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Chapter 2

Health-affecting compounds in Brassicaceae

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Abstract

Brassicaceae vegetables are considered to be a food in many areas all over the world. *Brassica* species are not only known for their high fat and protein contents for human and animal consumption, but Brassicaceae vegetables are also recognized as a rich source of nutrients such as vitamins (carotenoids, tocopherol, ascorbic acid, folic acid), minerals (Cu, Zn, P, Mg among others), carbohydrates (sucrose and glucose), amino acids (for example, L-alanine, L-aspartic acid, L-glutamic acid, L-glutamine, L-histidine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, and L-valine), and different groups of phytochemicals such as indole phytoalexins (brassinin, spirobrassinin, brassilexin, camalexin, 1-methoxyspirobrassinin, 1-methoxyspirobrassinol, and methoxyspirobrassinol methyl ether), phenolics (such as feruloyl and isoferuloylcholine, and hydroxybenzoic, neochlorogenic, chlorogenic, caffeic, *p*-coumaric, ferulic, and sinapic acids, anthocyanins, quercetin and kaempferol), and glucosinolates mainly including glucoiberin, glucoraphanin, glucoalyssin, gluconapin, glucobrassicanapin, glucobrassicin, gluconasturtiin, and neoglucobrassicin. All these phytochemicals contribute to the reported antioxidant, anticarcinogenic, and cardiovascular protective activities of *Brassica* vegetables. However, not all members of this family are equal from a nutritional viewpoint, since significant qualitative variations in the phytochemical profiles of *Brassica* species and varieties suggest differences in the health promoting properties among these vegetables. In this review, *Brassica* phytochemicals with their nutritional value and health promoting activities are discussed to give an overview of the literature for *Brassica* as a food crop.

Keywords: Brassicaceae, Health effects, Metabolites

1 Introduction

The Brassicaceae (Cruciferae) family is composed of 350 genera and about 3500 species,[42] including some crops of great economical importance such as *Brassica napus* L., *B. rapa* L., and *Sinapis alba* L.[43, 44] These species are used as food, spices, and as a source of vegetable oils.[45] The Brassicaceae vegetables represent a major part of the human diet[4] being consumed by people all over the world[46-48] and are considered important food crops in China, Japan, India, and European countries.[42, 49, 50] Over the past 3 decades *Brassica* production has grown steadily becoming an important source of oil and protein of plant origin for animal and human nutrition, respectively. Rapeseed (canola) ranks currently as the third source of vegetable oil (after soy and palm) and the third leading source of oil meal (after soy and cotton).[51] *Brassica* is an inexpensive though very nutritive source of food, providing nutrients and health-promoting phytochemicals such as phenolic compounds, vitamins,[52-55] phytic acid, fibre, soluble sugars,[56] glucosinolates,[57] minerals, polyphenols,[58] fat, and carotenoids (**Figure 1**).[59] There is currently much interest in identifying phytochemicals with useful biological activity in food[60] and any significant finding related to the presence of valuable compounds in *Brassica* species will be welcomed by the food industry.[51]

There is ever-increasing evidence that a higher consumption of *Brassica* vegetables, for example, broccoli, cabbage, kale, mustard greens, Brussels sprouts, and cauliflower, reduces the risk of several types of cancer.[61, 62] The anticarcinogenic effect of these vegetables has been attributed to decomposition products of glucosinolates, indoles, and iso-thiocyanates,[63] phytoalexins, and other antioxidants.[64, 65] Indole-3-carbinol, a natural component of *Brassica* vegetables[66] has an interesting anticarcinogenic potential, acting via different metabolomic and hormonal pathways.[65] It reduces the incidence of tumours in reproductive organs[66] and the growth of human breast cancer cells.[67]

Overall, to date, the most promising anticarcinogenic dietary compounds have been detected in cruciferous vegetables and further elucidation of their protective mechanisms and the identification of other active constituents may contribute to the development of highly health supporting *Brassica* varieties.[68]

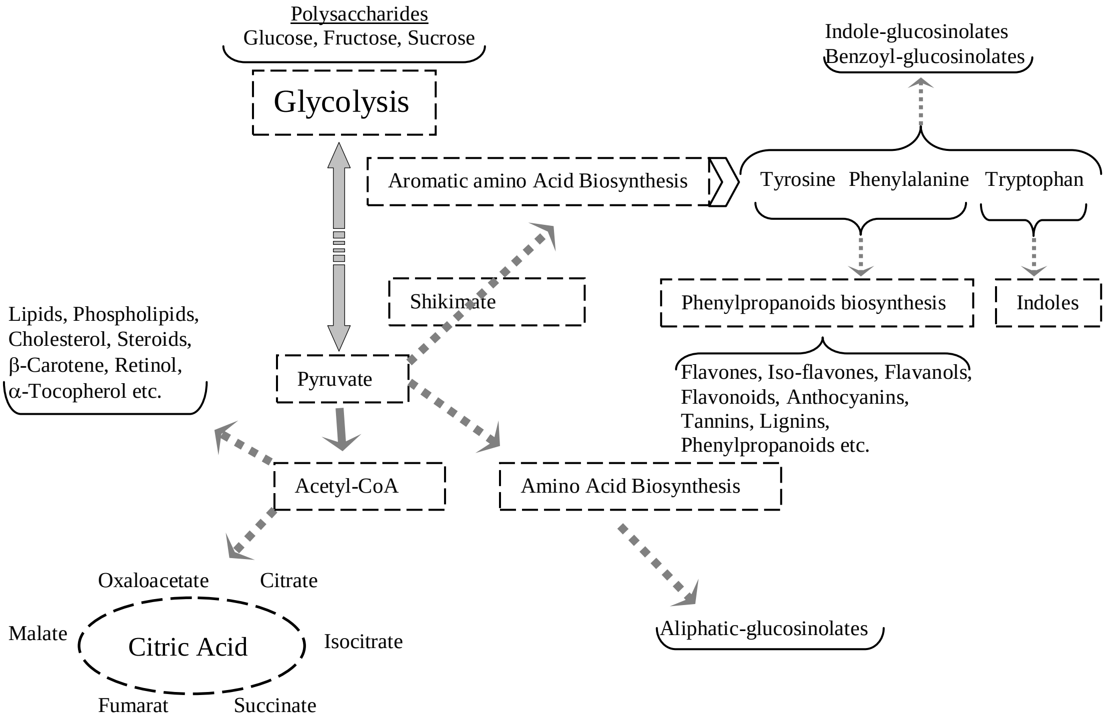

Figure 1 – General biosynthesis pathway for Brassicacae metabolites.[69-73]

