

713 The heat generated by cooking often leads to myrosinase inactivation at temperatures
714 >60°C [18], and is a barrier to increasing health benefits. In addition to this, high temperatures
715 (≥100°C) also cause GSL degradation when tissue water content is >34% [33]; this means
716 commercial produce would be severely affected. Boiling crops like watercress results in
717 severe GSL losses – probably through such thermal degradation [71].

718 Steaming of vegetables has produced some conflicting results. Papers have reported
719 GSL losses, some no-significant change, and others have observed significant increases [140]. A

720 study by Giallourou et al. [71] on the effects of cooking on watercress, found that steaming
721 significantly increased gluconasturtiin concentrations (from 1.8 to 2.0 $mg.g^{-1}$ dw), and
722 Gliszczyńska-Świgło et al. [141] reported a 1.2-fold increase in total GSLs in broccoli. In the
723 latter study, the authors hypothesised that this increase was time dependent, having seen no
724 significant effects before 3.5 minutes of steaming. Similarly with watercress, steaming for 2-
725 5 minutes saw no major losses in GSLs. This suggests there is an optimum time to steam in
726 order to increase or preserve GSL bioavailability and avoid their breakdown due to prolonged
727 heat. Another study looking at broccoli steaming found an increase in total GSL content [141],
728 however it is speculated that this is because cooking and heating increases compound
729 extractability [33]. This translates into greater bioavailability and benefits to the consumer [30],
730 and it has been demonstrated in simulated *in vitro* digestion of cauliflower that sinigrin
731 bioavailability is increased by 29.5% and 114.7% after steaming and boiling, respectively [142].
732 Ciska & Kozłowska [143] hypothesised that the disintegration of tissues by heat releases GSLs
733 which would otherwise be bound within cell walls; this would account for the relative
734 increases observed. But GSL bioavailability is of little significance for human health unless
735 there is a means by which they can be hydrolysed into ITCs/indoles.
736 Microwaving has been found to induce severe GSL losses in numerous studies. As
737 with steaming, it has been hypothesised that microwaves cause a cell structure collapse
738 leading to contact between GSLs and myrosinase [140]. No studies have determined if there is a
739 respective increase in ITCs as a result, or whether myrosinase is inactivated due to high
740 temperatures.
741 Matusheski et al. [144] have demonstrated that cooking chopped broccoli heads at 60°C
742 for 5 – 10 minutes increases and favors SFN production. It was hypothesised that the 60°C
743 heat inactivated ESPs leaving myrosinase active and free to convert GSLs to ITCs. Such
744 optimization methods for maximizing content signify that high SFN concentrations could be

745 ingested even after cooking, providing that heating is not too prolonged or intense. Breeding
746 efforts should therefore focus on selecting plant lines with greater myrosinase function and
747 stability ant higher temperatures.

748 **Condiment Selection**

749 There is some evidence to suggest that the condiment with which Brassicaceae are
750 ingested aids in ITC production and enhances absorption within the gastrointestinal tract
751 (studied in rats). Ippoushi et al. [145] have demonstrated that when raw, grated daikon radish is
752 prepared in oil, the ITC absorptive content was increased compared to water. This perhaps
753 suggests that oil stabilizes and preserves ITCs before ingestion.

754 The addition of exogenous myrosinase to cooked Brassicaceae has also been
755 suggested as a means to boost GSL conversion to ITCs [18]. This commonly means the addition
756 of mustard to foods, but many people find the pungency of this condiment too intense.

757 **Sensory Perceptions**

758 The effects of differing GSL content in produce on the consumer and their tastes are
759 very complicated [68]. It is known that not all consumers are the same in their preferences for
760 Brassicaceae vegetables due to differences in genotype and life experience [146]. Certain GSLs
761 and their hydrolysis products have been attributed with bitter tastes. The rejection of bitter
762 tastes by some consumers is a barrier to encouraging greater consumption [13], especially if
763 breeding goals are to increase quantities within tissues [102]. It has been demonstrated that
764 bitterness perceptions can be reduced or even masked [147] by enhancing relative sugar
765 concentrations within tissues [146]. Therefore, through selective breeding, health-beneficial
766 bitter compounds can be enhanced without negatively impacting on consumer acceptance.

767 Crop sensory improvement through plant breeding is perhaps even further behind
768 efforts to breed for health benefits. These two should go hand-in-glove, but often are not
769 considered together in published research papers. The trends seen in consumers preferring to

770 purchase more nutritious foods has not been mirrored by an improvement of the sensory
771 properties of the foods themselves [148]. This means that if this trend is to be expanded or
772 sustained, new varieties will need to be produced with enhanced sensory and nutritional
773 traits, not just one or the other.

774 **Gut Microflora**

775 Many cooking studies on Brassicaceae have reported significant increases in available
776 GSLs, but often omit that the temperatures involved would significantly or completely
777 inactivate myrosinases. This means that any GSL to ITC and indole conversion would be
778 reliant upon gut microflora. Some bacteria found within the human gut are known to possess
779 myrosinase-like enzymes. They act as a potential means by which humans can ingest ITCs,
780 even if cooking has inactivated plant myrosinase. It has been speculated that such bacteria
781 play a vital role in mediating the health benefits of ITCs, but the degree to which this occurs
782 is unclear and requires extensive study [106].

783 **Consumer Health Benefits – Evidence From Cell & Animal Studies**

784 The vast majority of knowledge accumulated around ITCs comes from cell and
785 animal studies. ITCs and indoles are classed as anticarcinogens and act as blocking agents
786 that increase cytochrome P450 activity [149]; see Figure 2 for chemical structures of the most
787 widely studied compounds. The prevailing mechanism of action suggested within studies is
788 phase II metabolic detoxification enzyme activation, such as glutathione-*S*-transferase (GST),
789 NAD(P)H:quinone oxidoreductase (NQO), and phase I enzyme inhibition [149–151]. Waste
790 metabolites produced by cells are excreted into the blood and converted by the liver into
791 mercapturic acid; this is then excreted in the urine [96].

792 SFN has been linked with detoxification pathway modification, which increases the
793 excretion of potential carcinogens from cells [30]. It is also linked with prostate cancer cell
794 apoptosis, and has been shown to act in a dose-dependent manner against kidney and

795 colorectal cancer cell lines by inhibiting histone deacetylation [150]. There is also evidence to
796 suggest that the increase in phase II detoxification enzymes by SFN could help reduce
797 damaging effects in basal ganglia, and protect dopaminergic neurons [10]; this has significant
798 implications for neurodegenerative diseases. For an excellent review of the neuroprotective
799 effects of SFN see Giacoppo et al. [10].