Extracts of the different species of the Brassicaceae family show antioxidant effects[74] and decrease oxidative damage,[75] while the juice of some *Brassica* species has been proved to protect human hepatoma cells from the genotoxic effects of carcinogens.[68] However, compounds such as glucosinolates and phytates may also have a negative effect on human and animal health. For example, glucosinolates and glucosinolate by-products can be toxic and are responsible for the bitter, hot, and pungent flavours of Brassicaceae vegetables.[76] Also, thiocyanates, isothiocyanates, and oxazolidine-2-thiones have been shown to be goitrogenic.[77] As Brassicaceae vegetables can be a good source of minerals, antinutrients such as phytates can decrease their bioavailability.[78]

The purpose of this review is to provide an overview of health-affecting compounds identified in the *Brassica* genus.

2 Vitamins

Brassica vegetables contain high levels of vitamins[58] including carotenes, tocopherols,[79] vitamin C, and folic acid[80] (**Table 1**). It is a well-known fact that the first 3 vitamins have the potential to prevent and treat malignant and degenerative diseases.[79] Broccoli (*Brassica oleracea*) extracts are protective against reactive oxygen species (ROS) presumably due to the presence of vitamin C, quercetin, kaempferol, lutein, zeaxanthin,[81] α-tocopherol, γ-tocopherol, and β-carotene.[82] Bioavailabilty is a critical feature in the assessment of the role of these compounds in human health. When 200 g of broccoli was consumed by healthy volunteers, significant changes in serum of both men and women were observed for lutein, whereas for γ-tocopherol a significant change was detected in women only, whereas no changes were observed for α-tocopherol, β-carotene, and retinol.[83]

- **Carotenoids**

In some *Brassica* species, carotenoid content is two-fold higher than in spinach.[84] Sixteen carotenoids were identified in *B. chinensis*, *B. parachinensis*, and *B. pekinensis*, out of which lutein and β-carotene were the most abundant.[85, 86] Lutein has also been isolated from extracts of fresh raw kale (*Brassica oleracea* var. *acephala*)[87] and high levels of other carotenoids, mainly β-carotene, were also detected.[76, 79, 88] Two other vegetables, Brussels sprouts and green cabbage, have been reported to contain significant amounts of *trans*-β-carotene and *cis*-β-carotene.[89] Carotenoids present in dark green leafy vegetables might be involved in the prevention of several diseases related to oxidative stress.[86]

- **Tocopherols**

The predominant tocopherol in all *Brassica* vegetables is α-tocopherol with the exception of cauliflower, which predominantly contains γ-tocopherol.[90] The tocopherol content of rapeseed oil consists of 64% γ-tocopherol, 35% α-tocopherol, and less than 1% is the mixture of δ-tocopherol and plastochromanol-8.[91]

- **Vitamin C**

High levels of vitamin C have been reported in Chinese cabbage, broccoli, cauliflower and cabbage[92] (**Table 1**). The content of this vitamin in different cultivars of cabbage (*Brassica oleraceae* L.) ranges from 12.0 to 112.5 mg/100 g.[93]

- **Folic acid**

Raw broccoli,[94] cauliflower[95] and cabbage contain folic acid[96] (**Table 1**), a scarce and important vitamin which acts as a coenzyme in many single carbon transfer reactions in the synthesis of DNA, RNA, and protein components.[97] Folic acid reduces the risk of neural tube defects and may be associated with the reduced risk of vascular disease and cancer,[98, 99] while low folate intake has been identified as a main cause of anaemia.[100]

Table 1 – Variation of vitamins (μg/g) among different Brassicaceae vegetables on fresh weight basis.

	Ascorbic acid	α-Carotene	β-Carotene	α-Tocopherol	Folate
Broccoli	748 ± 62 [a]	0.3 [b]	8.9 [b]	16.2 [b]	1.771 [d]
Kale	186 [e]	0.6 [b]	48.6 [b]	19.2 [b]	–
Cauliflower	499 ± 53 [a]	–	72 ± 0.5 [g]	1.7 [b]	0.53 [e]
Chinese cabbage	253 [a]	–	0.1 [c]	0.8 [c]	0.81 [f]
White cabbage	188 ± 13 [a]	0.02 [b]	0.8 [b]	1.7 [b]	–
Brussels sprouts	158 [c]	–	1.4 [c]	1.5 [c]	–

a = [92]; b = [79]; c = [101]; d = [94]; e = [95]; f = [97]; g = [102].