800 ITCs such as PEITC (abundant in watercress) and AITC (abundant in mustards) have
801 been shown in cell studies to inhibit tumorigenesis, protect DNA from damage, and induce
802 apoptosis. The specific structure and length of the alkyl chain an ITC has is linked to its
803 efficacy in inhibiting tumor formation. Phenylhexyl ITC (C_6; PHITC) is 50 – 100 times more
804 efficacious in this respect than PEITC [150] in studies focused on reducing the effects of
805 smoking. The dose used however was 5 μmol (1.1 mg) per mouse for four days – far in
806 excess of what an equivalent human could realistically ingest [152].

807 The juice extracts from Brassicaceae plants such as 'salad' rocket [63], garden cress [153]
808 and radish [61], and their application to cancerous cell lines, such as colon cancer (HT-29) or
809 hepatoma (HepG2) cells, are used to establish antigenotoxic, detoxification or
810 antiproliferative effects. In rocket, it has been shown that extracts have protective effects
811 against DNA damage in comet assays [63]. ITCs and their cysteine conjugates have shown
812 efficacy in inhibiting HL-60 leukemia cells at concentrations as low as 0.8 $\mu mol.L^{-1}$ [150]. In the
813 use of other cell lines, the results are more mixed: some respond with an increase in CYP
814 activity when exposed, whereas others do not [149].

815 Similar effects have been associated with indolic-GSL breakdown products, such as
816 I3C and 3,3'-diindolylmethane (DIM). Dietary studies conducted in rats have found that
817 phase II detoxification enzymes are enhanced in the stomach, liver and small intestine after
818 consumption of these compounds. Indoles are thought to act somewhat differently to ITCs
819 however, inhibiting cancer cells through cytostatic mechanisms, rather than apoptosis [96].

820 **Consumer Health Benefits – Evidence From Human Clinical Trials & Epidemiology**

821 The increase in consumption of fruits and vegetables is accepted to be beneficial to
822 human health [154], but the compounds responsible and the interactions with genotype are not
823 clear. It is assumed that what is beneficial for one person to consume, is beneficial for all
824 people. This is not the case for many food types, and some evidence suggests it is the same
825 for Brassicaceae vegetable consumption. It is known that human metabolic genotypes vary in
826 the degree of beneficial effects that they will impart after ingestion of phytochemical
827 compounds [155], and adds an additional layer of complexity to producing Brassicaceae with
828 enhanced GSL/ITC traits [75].

829 The quantities required to elicit benefits in humans (both acute and chronic) are
830 difficult to define due to variations in bioavailability within Brassicaceae food matrices and
831 GSL-metabolism by gut microbiota in subjects [156]. The experimental quantities used in
832 clinical research trials frequently do not translate into realistic or sustainable amounts that the
833 average person can achieve. A study by Bogaards, Verhagen, & Willems [157] demonstrated
834 that after human males consumed 300 g of Brussels sprouts per day, there was a significant
835 increase in GST products in the blood compared to those on a GSL-free diet. While
836 indicative of an underlying metabolic mechanism for ITC degradation, few people would be
837 willing or able to consume such large Brussels sprout quantities on a daily basis. The
838 impracticality of studies in the 'real world' and to ordinary people often detracts from the
839 importance of the mechanistic findings. Doses are also often administered in a form that
840 would not regularly be consumed (i.e. as a drink or powder supplement) [158], which limits the
841 relevance of results and the conclusions drawn. This raises the question: are the beneficial
842 effects seen in trials 'real-world' effects, or just ones induced by extreme acute consumption?

843 Epidemiological studies looking at cancer risk vs. Brassicaceae vegetable
844 consumption have reported mixed results. Studies in patients with prostate cancer, for

example, have found both significant inverse associations and no significant associations. For other cancers, such as endometrial, the risk reductions reported are moderate [151]. Data are encouraging, but do not identify or distinguish the modes of action that are responsible [106]. ITCs and indoles are strong candidates, but other compounds such as flavonoids, carotenoids and anthocyanins are also present in Brassicaceae. It is unlikely that these compounds act in isolation within the human body, and it may be the combined effect of ingesting a diverse range of phytochemicals contributes towards such risk reductions [63].

Genetic studies on humans have identified several genes that play a role in ITC metabolism. GST loci and the associated *GSTM1*, *GSTT1* and *GSTP* genotype polymorphisms impact the relative protective effects of ITCs that an individual will receive. Individuals that are *GSTT1*-null and *GSTM1*-null are at higher risk of developing some cancers, such as renal cell carcinoma. Those who carry present copies of both *GSTT1* and *GSTM1,* and have only a low Brassicaceae intake, are still at a lower risk than null individuals by comparison [151]. It has been estimated that up to 40% of the population may benefit from increased Brassicaceae consumption due to the elevated risk associated with some null genotypes [13]. Breeding goals selecting for certain GSLs/ITCs have not considered consumer genotype as a variable, but in future this must be an expressed goal if populations are to gain full benefits of newly developed varieties [75]. This means that selection and enhancement for other compounds such as flavonoid glycosides, anthocyanins and carotenoids may be practical way of ensuring an 'all-round' health benefit to Brassicaceae crops.

It is well documented in clinical studies of raw vs. cooked vegetables that cancer risk (of multiple types) decreases with raw plant matter ingestion [159]. Consuming uncooked species (such as rocket or watercress) increases the contact between GSLs and myrosinase and the amounts of ITCs absorbed [18]. Due to the detrimental effects of cooking on GSLs and

myrosinase, *B. oleracea* crops may not be as effective/efficient as uncooked species at eliciting such reductions in overall risk.

The reported anticancer effects of Brassicaceae in the diet are poorly substantiated by empirical quantification of the total GSL/ITC amounts that are ingested and absorbed by the body, due to the potential variables previously outlined. A review of the health promoting properties of broccoli by Ares et al. [160] concluded that even with high broccoli intake, it is likely to be insufficient to stimulate anticancer effects at doses outlined in clinical studies. Broccoli varieties bred for high glucoraphanin content have showed promise however. It has been observed that doubling the level of glucoraphanin in florets can produce a three-fold increase in sulforaphane metabolites within the bloodstream compared with a standard variety [155]. This is supported by some excellent and rigorous human clinical studies with *Beneforté* broccoli, and have shown encouraging results [161–163]

SUMMARY

Cell and animal studies have shown that ITCs and indoles have strong protective effects against some cancers [164]. Epidemiological evidence also suggests that vegetables containing GSLs are associated with reduced risks of developing cancer, heart disease [165] and neurodegenerative diseases [10]. These two kinds of studies are measuring very different things however. *In vitro* and *in vivo* animal studies often use ITC compounds in isolation and at high doses [166] measuring only acute effects. Epidemiological research often takes place over several years, and does not account for compounds acting in isolation (i.e. the beneficial effects cannot be wholly attributed to GSLs/ITCs) [55]. Flavonols, anthocyanins and carotenoids are but a few of the other classes present in these crops, and all have similar reported effects attributed to them [96,167].