3 Minerals

Brassica plants have been found to be rich in many minerals including calcium and iron.[84] Among the green leafy vegetables, *B. oleracea* L. *acephala* (kale) is an excellent source of minerals,[76] accumulating high levels of P, S, Cl, Ca, Fe, Sr, and K (**Table 2**).[103] Broccoli accumulates Se to concentrations many times above that found in soil, which may greatly enhance its health-promoting properties.[104] Different *Brassica* vegetables such as cauliflower, bok choy (*B. rapa*) stems and leaves, broccoli (*B. oleracea v. botrytis*), and kale (*B. oleracea v. acephala*) are reported to have high mineral contents[96] (**Table 2**). Interestingly, all these *Brassica* vegetables exhibit excellent calcium bioavailability.[105] Cabbage leaf (*B. oleracea* var. *capitata*) also contains potentially useful amounts of copper, zinc, iron, and a number of other essential minerals and trace elements.[106]

Brassica can be cultivated under hydroponic conditions that lead to high levels of nutritionally important minerals such as Cr, Fe, Mn, Se, and Zn. Owing to reproducible and high concentration of minerals in the edible plant tissue small quantities of this enriched plant can be processed to make capsules or tablets that supply 100% of the recommended daily intake of these elements, with the advantage of using a natural plant source.[107] However, the bioavailability of some of these minerals might be reduced by the presence of glucosinolates, phytates, and phenolics.[78]

Table 2 – Variation of minerals (μg/g) among different Brassicaceae vegetables on fresh weight basis

	Broccoli	Kale	Cauliflower	Chinese cabbage	White cabbage	Brussels sprouts
Ca	272 ± 20 [a]	2860 ± 430 [b]	175 ± 17 [a]	470 ± 60 [b]	440 ± 60 [b]	356 ± 13 [a]
Fe	8.7 ± 0.5 [a]	4 ± 2 [b]	5.0 ± 0.3 [a]	2 ± 0.3 [b]	1.4 ± 0.3 [b]	7.6 ± 0.2 [a]
Cu	0.94 ± 0.07 [a]	0.4 ± 0.2 [b]	0.56 ± 0.07 [a]	0.4 ± 0.2 [b]	0.5 ± 0.5 [b]	0.9 ± 0.09 [a]
Mg	181 ± 8 [a]	510 ± 40 [b]	145 ± 22 [a]	130 ± 30 [b]	140 ± 20 [b]	207 ± 12 [a]
K	2890 ± 70 [a]	7120 ± 5170 [b]	2210 ± 140 [a]	2280 ± 1120 [b]	2660 ± 870 [b]	4250 ± 250 [a]
Zn	9.5 ± 0.3 [a]	2.9 ± 0.5 [b]	6.4 ± 0.3 [a]	2.3 ± 0.4 [b]	2 ± 1 [b]	5.8 ± 0.4 [a]
Na	180 ± 6 [a]	120 ± 40 [b]	192 ± 27 [a]	50 ± 20 [b]	30 ± 10 [b]	107 ± 7 [a]
Mn	1.92 ± 0.09 [a]	3 ± 1 [b]	1.31 ± 0.07 [a]	0.5 ± 1.4 [b]	2 ± 1 [b]	2.31 ± 0.13 [a]

a = [108]; b = [109].

Heavy metals (for example, Mo, B, Co, Se, Cd, Pb, Cr, Ni, Hg, and As) and others such as Cu, Zn, Mn, Fe may be found in high concentration in contaminated soils and have toxic effects on plants, animals, and human beings.[110] The use of metal-accumulating plants to remove toxic metals from soil is known as phytoremediation[22] and *Brassica* species such as *B. oleracea* and *B. napus*, known for their metal accumulator properties, are used for this purpose.[111] However, this characteristic, which constitutes an advantage for the former use entails, an important toxicological risk if these fruits and vegetables grown in contaminated soils are ingested.[112]

4 Lipids

Rapeseed oil is one of the most common edible oils in the world. Its nutritive value is excellent due to its unsaturated fatty acid content.[113] Mustard oil is also a significant source of unsaturated fatty acids containing about 94.2%, and only 5.4% saturated fatty acids. These are recognized as essential dietary elements with important effects on human health.[114] Mustard oil contains linolenic acid 21.4% (omega – 3), palmitic acid 2.9%, palmitoleic acid 0.2%, stearic acid 1.0%, oleic acid 19.4%, linoleic acid (omega – 6) 9.7%, and erucic acid 44.4% [115], showing an inhibition of mutagenicity.[114] Oil content in seeds of different *B. campestris* genotypes varies from 38.9% to 44.6% and major fatty acids found are oleic, linoleic, linolenic, eicosaenoic, erucic acid ranging from 10.1% to 17.3%, 5.9% to 14.5%, 5.2% to 15.0%, 7.7% to 13.7% and 39.6% to 59.9%, respectively.[116] Canola seed oil is one of the richer sources of omega-3-unsaturated fatty acids[65] and in particular of α-linolenic acid.[117] The oil of commercial *B. napus* L. is rich in oleic acid and contains moderate levels of linoleic and linolenic acid.[118]

Cauliflower is considered to be a food of high nutritional value and some authors relate its quality to the stability of its fatty acids. Environmental stress may enhance the fatty matter content (linolenic acid) and polyphenols.[119]

The essential oil of *B. rapa* var. *perviridis* leaves was found to contain 48 volatile components, representing 94.0 – 96.6% of the oil. The main constituents were found to be 3-butenyl-isothiocyanate (1.4 – 29.2%), 4-pentenyl isothiocyanate (8.2–23.5%), 2-methyl 5-hexenenitrile (1.3–16.8%), 2-phenylethyl isothiocyanate (7.0– 13.7%), and phytol (6.1–23.5%).[84] Volatile chemicals emitted by rape seed oil also contain monoterpenes (limonene, sabinene, β-myrcene, and *cis*-3-hexen-1-ol acetate), sesquiterpenes, short-chain aldehydes and ketones, other green leaf volatiles and organic sulfides including the respiratory irritant, dimethyl disulfide.[120] The emission of volatiles from cabbage, consisted mainly of monoterpenes (sabinene, limonene, α-thujene, 1,8-cineole, β-pinene, myrcene, α-pinene, and γ-terpinene). (Z)-3-Hexenyl acetate, sesquiterpene (E, E)-α-farnesene, and homoterpene (E)-4, 8-dimethyl-1, 3, 7-nonatriene were emitted mainly from herbivore-damaged plants.[121]