894 The health benefits a consumer receives from long-term Brassicaceae ingestion
895 depends on the type and abundances of GSLs/ITCs/indoles within tissues. It depends on the
896 environment in which these crops were grown, and their genetic predisposition for producing
897 certain myrosinase breakdown products over others (i.e. ITC: nitrile ratio). It depends on how
898 the crop is stored, prepared and cooked; it even depends on the metabolic genotype of the
899 individual consumer. This therefore means that GSL measurement at harvest, as a proxy for
900 ITCs/indoles at the time of consumption is extremely tenuous. It makes suggesting how much
901 Brassicaceae should be consumed difficult and filled with caveats that are specific to the
902 species in question and the person consuming it.
903 In order to breed new Brassicaceae varieties with enhanced health benefits, the
904 concentrations and relative myrosinase hydrolysis product abundances must be considered [75].
905 The literature is plentiful in studies analysing and reporting GSL concentrations, but is
906 lacking in corresponding ITC, nitrile and indole measurements. The predominant reason for
907 this is that these compounds are difficult to extract, identify and quantify, due to their
908 volatile/unstable nature and reactivity [168]. Simple methods have now been developed
909 however, which give robust and informative results [98,169]. While the extraction methods take
910 longer than a crude methanol GSL extraction, it is possible to analyse ITCs/nitriles easily by
911 GC-MS. The information about these compounds will be vital to breeders in making
912 informed selections for any possible health benefits. GSLs are a convenient proxy
913 measurement for the types of breakdown products, but are not in-and-of themselves a good
914 indicator of ITC:nitrile ratios, total abundances, or myrosinase activity.
915 In conclusion, the future of breeding for enhanced GSL/ITC Brassicaceae crops is
916 positive due to the abundance of phenotypic variation available for selection by breeders, and
917 the increased interest in developing health-beneficial products for the consumer. Consumers
918 themselves are actively looking for such products, and are more aware about the long-term

919 effects of bad dietary habits [170]. As the development of *Beneforté* broccoli has demonstrated,
920 breeding in this way is achievable for commercial Brassicaceae crops, but must be done in a
921 holistic way which accounts for every stage of varietal development, commercial production,
922 agronomic, and environmental factors – as well as the tastes, preferences and genotypes of
923 the end consumer [75]. This may take decades to achieve, but a roadmap has been established.
924

925 **References**

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Figure legends

Figure 1. A schematic of the most important factors for consideration when breeding for improved glucosinolate/isothiocyanate profiles of Brassicaceae species.

Figure 2. Molecular structures of isothiocyanates and indole compounds with known health-beneficial properties.

Table 1. Summary examples of glucosinolate content of edible crop species. Concentrations are expressed as $mg.g^{-1}$ dw of sinigrin. Values presented represent the average control concentration or raw material at the point of harvest unless otherwise stated. Values for leaves, sprouts, florets, stems and roots are presented separately.

Common name	Species	No. of cultivars tested	Environment	Glucoiberin	Progoitrin	Glucoraphenin	Glucoraphanin	Sinigrin	Glucoalyssin	Gluconapin	Diglucothiobeinin	Glucoiberverin	Glucosativin	4-hydroxyglucobrassicin	Glucolepiidin	Glucobrassicanapin	Gluconapoleiferin	Glucotropaeolin	Dimeric glucosativin	Glucoerucin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Total	References
Leaves																										
Ezo-wasabi	*Cardamine fauriei*	1	H	**13.0**						**47.0**											**3.0**				**63.0**	50
Chinese cabbage	*Brassica rapa* var. *chinensis*	1	CE		nd		0.4	0.2	nd	0.7				nd		nd	nd			nd	<0.1	<0.1	nd	nd	1.9	36
		23	F		0.6		nd	nd	nd	nd				nd		nd	nd			nd	0.8	1.7	nd	0.1	3.3	43
		7	G		0.4		nd	<0.1	0.4	1.0				0.2		nd	nd			nd	0.5	0.1	0.8	<0.1	4.8	45
	Brassica rapa var. *pekinensis*	23	G		0.4		nd	nd	nd	nd				nd		nd	nd			nd	0.5	2.1	nd	0.7	3.3	43
		12	G		0.5		0.1	<0.1	0.5	nd				0.1		1.4	1.0			0.1	0.8	nd	1.3	0.1	5.9	44
		1	?		nd		nd	nd	nd	nd				nd		nd	nd			nd	0.5	0.2	0.6	<0.1	1.4	31, 32
	Average	-	-		**0.3**		**0.1**	**<0.1**	**0.2**	**0.3**				**0.1**		**0.2**	**0.2**			**<0.1**	**0.5**	**0.7**	**0.5**	**0.2**	**3.4**	
Salad rocket	*Eruca sativa*	28	CE		nd	<0.1	0.2		<0.1		<0.1	<0.1	3.9	<0.1	<0.1			<0.1	2.2	0.2					6.7	62
		1	CE		nd	nd	0.3		nd		1.4	0.7	4.2	nd	nd			nd	nd	<0.1					6.6	63
		1	CE		nd	nd	4.6		nd		nd	nd	10.8	nd	nd			nd	nd	2.9					18.3	64
		21	G		0.8	nd	2.8		0.6		6.8	nd	3.3	nd	nd			nd	9.8	5.4					29.5	65
	Average	-	-		**0.2**	**tr**	**2.0**		**0.2**		**2.1**	**0.2**	**5.6**	**tr**	**tr**			**tr**	**3.0**	**2.1**					**15.3**	