In *Brassica* oils, triacylglycerols are the main constituents making up about 98% of the oils. The remaining nonglyceridic fraction consists of different lipophylic phytochemicals such as tocopherols, sterols, and sterol esters.[122] Similarly, in *Brassica* oils the remaining 2% consists of sterols, phospholipids, and sphingolipids. The major sterols were identified as stigmasterol,[123] sitosterol, campesterol, and cholesterol,[122] the phospholipids as phosphatidylethanolamine and phosphatidylcholine, and the sphingolipids as cerebrosides.[124]

For the purpose of human nutrition, a high ingestion of oleic acid, linoleic and linolenic acids is advantageous.[118] All polyunsaturated fatty acids including both linoleic and linolenic acids are essential because they cannot be synthesized by humans.[125] However, the type of fatty acids in the dietary fat is very important, being considered, for example, as one of the detrimental factors in colon cancer development. Fats containing omega 6-polyunsaturated fatty acids were found to enhance chemically induced colon cancer,[115] while omega-3-polyunsaturated fatty acids reduce it.[75, 115] Consumption of diet rich with canola fat may also alter the fatty acid composition of lipids of adipose tissue, muscle, kidney, and liver.[126] A diet high in *trans*–α–linolenic acid may increase plasma LDL/HDL cholesterol and total cholesterol/HDL-cholesterol ratios. Careful deodorization prevents the formation of *trans*-α-linolenic acid and may help to improve the diet.[117]

5 Carbohydrates

The type and concentration of free sugars influence the flavour of *Brassica* products.[127] Fructose, glucose, and sucrose are the major soluble sugars found in *Brassica*.[128] A comprehensive evaluation of the nutritive profiles of *Brassica* seed meals of yellow-seeded types (*B. napus*, *B. rapa*, *B. juncea*, and *B. carinata*) and conventional brown-seeded (canola) type showed that all contain sucrose (7.5% – 8.7%), oligosaccharides (2.3% – 2.5%), ash (6.9% – 7.0%), and non-starch polysaccharides (20.4% – 19.7%).[129] Fructose is the major sugar in the different types of *Brassica*, representing between 48.8 and 56.9% of the total sugar content in broccoli cvs. *Marathon* and *Senshi*, respectively, 48.7% (cv. *Mirandela*) and 53.8% (cv. *Murciana*) in the other cabbages. Glucose is the second major sugar, while sucrose represents a maximum of 20.5% in broccoli cv. *Shogun* and 11.1% in cv. *Murciana*.[127]

- **Dietary Fibre**

It is composed of non-starch polysaccharides[130] and is an important constituent in Brassicaceae vegetables, contributing to prevent colon cancer.[131] In white cabbage (*B. oleracea* var. *capitata*) dietary fibre represents one-third of the total carbohydrate content, the other two-third being low-molecular weight carbohydrates, including glucose (37%), uronic acid (32%), arabinose (12%), and galactose (8%).[132, 133] The dietary fibre content of 6 cultivars of white cabbage (*B. oleracea* var *capitata*) was evaluated finding that of the average total dietary fibre of

241 mg/g of dry matter, approximately 25% was soluble.[132] Dietary fibre content of other species was found to vary between 271 and 352 mg/g for the yellow-seeded *B. napus* and brown-seeded *B. napus*, respectively,[134] with intermediate values in other species, such as cauliflower (302 mg/g of D.W.), broccoli (330 mg/g of D.W.), and cabbage (226 mg/g of D.W.).[96]

6 Protein and Free Amino Acids

The defatted meal of *Brassica* oilseeds is a valuable source of protein for the livestock feed industry[135] and may constitute an important protein source for human nutrition thereby increasing the value of *Brassica* crops. However, the high temperatures and organic solvents used during the oil extraction process cause denaturation of proteins in *Brassica* meal.[56] Protein and free amino acid content in rapeseed meal have a high nutritive value, but the utilization of rapeseed/canola as a source of food-grade proteins for human consumption is still limited due to the presence of antinutrients such as glucosinolates, phytates, and phenolics.[113, 136, 137] Therefore, it is used only for animal feeding.[138] There is a variation in protein content in different groups of *Brassica*, such as *B. napus* seeds have higher protein solubilities than meals from *B. rapa* seeds. Meals with higher protein solubility values also have higher foaming capacity values.[139] Seeds of rape, *B. napus*, and related cruciferous oilseed crops, such as *B. campestris*, *B. juncea*, *B. carinata*, and *B. nigra* are rich sources of edible protein and rapeseed/canola meal, the by-product of the oil-extraction process, contains up to 42.7% – 50% protein.[129, 140]

The rape seed (*B. napus*) meal contains napin and cruciferin as storage proteins and oleosin as a structural protein associated with oil bodies.[138, 140] The 2S albumins or napins in oilseed rape and turnip rape are potential food allergens.[141] Free amino acids are involved in secondary plant metabolism and in the production of compounds which directly or indirectly play an important role in plant-environment interactions and human health. A total of 17 amino acids were identified (L-alanine, L-arginine, L-asparagine, L-aspartic acid, glycine, L-glutamic acid, L-glutamine, L-histidine, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine) in *B. oleracea var italica*.[5, 125] *S*-methylcysteine sulfoxide, a naturally occurring *S*-containing amino acid, is contained at high concentrations in *Brassica* vegetables such as broccoli and cabbage. Its cholesterol-lowering effects have been demonstrated in animals, observing a significant decrease of the serum level of LDL-C (14% decrease) following the oral administration of broccoli (*B. oleracea L.* var. *botrytis L.*) and cabbage (*B. oleracea L.* var. *capitata L.*).[142]

7 Indoles

Plants may respond to pathogen attack by producing phytoalexins.[143] Phytoalexins are a group of structurally diverse molecules[144, 145] that are generally non-specific in their antimicrobial activities.[145 145, 146] A number of phytoalexins have been isolated from crucifers (**Figure 2**).[147]

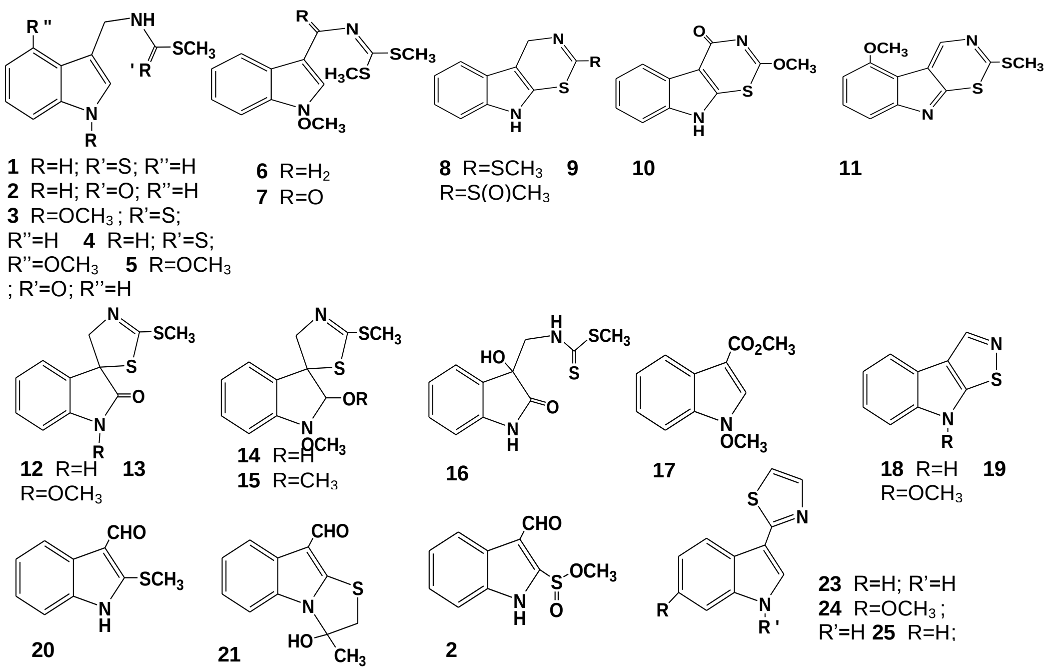

Figure 2 – Structures of cruciferous phytoalexins: **1:** brassinin, **2**: brassitin, **3:** 1-methoxybrassinin, **4:** 4-methoxybrassinin, **5:** 1-methoxybrassitin, **6:** 1-methoxybrassenin A, **7:** 1-methoxybrassenin B, **8:** cyclobrassinin, **9:** cyclobrassinin sulfoxide, **10:** cyclobrassinone, **11:** dehydro-4-methoxycyclobrassinin, **12:** spirobrassinin, **13:** 1-methoxyspirobrassinin, **14:** 1-methoxyspirobrassinol, **15:** 1-methoxyspirobrassinol methyl ether, **16:** dioxibrassinin, **17:** methyl 1-methoxyinodole-3-carboxylate, **18:** brassilexin, **19:** sinalexin, **20:** brassicanal A, **21:** brassicanal B, **22:** brassicanal C, **23:** camalexin, **24:** 6-methoxycamalexin, **25:** 1-methylcamalexin [147].

In *Brassica* indole phytoalexin (camalexin) synthesis is induced as a response to pathogen attack and ROS generating abiotic elicitors.[148, 149] These phytoalexins inhibit the growth of human cancer cells and thus may have a potential use as chemopreventive agents.[64] Several indole phytoalexins found in *Brassica* vegetables, brassinin, spirobrassinin, brassilexin, camalexin, 1-methoxyspirobrassinin, 1-methoxyspirobrassinol, and methoxyspirobrassinol methyl ether, have been found to possess significant antiproliferative activity against various cancer cells, while others, such as cyclobrassinin, spirobrassinin, brassinin also exhibited chemopreventive activity in models of mammary and skin carcinogenesis.[150]

Brassicaceae species contain a range of signalling and regulatory compounds known to be involved in general defence mechanisms activated by pathogen and herbivore attacks on plants.[151] These include salicylic acid, ethylene, H_2O_2, and jasmonic acid (an acid-derived oxylipin)[79] and signal peptides, such as systemin.[152-154] Some of these are bioactive compounds which exhibited anticancer activity in animals when added to experimental diets.[79] In particular, jasmonic acid and its derivatives, which represent the best characterized class of signal compounds, mediating the defence responses to wounding and herbivore attack in Brassicaceae,[155-159] have been proved to inhibit the proliferation of human prostate cancer cells, while not affecting normal human blood cells.[64, 160]

8 Phenylpropanoids, Flavonoids and Tannins

Phenylpropanoids, flavonoids and other minor compounds (**Table 3**) are considered to be among the health promoting compounds in Brassicaceae species.[161] Plant polyphenols are multifunctional, having diverse biological activities apart from acting as reducing agents.[162] Phenolics also contribute to the bitter, astringent, and unpleasant flavour of rapeseed, though the threshold of this unpleasant flavour is higher for individual phenolic compounds than for the mixture.[113] In spite of this they are considered to be beneficial and harmless components of rapeseed meal.