Table 1. Continued

Common name	Species	No. of cultivars tested	Environment	Glucoiberin	Progoitrin	Epi-progoitrin	Glucoraphenin	Glucoraphanin	Sinigrin	Glucoalyssin	Gluconapin	Diglucothiobeinin	Glucoiberverin	Glucosativin	4-hydroxyglucobrassicin	Glucolepidin	Glucotropaeolin	Dimeric glucosativin	Glucoerucin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Total	References
Wild rocket		7	CE		nd		<0.1	0.2		nd		nd	<0.1	2.4	<0.1	<0.1	<0.1	4.7	0.2					7.7	62
	Diplotaxis tenuifolia	1	CE		nd		nd	0.4		nd		1.1	0.9	3.6	nd	nd	nd	nd	0.8					6.8	63
		16	G		0.4		nd	4.6		0.8		3.5	nd	2.0	nd	nd	nd	5.5	2.2					19.0	65
	Average	-	-		**0.1**		**tr**	**1.7**		**0.3**		**1.5**	**0.3**	**2.7**	**tr**	**tr**	**tr**	**3.4**	**1.1**					**11.2**	
Kale		1	CE	2.0	nd			0.4	0.4		nd				0.2				nd	2.1	<0.1	<0.1	0.2	5.3	52
		153	F	3.2	0.3			0.1	3.9		nd				nd				nd	2.9	<0.1	nd	0.3	10.7	37
	Brassica oleracea var. *acephala*	5	F	1.3	3.1			0.6	0.6		0.1				nd				<0.1	2.9	0.4	nd	nd	15.1	18
		2	G	1.1	<0.1			0.1	0.4		nd				0.1				nd	1.8	nd	0.1	0.3	3.9	19
	Average	-	-	**1.9**	**0.9**			**0.3**	**1.3**		**<0.1**				**0.1**				**tr**	**2.4**	**0.1**	**<0.1**	**0.2**	**8.8**	
Cabbage		1	CE	nd	nd	nd		0.1	1.1	nd	0.1		nd		nd					0.1	<0.1	nd	nd	1.8	36
		26	F	2.7	0.3	0.3		<0.1	1.0	<0.1	<0.1		<0.1		<0.1					2.5	nd	nd	0.2	7.2	37
	Brassica oleracea var. *capitata*	6	F	0.2	0.6	nd		0.2	0.6	nd	0.2		<0.1		nd					0.8	nd	0.1	<0.1	2.5	1
		2	G	1.6	0.3	nd		1.1	1.7	nd	0.2		nd		0.1					2.6	nd	0.3	0.3	8.8	25
		1	?	2.9	0.1	nd		0.1	4.1	nd	nd		nd		nd					2.7	nd	0.5	nd	10.3	
	Brassica oleracea var. *capitata* f. Savoy	1	?	1.7	0.1	nd		0.1	1.7	0.2	0.2		nd		0.2					1.0	nd	0.7	<0.1	5.8	31, 32
	Brassica oleracea var. *capitata* f. Oxheart	1	?	0.3	0.1	nd		0.1	<0.1	0.4	nd		nd		<0.1					0.4	nd	<0.1	nd	1.4	
	Average	-	-	**1.3**	**0.2**	**<0.1**		**0.2**	**1.5**	**0.1**	**0.1**		**tr**		**<0.1**					**1.4**	**tr**	**0.2**	**0.1**	**4.1**	

Table 1. Continued

Common name	Species	No. of cultivars tested	Environment	Glucoiberin	Progoitrin	Glucoraphanin	Sinigrin	Glucoalyssin	Gluconapin	Glucoiberverin	4-hydroxyglucobrassicin	Glucobrassicanapin	Glucoerucin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Total	References
Red cabbage	*Brassica oleracea* var. *capitata* f. *rubra*	4	F	0.1	0.6	0.3	0.2	nd	0.3	<0.1	nd		nd	1.7	<0.1	0.1	0.2	3.4	1
		1	G/F	1.5	3.6	0.6	1.6	nd	1.4	nd	0.3		0.3	1.2	0.1	1.9	nd	18.4	39
		1	?	0.6	0.5	<0.1	1.1	0.1	0.2	nd	<0.1		nd	1.5	nd	0.1	nd	4.1	31, 32
		1	?	0.4	0.7	1.3	0.6	nd	1.3	nd	0.1		nd	0.2	nd	0.1	nd	4.7	40
		1*	?	nd	nd	1.1	1.3	nd	nd	nd	0.2		nd	0.3	nd	0.3	nd	3.0	41
	Average	-	-	**0.5**	**1.1**	**0.7**	**1.0**	**<0.1**	**0.6**	**tr**	**0.1**		**0.1**	**1.0**	**<0.1**	**0.5**	**<0.1**	**6.7**	
White cabbage	*Brassica oleracea* var. *capitata* f. *alba*	?	?	1.2	0.3	nd	1.1	nd						nd		nd	nd	2.6	38
		1	?	2.7	0.1	0.1	1.7	<0.1						1.4		0.2	<0.1	6.1	31, 32
	Average	-	-	**2.0**	**0.2**	**0.1**	**1.4**	**tr**						**0.7**		**0.1**	**tr**	**4.4**	
Collards	*Brassica oleracea* var. *sabellica*	5	F	**1.0**	**2.9**	**0.3**	**6.5**		**0.7**					**4.6**	**0.1**			**18.2**	18, 19
Mustard greens	*Brassica juncea*	1	CE			nd	3.9		0.2					<0.1	0.1			4.3	36
		2	F			<0.1	29.3		0.2					0.3	0.3			47.4	18, 19
	Average	-	-			**tr**	**16.6**		**0.2**					**0.2**	**0.2**			**25.9**	
Leaf rape	*Brassica napus* var. *pabularia*	36	G		**2.2**			**0.4**	**1.1**			**3.2**		**0.4**				**7.9**	53

* = Cultivars were purchased from multiple supermarkets but treated as one sample

Table 1. Continued

Common name	Species	No. of cultivars tested	Environment	Glucoiberin	Progoitrin	Glucoraphanin	Sinigrin	Glucoalyssin	Glucosinalbin	Gluconapin	Diglucothiobeinin	Glucoiberverin	Glucosativin	4-hydroxyglucobrassicin	Gluconapoleiferin	Dimeric glucosativin	Glucoerucin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Glucomoringin	Acetyl glucomoringin (I, II, III)	3-hydroxypropyl GSL	Total	References
Watercress	*Nasturtium officinale*	1	CE											nd				0.2	6.6	nd					7.1	36
		1	H$											0.2				0.5	1.8	0.3					2.8	71
	Average	-	-											**0.1**				**0.4**	**4.2**	**0.2**					**5.0**	
Chinese kale	*Brassica oleracea* var. *alboglabra*	1	F	**0.1**	**1.9**	**4.0**	**0.1**			**7.6**		**<0.1**		**0.2**	**0.1**		**0.1**	**0.6**		**0.1**	**0.2**				**14.9**	21
Turnip rape	*Brassica napus*	1	CE				**<0.1**			**4.8**								**0.1**	**0.7**						**5.6**	36
Dogmustard	*Erucastrum* spp.	1	G		**<0.1**	**1.9**		**0.9**			**<0.1**		**<0.1**			**0.2**	**0.6**								**3.6**	65
Annual wall-rocket	*Diplotaxis muralis*	2	G		**0.3**	**3.2**		**0.4**			**4.0**		**0.9**			**5.9**	**2.8**								**17.4**	
White mustard	*Sinapis alba*	1	G				**2.0**		**27.1**																**29.1**	24
Moringa	*Moringa oleifera*	6	F∨																			50.2	9.3		**59.5**	60
		30	F																			12.0	12.0		**24.0**	57
		1	G/F																			17.1	11.8		**28.9**	59
		6	G																			48.5	34.2		**82.7**	58^
	Average	-	-																			**29.3**	**14.1**		**43.4**	
Spider plant	*Cleome gynandra*	6	F																					**3.1**	**3.1**	69
Ethiopian mustard	*Brassica carinata*	2	CE		<0.1		6.9	<0.1		<0.1				<0.1				0.1	nd	<0.1	<0.1				**7.1**	48
		1	G		nd		1.3	nd		0.1				nd				0.2	<0.1	<0.1	<0.1				**1.7**	49
	Average	-	-		**tr**		**4.1**	**tr**		**0.1**				**tr**				**0.2**	**tr**	**<0.1**	**<0.1**				**4.4**	
Sprouts																										
Broccoli	*Brassica oleracea* var. *italica*	1	CE	1.1	tr	18.3		<0.1		tr				4.0			3.9	5.5	tr	2.9	3.1				38.8	11
		1	CE	2.9	nd	7.7		nd		nd				1.5			0.3	1.4	nd	3.5	1.6				18.9	15
	Average	-	-	**2.0**	**tr**	**13.0**		**tr**		**tr**				**2.75**			**2.1**	**3.5**	**tr**	**3.2**	**2.4**				**28.9**	

$ = cultivars were grown commercially in outdoor water beds; ∧= concentrations determined from reported % of total; ∨ = grown in various geographical locations.

Table 1. Continued

Common name	Species	No. of cultivars tested	Environment	Glucoiberin	Progoitrin	Epi-progoitrin	Glucoraphenin	Glucoraphanin	Sinigrin	Glucoalyssin	Gluconapin	Glucoiberverin	4-hydroxyglucobrassicin	Glucobrassicanapin	Gluconapoleiferin	Glucoerucin	Glucoraphasatin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Total	References
Turnip	*Brassica rapa* var. *rapa*	1	CE		**4.2**					**0.1**	**0.8**		**2.4**	**tr**	**tr**			**2.3**		**2.2**	**2.4**	**15.0**	
Rutabaga	*Brassica napus* var. *rapifera*	1	CE		**18.5**						**1.6**		**1.7**	**tr**	**tr**			**3.0**		**3.8**	**3.4**	**31.9**	11
China rose radish	*Raphanus sativus*	1	CE				**3.3**						**1.5**			**0.2**	**41.1**			**2.7**		**48.8**	
Radish		1	CE				**16.7**						**2.7**				**17.2**			**tr**		**36.6**	
Chinese kale	*Brassica oleracea* var. *alboglabra*	1	H	1.0	28.7			1.7	16.7		45.9		0.6		0.4	0.5		0.9		1.8	0.2	98.2	46
		2	?	1.2	11.9			nd	nd		15.9		nd		nd	nd		0.6		3.0	nd	32.8	47
	Average	-	-	**1.1**	**20.3**			**0.9**	**8.4**		**30.9**		**0.3**		**0.2**	**0.3**		**0.8**		**2.4**	**0.1**	**65.5**	
Florets/Buds																							
Broccoli	*Brassica oleracea* var. *italica*	1	CE/F	1.7	nd	nd		17.4	nd	nd	nd	nd	1.8	nd	nd	nd		4.0	nd	0.8	1.2	26.9	16
		10	F	nd	0.4	nd		4.0	<0.1	nd	nd	nd	nd	nd	nd	nd		nd	nd	nd	nd	6.4	17
		6	F	0.3	0.1	nd		2.3	<0.1	nd	<0.1	nd	nd	nd	nd	0.1		2.3	<0.1	nd	nd	7.3	18, 19
		6	F	0.4	nd	nd		2.2	nd	nd	nd	nd	0.1	nd	nd	nd		1.4	nd	0.6	0.2	4.9	20
		4	F	0.2	0.3	nd		1.8	<0.1	nd	<0.1	nd	<0.1	nd	0.1	<0.1		0.9	nd	0.1	0.3	3.9	21
		1	F	0.3	nd	nd		1.7	nd	nd	nd	nd	0.1	nd	nd	nd		0.6	nd	0.1	0.3	3.1	22
		1	F	0.7	nd	nd		4.6	nd	nd	nd	nd	0.1	nd	nd	nd		1.7	nd	3.8	0.2	11.1	23