The contribution of *Brassica* vegetables to health improvement has generally been associated with their antioxidant capacity and, undoubtedly, phenolic compounds are the major antioxidants of *Brassica* vegetables.[89, 163, 164] Phenolics is a generic term which refers to a large number of compounds that can be classified in groups, namely, phenolic acids, flavonoids, isoflavonoids, lignans, stilbenes, and complex phenolic polymers.[11, 165]

As mentioned above, these antioxidants have proved to be good for human health and also useful as food preservatives.[166] Mustard seeds have a chemopreventive potential and enhance the antioxidant defence system. Their inclusion in the diet may very probably contribute to reducing the risk of cancer incidence in the human population.[167] A rapeseed phenolic extract has shown a stronger antioxidant activity than many artificial antioxidants[168] and exhibited a greater efficiency on a mole-to-mole basis than natural antioxidants such as vitamin C, vitamin E, and β-carotene.[162]

Table 3 – Variation of phenolics (μg/g) among different Brassicaceae vegetables on fresh weight basis

	Quercetin	Kaempferol	Apigenin	Lutein
Brassica oleracea L var. *italica* (Broccoli)	137 [a]	46 [a]	–	6.8 [c]
Brassica oleracea L var *botrytis* L (Cauliflower)	39 [a]	12 [a]	2 [a]	1.3 [c]
Brassica campestris var *chinensis* (Chinese cabbage)	390 [a]	96 [a]	45 [a]	0.2 [c]
Brassica rapa L. *subsp. sylvestris*	102 [b]	334 [b]	–	–
Brassica oleracea var *capitata* (white cabbage)	51 [a]	–	8 [a]	1.4 [c]

a = [92]; b = [161]; c = [101].

Species of the Brassicaceae family are generally rich in polyphenols. *Brassica rapa*[113] and *B. oleracea* L. var. *botrytis* contain a high amount of phenolic compounds.[169] Phenolic contents of several species have been reported, such as Chinese cabbage (1189±125µg/g), broccoli (822±89 µg/g), cauliflower (278±15µg/g), and white cabbage (153±21µg/g) on a fresh weight basis.[92, 168] In the case of broccoli, phenylpropanoids such as ferulic, sinapic, caffeic, and protocatechuic acid were reported to be the most abundant and important bioactive compounds.[170, 171] Four phenylpropanoids (caffeic, *p*-coumaric, ferulic, and sinapic acid) were identified in the water-soluble phenolic fraction of the leaves of oilseed rape (*Brassica napus* L. var. *oleifera*)[172] and gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, syringic, salicylic, *p*-coumaric, caffeic, ferulic, and sinapic acid were identified in kale (*B. oleraceae* L. var. *acephala* DC.).[173]

The main phenolics in rapeseed meal were determined to be sinapic acid which constitutes over 73% of its free phenolic acid content,[51] while apart from sinapic acid, rapeseed oil also contains vinylsyringol.[174, 175] An efficient peroxynitrite scavenger activity has been described for sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid), which has shown to contribute to the cellular defence against this powerful cytotoxic free radical, thus avoiding peroxynitrite-mediated disorders.[176] Besides the typical seed constituent sinapic acid, large amounts of choline esters of other phenolic acids have been detected in Brassicaceae species, for example, feruloyl- and isoferuloylcholine and hydroxybenzoic acid.[177] Brassicaceae plants accumulate glucose esters (1,6-di-*O*-sinapoylglucose), gentiobiose esters (1-*O*-caffeoylgentiobiose and 1,2,6-tri-*O*-sinapoylgentiobiose) of phenolic acids and kaempferol conjugates.[178]

Flavonoids are one of the most common and widely distributed groups of plant phenolics. Over 5000 different flavonoids have been described to date and they are classified into at least 10 chemical groups. Among them, flavones, flavonols, flavanols, flavanones, anthocyanins, and isoflavones are particularly common in the human diet.[179] As these compounds have interesting biological activities, these are being used in numerous medical treatments,[180, 181] connected to cancer-prevention[182, 183] and cardiovascular system protection, including inhibition of oxidative damage.[184, 185] At higher doses, however, flavonoids may act as mutagens, pro-oxidants that generate free radicals, and as inhibitors of key enzymes involved in hormone metabolism.[186]

Flavones are involved in various interactions with other organisms, microbes as well as insects and other plants.[187] Pharmacological activities have been described for various flavonoids (for example, quercetin, apigenin, catechins) which have shown an anti-inflammatory action by inhibiting cycloxygenase-2 and inducible nitric oxide synthase.[188] The flavonols quercetin, kaempferol, and isorhamnetin are among the flavonoid derivatives present in *Brassica* species.[53, 189-191] Two main flavonol glycosides, quercetin 3-*O*-sophoroside and kaempferol 3-*O*-sophoroside, are present in broccoli florets. Three minor glucosides of quercetin and kaempferol, isoquercitrin, kaempferol 3-*O*-glucoside, and kaempferol diglucoside, have also been detected. The quercetin and kaempferol glycosides were present in florets at a level of 43 μg/g and 94 μg/g of dry weight, respectively.[192] Glycosylated kaempferol derivatives from the external leaves of tronchuda cabbage (*B. oleracea* L. var. *costata* DC) have been reported.[193]

Total flavonoid content in Chinese cabbage, broccoli, cauliflower, and white cabbage is 944 µg/g, 316 µg/g, 172 µg/g, and 102 µg/g, on a fresh weight basis, respectively.[92] The accumulation of derivatives of flavonols such as quercetin in *Camelina sativa*; quercetin and kaempferol in *Crambe hispanica* var. *glabrata*; quercetin, kaempferol, and isorhamnetin in *Brassica napus*; kaempferol and isorhamnetin in *Sinapis alba* is reported.[190] The constitutive flavonoids of *B. napus*, isorhamnetin-3-sophoroside-7-glucoside and kaempferol-3,7-diglucoside, are effective deterrents of armyworm.[44] Analysis of *B. alba* extracts revealed the presence of 3,5,6,7,8-pentahydroxy-4-methoxy flavone in shoots, as well as 2,3,4,5,6-pentahydroxy chalcone and 3,5,6,7,8-pentahydroxy flavone in roots and root exudates. Apigenin was also found in the shoots and roots.[194]

Anthocyanins are potent antioxidants and consequently may be chemoprotective.[195] Brassicaceae plants provide a variety of anthocyanins. Cauliflower and red cabbage showed differences in their anthocyanin profiles: cyanidin-3,5-diglucoside was absent in cauliflower, while it was well represented in red cabbage, together with the characteristic anthocyanin of *Brassica* genus, cyanidin-3-sophoroside-5-glucoside. The *p*-coumaroyl and feruloyl esterified forms of cyanidin-3-sophoroside-5-glucoside were predominant in cauliflower, while the sinapoyl ester was mostly present in red cabbage.[196] Red pigmentation of red cabbage is caused by anthocyanins. Red cabbage contains more than 15 different anthocyanins which are acylglycosides of cyaniding.[197] Red radish (*Raphanus sativus* L.) contains significant amounts of anthocyanins of which 12 acylated anthocyanins were isolated and analyzed spectroscopically to determine their structure. Six of these were identified as anthocyanin glycosides with 1 or 2 phenylpropanoids.[198] Total proanthocyanidins content in brocolli was found to be 12 µg/g and 7 µg/g in cauliflower, calculated over fresh weight. [92]

Five lignans, 5 neolignans, 2 sesquilignans, and 1 dilignan were identified in a phytotoxic extract of *Brassica fruticulosa*.[199] These compounds exhibited interesting antimicrobial, antifungal, and/or herbicidal activities that are believed to participate in plant defence mechanisms.[200] These compounds also have cancer-preventive effects.[65]

Tannins have an adverse effect on the nutritive value of rapeseed meal proteins or isolated proteins.[201] These compounds suppress the availability of essential amino acids[202] and may form complexes with essential minerals, proteins, and carbohydrates.[203] Tannins have also a profound inhibitory effect on the digestion of carbohydrates and proteins in particular.[204] In Brassicaceae vegetables different amounts of tannins have been reported.[58] Inositol hexa-phosphate (phytic acid) and condensed tannins are reported in *B. carinata*,[78] both of which play an important role in iron binding.[203] Cabbage and turnip contain various amounts of phytic acid, tannic acid, and/or oxalic acid. Tannic acid was found at 12.66 mg/g (fresh weight basis) in cabbage. Levels of both tannic acid and phytic acid can be significantly ($p < 0.05$) reduced by different blanching methods.[205] The total amount of tannins in rapeseed/canola hulls ranged from 19.13 to 62.13 mg/g of oil-free hulls. Insoluble tannins predominated in canola/rapeseed hulls and comprised from 70 to 95.8% of total tannins present. The amounts of sodium–dodecyl-sulphate-extractable tannins were comparable to those of soluble tannins but constituted only 4.7–14.1% of insoluble tannins present.[206]

9 Glucosinolates

Sulfur-containing phytochemicals of 2 different types are present in *Brassica* (Cruciferae) vegetables (cabbage, broccoli, etc.): glucosinolates and *S*-methyl cysteine sulfoxide. Glucosinolates (**Figure 3**) are thioglucosides containing a cyano group and a sulfate group.[207]

Glucosinolates are derived from amino acid biosynthesis (**Figure 1**) and are important secondary metabolites in Brassicaceae family, involved in plant defence against pests and diseases.[207] For example glucoiberin, glucoraphanin, glucoalyssin, gluconapin, glucobrassicanapin, glucobrassicin, gluconasturtiin, and neoglucobrassicin are health promoting compounds found in broccoli inflorescences (*B. oleracea* L., var. *italica,* cv. Marathon).[55]

These compounds have both positive and negative nutritional effects,[208] appearing to possess anticarcinogenic properties, but also quite different toxic effects.[10] The effects of specific glucosinolate degradation products on individual organisms vary and are not always known. If used in excessive quantity, many of these compounds can be highly toxic.[209]

Glucosinolates and their concentrations vary among the different groups of Brassicaceae (**Table 4**).[210] In Brussels sprouts, cabbage, cauliflower, and kale, the predominant glucosinolates were found to be sinigrin and glucobrassicin. Brussels sprouts also had significant amounts of gluconapin.[211] The predominant glucosinolates in broccoli are 4-methylsulfinylbutyl glucosinolate (glucoraphanin),[212] 3-butenyl glucosinolate (gluconapin), and 3-indolylmethyl glucosinolate (glucobrassicin).[211] Cruciferous vegetables of the *Brassica* genus (for example, Brussels sprouts, cauliflower, and broccoli) contain high levels of an indolylmethyl glucosinolate commonly known as glucobrassicin.[213] A great number of glucosinolates have been identified in *B. oleracea* var. *capitata f. alba*, namely glucoiberin, progoitrin, epiprogoitrin, sinigrin, glucrafanin, gluconapoleiferin, glucoalysin, gluconapin, 4-hydroxybrassicin, glucobrassicanapin, glucobrassicin, gluconasturein, methoxyglucobrassicin, and neoglucobrassicin.[49]

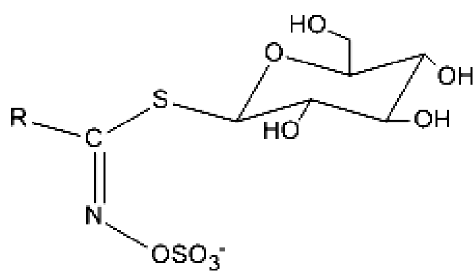

Figure 3 – Basic structure of glucosinolates

The major glucosinolates detected in different varieties of *B. oleracea* were 2-propenyl, 3-methyl-sulphinylpropyl and indol-3-yl-methyl, which accounted for an average of 35, 25, and 29%, respectively of the total glucosinolate content, while in *B. rapa*, but-3-enyl represented 86% of the total, with pent-4-enyl and 2-phenylethyl as the other major glucosinolates. The average total glucosinolate content of the flower buds was determined to be 2518 μmol 100 g^{-1} dry wt. in troncha (*B. oleracea* var. *tronchuda*) and 4979 μmol 100 g^{-1} dry wt in nabo (*B. rapa*), which is much higher than the highest amounts reported for broccoli (*B. oleracea* var. *italica*).[214] As in other Brassicaceae seeds and plants, rapeseed contains up to 5% of glucosinolates, which are partially decomposed during rapeseed processing or storage.