Table 1. Continued

Common name	Species	No. of cultivars tested	Environment	Glucoiberin	Progoitrin	Epi-progoitrin	Glucoraphanin	Sinigrin	Glucoalyssin	Gluconapin	Glucoiberverin	4-hydroxyglucobrassicin	Glucobrassicanapin	Gluconapoleiferin	Glucotropaeolin	Glucoerucin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Total	References
		1	F	nd	nd	nd	0.9	nd	nd	nd	nd	nd	nd	nd		nd	nd	<0.1	nd	nd	1.0	24
		2	G	nd	nd	nd	4.6	nd	nd	nd	nd	0.2	nd	nd		nd	2.6	nd	0.3	1.9	9.3	25
		148	G	0.1	0.9	0.6	1.4	<0.1	<0.1	0.1	nd	0.1	nd	nd		0.1	2.0	nd	0.3	0.7	5.8	87
		50	G/F	<0.1	0.1	nd	2.8	<0.1	0.1	0.4	nd	0.1	0.1	0.3		nd	0.4	0.2	0.2	0.1	5.1	26, 27
		-	M	1.6	3.2	<0.1	7.7	<0.1	1.2	0.2	<0.1	0.1	<0.1	0.2		<0.1	6.9	0.1	0.4	4.0	25.6	28+
Broccoli (continued)	*Brassica oleracea* var. *italica*	2	?	nd	nd	nd	8.3	nd	nd	nd	nd	nd	nd	nd		nd	nd	nd	nd	nd	8.3	29
		-	?	nd	nd	nd	1.9	nd	nd	nd	nd	nd	nd	nd		nd	nd	nd	nd	nd	2.4	30
		1	?	0.2	2.3	nd	0.1	nd	2.3	0.2	nd	nd	nd	nd		nd	0.8	nd	0.1	0.4	6.6	31, 32
		1	?	1.3	nd	nd	3.2	nd	nd	nd	nd	nd	nd	nd		nd	0.4	nd	0.1	0.6	5.6	33
		1	?	0.2	nd	nd	0.9	nd	nd	nd	nd	0.1	nd	nd		nd	0.7	nd	0.4	0.2	2.5	34
		1	?	0.3	nd	nd	2.8	nd	nd	nd	nd	0.1	nd	nd		nd	2.3	nd	0.3	0.9	6.9	14
	Average	-	-	**0.4**	**0.4**	**<0.1**	**3.8**	**0.4**	**0.2**	**<0.1**	**tr**	**0.1**	**<0.1**	**<0.1**		**<0.1**	**1.5**	**<0.1**	**0.4**	**0.5**	**7.9**	
		6	F	0.4	0.5		0.5	0.5	nd	0.3	<0.1	nd			nd	<0.1	15.8	<0.1	nd	nd	22.4	18, 19
		1	F	0.1	2.1		nd	10.5	nd	0.9	nd	nd			0.4	nd	2.6	0.2	0.7	0.1	17.6	35
Brussels sprouts	*Brassica oleracea* var. *gemmifera*	2	G	1.4	0.8		0.6	0.9	nd	0.3	nd	0.2			nd	nd	4.4	nd	0.4	0.2	10.3	25
		1	?	1.2	3.0		0.1	3.3	0.8	2.8	nd	0.5			nd	nd	1.8	0.1	0.6	nd	13.9	31, 32
		1	?	0.9	nd		0.2	nd	nd	nd	nd	0.1			nd	nd	0.9	nd	nd	0.1	2.2	34
	Average	-	-	**0.8**	**1.3**		**0.3**	**3.0**	**0.2**	**0.9**	**tr**	**0.2**			**0.1**	**tr**	**5.1**	**0.1**	**0.3**	**0.1**	**13.3**	

+ = Median values taken from range data

Table 1. Continued

Common name	Species	No. of cultivars tested	Environment	Glucoiberin	Progoitrin	Glucoraphanin	Sinigrin	Glucoberteroin	Glucoalyssin	Glucosinalbin	Gluconapin	Glucoiberverin	4-hydroxyglucobrassicin	Glucobrassicanapin	Gluconapoleiferin	Glucotropaeolin	Glucolimnathin	Glucoerucin	Glucoraphasatin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Glucomoringin	Acetyl glucomoringin (I, II, III)	3-hydroxypropyl GSL	Total	References
Cauliflower	*Brassica oleracea* var. *botrytis*	5	F	0.5	nd	<0.1	0.3				<0.1	0.2	nd		nd			<0.1		2.5	<0.1	nd	nd				4.0	18, 19
		4	F	0.9	0.1	0.4	0.1				<0.1	0.1	<0.1		<0.1			<0.1		0.9	nd	0.1	0.1				2.9	21
		1	F	0.7	0.1	0.1	0.7				nd	nd	nd		nd			nd		0.9	nd	<0.1	<0.1				2.5	22
		2	G	0.6	0.1	<0.1	0.8				<0.1	nd	0.2		nd			nd		2.3	nd	0.2	1.5				5.8	25
		5	G/F	1.3	2.5	0.9	0.6				nd	nd	0.7		nd			0.1		4.1	nd	0.7	0.5				11.4	42
		1	?	0.1	nd	0.1	0.1				nd	nd	<0.1		nd			nd		0.3	nd	0.1	<0.1				0.7	31, 32
		1	?	0.4	nd	<0.1	nd				nd	nd	<0.1		nd			nd		0.7	nd	0.2	0.1				1.5	34
	Average	-	-	**0.6**	**0.4**	**0.2**	**0.4**				**tr**	**<0.1**	**0.1**		**tr**			**<0.1**		**1.7**	**tr**	**0.2**	**0.3**				**4.1**	
Stem																												
Kohlrabi	*Brassica oleracea* var. *gongylodes*	1	F	0.1	<0.1	0.2			nd			0.4	nd					1.3		1.1	0.1	nd	nd				3.4	18, 19
		1	?	0.1	nd	<0.1			0.1			nd	<0.1					nd		0.5	nd	<0.1	0.2				1.0	31, 32
	Average	-	-	**0.1**	**tr**	**0.1**			**0.1**			**0.2**	**tr**					**0.7**		**0.8**	**0.1**	**tr**	**0.1**				**2.2**	
Moringa	*Moringa oleifera*	1	F																					**16.3**	**4.8**		**21.1**	60
Spider plant	*Cleome gynandra*	8	F																							**7.6**	**7.6**	69
Ethiopian mustard	*Brassica carinata*	1	G				**2.8**				**0.4**		**0.1**							**0.2**	**2.3**	**0.1**	**0.1**				**6.0**	49
Root																												
Rutabaga	*Brassica oleracea* var. *rapifera*	1	CE		2.8	nd		1.2	0.2				0.1	0.1	0.4			0.4		0.2	nd	0.1	0.1				5.6	68
		1	?		0.9	0.3		nd	nd				0.1	nd	nd			nd		0.4	1.2	0.1	0.4				3.5	31, 32
	Average	-	-		**1.9**	**0.2**		**0.6**	**0.1**				**0.1**	**0.1**	**0.2**			**0.2**		**0.3**	**0.6**	**0.1**	**0.3**				**4.6**	
Maca	*Lepidium meyenii*	3	F						**0.1**	**0.2**						**6.9**	**1.5**										**8.6**	54
Radish	*Raphanus sativus*	1	?	**0.1**		**0.1**													**1.9**	**0.1**	**0.1**	**0.2**					**2.8**	31, 32
Moringa	*Moringa oleifera*	3	F[V]																					**8.6**	**10.2**		**18.8**	60

Table 2. Summary of factors that influence glucosinolate composition of Brassicaceae plants during cultivation

	Species			
Variable	**Broccoli**	Reference	**Cauliflower**	Referer
Genotype	↑ Indole GSLs ↑ Alkyl GSLs	86	↑Indole GSLs	
	Significant differences in total GSLs, indole GSLs & glucoraphanin	25	Significant differences in total GSLs, indole GSLs & glucoraphanin	
	Significant differences among cultivars for alkyl, alkenyl, indole and total GSLs.	21	Significant differences among cultivars for alkyl, alkenyl, indole and total GSLs.	
	Significant differences between individual GSL concentrations between cultivars	87		
Environmental temperature	↑ Total GSLs at low temp. ~14°C	86	↑Total GSLs at low temp. ~14°C	
	↓Total GSLs with increasing temperature	25	↓Total GSLs with increasing temperature	
	Variability of individual GSLs according to temp.	16		
Light intensity	↑Total GSLs at high light levels (450 µmol $m^{-2}s^{-1}$)	86	↑Total GSLs at high light levels (450 µmol $m^{-2}s^{-1}$)	
	Total & indole GSLs influenced by day length & light intensity ↓Glucoraphanin with high light at harvest	25		
	↑Total GSLs with light	115	Total & indole GSLs influenced by day length & light intensity ↓Glucoraphanin with high light at harvest	
	Variability of individual GSLs according to day length	16		

↑Increase; ←→no-effect; ↓decrease

Table 2. Continued

	Species					
Variable	**Broccoli**	Reference	**Cauliflower**	Reference	**Radish**	Reference
Sulfur application	↑Alkyl & indole GSLs (600 mg S per plant)	86	-	-	↑Alkenyl GSLs (30 mg S per plant)	86
	←→(150 kg S ha^{-1})	115				
	←→Low S (15 kg S ha^{-1})					
	↑Aliphatic & total GSLs (>15 $mg.L^{-1}$)	15				
Nitrogen application	↑Total GSLs with reduced N	86	↑Total GSLs with reduced N	86	↑Total GSLs with reduced N	86
	↑Total GSLs with reduced N (1g N per plant)	115				
Selenium application	↑Total GSLs (5.2 mM Se)	115	-	-	↑Total GSLs & glucoraphanin in soil ↓Total GSLs in hydroponics	129
	←→	127				
Water availability	↑Total GSLs with reduced water	86	↑Total GSLs with reduced water	86	↑Total GSLs with reduced water	86
	↑Total GSLs with severe drought	115				
Soil salinity	↑Total GSLs (40, 80mM)	115	-	-	-	-
Season	↑Total GSLs in spring & autumn	86	↑Total GSLs in spring & autumn	86	←→	86
Amino acid supplementation	↑Alkyl GSLs with methionine	86	-	-	↑Alkenyl GSLs with methionine	86
Developmental stage	↑Indole GSLs in immature florets	85	↑Glucoraphanin between transplanting & harvest	25	←→	86
	↑Glucoraphanin between transplanting & harvest	25				

↑Increase; ←→no-effect; ↓decrease

Table 2. Continued

	Species					
Variable	**Cabbage**	Reference	**Brussels sprouts**	Reference	**Wild rocket**	Reference
Genotype	↑Sinigrin content in some varieties	37	Significant differences in total GSLs, indole GSLs & glucoraphanin	25	Significant differences between genotypes for aliphatic and total GSLs	62
	Significant differences in total GSLs, indole GSLs & glucoraphanin	25				
Environmental temperature	↓Total GSLs with increasing temperature	25	↓Total GSLs with increasing temperature	25	-	-
	↑Total GSLs at 32°C	115				
Light intensity	Total & indole GSLs influenced by day length & light intensity ↓Glucoraphanin with high light at harvest ↑Total GSLs during the night ↓Total GSLs during the day	25 115	Total & indole GSLs influenced by day length & light intensity ↓Glucoraphanin with high light at harvest	25	←→	63
Selenium application	←→	127	-	-	-	-
Water availability	↑Total GSLs with severe drought ↓Total GSLs under mild and severe drought	115	←→No effect under mild drought	25	-	-
Season	↑Glucoiberin & glucobrassicin in spring ↑Total GSL in spring	37	-	-	-	-
	↑Total GSL in spring ↑Indolic GSLs in fall	1				
Developmental stage	↑Glucoraphanin between transplanting & harvest	25	↑Glucoraphanin between transplanting & harvest	25	-	-

↑Increase; ←→no-effect; ↓decrease

Table 2. Continued

	Species					
Variable	**Ezo-wasabi**	Reference	**Salad rocket**	Reference	**Kale**	Reference
Genotype	-	-	Significant differences between genotypes for aliphatic and total GSLs	62	Significant differences in total GSLs, indole GSLs & glucoraphanin	25
Environmental temperature	-	-	-	-	↓Total GSLs with increasing temperature ↓Total GSLs at cold temperatures (9-15°C)	25 52
Light intensity	↑Total GSLs; red+blue light ↑Indolic:aliphatic GSL ratio; red or green light ↑Aliphatic, ↓ indolic GSLs; blue light	50	←→	63	Total & indole GSLs influenced by day length & light intensity ↓Glucoraphanin with high light at harvest	25
Developmental stage	-	-	-	-	↑Glucoraphanin between transplanting & harvest	25
Variable	**Turnip**	Reference	**Ethiopian mustard**	Reference	**Thale cress**	Reference
Light intensity	-	-	-	-	↑Total GSLs with light ↓Total GSLs in the dark	115
Sulfur application	↑Total GSLs (60 kg S ha^{-1})	115	-	-	-	-
Potassium application	↓Total GSLs with K deficiency	-	-	-	↑Total GSLs with K deficiency	115
Water availability	↑Total GSLs with mild drought	115	←→ No effect under mild drought ↑Total GSLs with severe drought	86, 115	↓Total GSLs under mild and severe drought	115

↑Increase; ←→no-effect; ↓decrease

Table 2. Continued.

	Species					
Variable	**Swede**	Reference	**Chinese cabbage**	Reference	**Rapeseed**	Reference
Genotype	-	-	Significant differences between genotypes for glucobrassicin and gluconasturtiin Total and indolic glucosinolates vary between genotypes	43 44	-	-
Environmental temperature	↑Progoitrin & glucoberteroin at 21°C	68	↑Total GSLs between 21-34°C ↓Total GSLs between 15-27°C	115	-	-
Water availability	-	-	-	-	↑Total GSLs with severe drought ←→ No effect under mild drought	115
Soil salinity	-	-	↑Total GSLs (40, 80mM)	115	-	-
Variable	**White mustard**	Reference	**Chinese kale**	Reference		
Genotype	-	-	Significant differences among cultivars for alkyl, alkenyl, indole and total GSLs.	21		
Light intensity	-	-	↓Gluconapin under blue light ↑Glucoraphanin under blue light	46		
Selenium application	←→	127	-	-		

↑Increase; ←→no-effect; ↓decrease

Figure 1

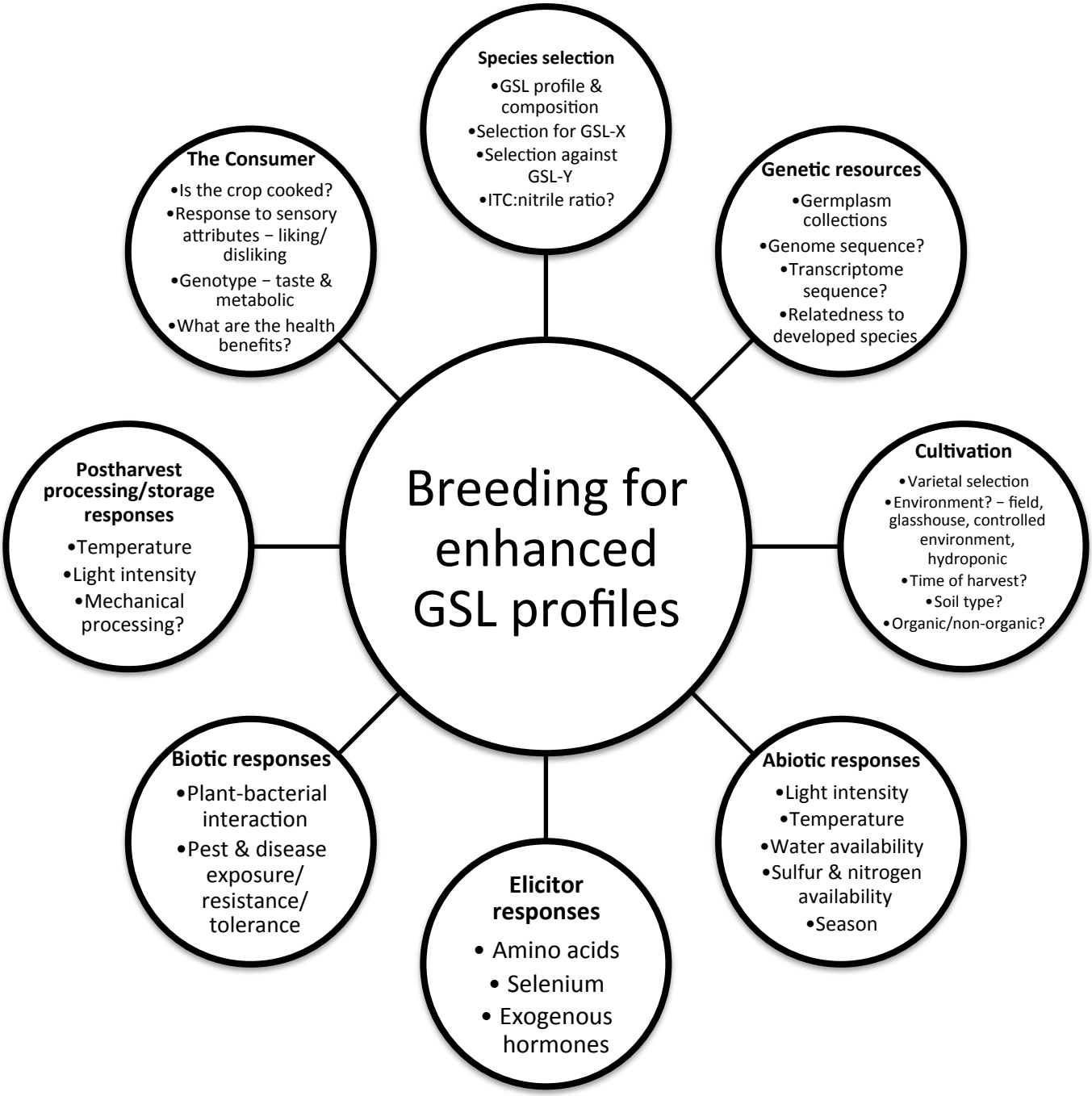
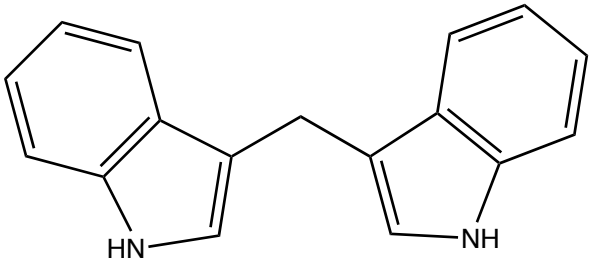
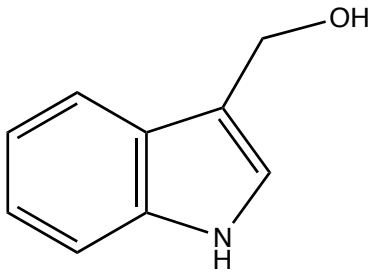

Breeding for enhanced GSL profiles
Species selection
•GSL profile & composition
•Selection for GSL-X
•Selection against GSL-Y
•ITC:nitrile ratio?
Genetic resources
•Germplasm collections
•Genome sequence?
•Transcriptome sequence?
•Relatedness to developed species
Cultivation
•Varietal selection
•Environment? – field, glasshouse, controlled environment, hydroponic
•Time of harvest?
•Soil type?
•Organic/non-organic?
Abiotic responses
•Light intensity
•Temperature
•Water availability
•Sulfur & nitrogen availability
•Season
Elicitor responses
• Amino acids
• Selenium
• Exogenous hormones
Biotic responses
•Plant-bacterial interaction
•Pest & disease exposure/ resistance/ tolerance
Postharvest processing/storage responses
•Temperature
•Light intensity
•Mechanical processing?
The Consumer
•Is the crop cooked?
•Response to sensory attributes – liking/ disliking
•Genotype – taste & metabolic
•What are the health benefits?

Figure 2.

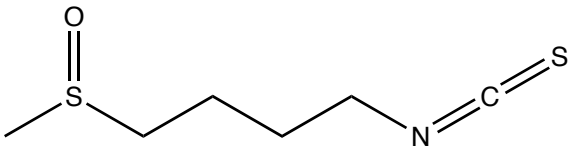
3,3'-diindolylmethane (DIM)



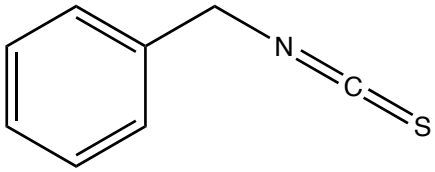
Indole-3-carbinol (I3C)



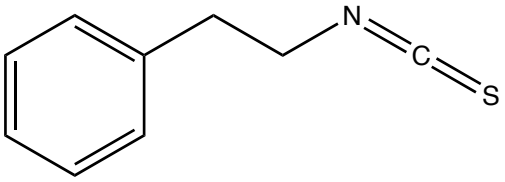
Sulforaphane (SFN)



Benzyl isothiocyanate (BITC)



Phenethyl isothiocyanate (PEITC)



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