When plant material is crushed, as in food preparation or chewing, a thioglucosidase–mediated autolytic process is initiated, generating indole-3-carbinol, glucose, and thiocyanate.[215] These, together with other important degradation products, such as isothiocyanates, vinyl-oxazolidinethione, and nitriles, contaminate the crude rapeseed oils, impairing their hydrogenation and transesterification and ultimately may be harmful to human consumption.[216]

The main glucosinolate breakdown products of *Brassica* vegetables are the sinigrin breakdown product 1-cyano-2,3-epithiopropane, the gluconapin hydrolysis product 3-butenyl isothiocyanate, the glucobrassicin metabolite ascorbigen and low concentrations of other indole glucosinolate-derived hydrolysis products such as neoascorbigen and 3,3`-di-indolylmethane.[217] Rapeseed meal, a by-product of rapeseed oil production, also contains glucosinolates which together with phytic acid contribute to its anti-nutritional properties.[218, 219]

Table 4 – Variation of glucosinolate contents (μg/g) among different Brassicaceae vegetables on dry weight basis

	Cabbage	Broccoli	Brussels sprouts	Cauliflower	Kale
Glucoiberin	2289 ± 380 [a]	697 ± 127 [a]	42 ± 84 [c]	–	3455 ± 591 [d]
Glucoraphanin	17 [a]	3208 ± 528 [a]	3099 [c]	218 ± 131 [c]	1361 [b]
Progoitrin	452 ± 20 [a]	1017 ± 68 [a]	2922 [c]	120 ± 40 [c]	524 [b]
Gluconapin	472 ± 26 [a]	96 ± 37 [a]	4654 [c]	111 ± 74 [c]	372 ± 37 [c]
Sinigrin	3443 ± 939 [a]	35 ± 143 [c]	3261 [c]	3332 ± 36 [c]	3400 ± 322 [d]
Glucoalysin	–	90 ± 45 [c]	90 ± 45 [c]	–	–
Glucoerucin	–	–	–	–	1206 [b]
Glucobrassicin	1315 ± 13 [a]	1566 ± 130 [a]	1431 ± 89 [c]	715 ± 716 [c]	353 ± 1029 [c]
Neoglucobrass-icin	38 ± 19 [a]	458 ± 29 [a]	95 ± 48 [c]	95 ± 95 [c]	353 [d]
4-Methoxyglu-cobrassicin	214 ± 24 [a]	124 ± 5 [a]	–	–	–

a = [221]; b = [222]; c = [211]; d = [223].

* Calculation is made by conversion of μmol to μg on dry weight basis.

Goitrin, a naturally occurring compound in cruciferous vegetables, can easily be nitrosated if in contact with nitrites in gastrointestinal conditions, yielding the mutagenic compound N-nitroso-oxazolidone, with loss of sulphur.[220] Additionally, goitrin which is a decomposition product of progoitrin (**Figure 4**) is known to be strongly goitrogenic, inhibiting the synthesis of thyroid hormones, thyroxine, and tri-iodine-thyronine by a selective binding of iodine which prevents iodine intake by the thyroid gland.[63]

The other decomposition products of glucosinolates, as mentioned before, are thiocyanates, isothiocyanates and oxazolidine-2-thiones (**Figure 5**),[50, 224] and have also been shown to be goitrogenic. The benzyl-, phenethyl-, allyl-isothiocyanate, and sulforaphane are formed through the hydrolysis of their naturally occurring precursor glucosinolates, glucotropaelin, gluconasturtiin, sinigrin and glucoraphanin, respectively, by myrosinase.[225]

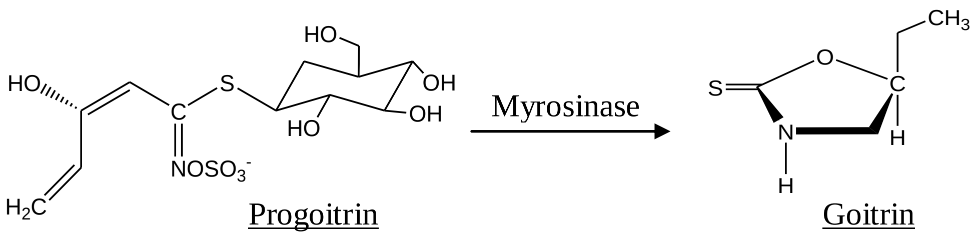

Figure 4 – Conversion of progoitrin to goitrin by myrosinase

However, under certain conditions, the glucosinolate aglycones may yield a nitrile rather than an isothiocyanate. Nitriles such as *S*-l-cyano-2-hydroxy-3-butene and 1-cyano-2-hydroxy-3,4-epithiobutane, are the most toxic of the normal glucosinolate hydrolysis products, with a human lethal dose of 170 and 178 mg/kg, respectively.[218] These negative effects of glucosinolates have led to research directed at finding methods to reduce the glucosinolate content in the seeds of some *Brassica* crops.[47] Other processes intended to avoid toxicity of the meal include heat treatment of the seeds prior to removal of the oil.

This inactivates myrosinase and subsequent breakdown of glucosinolates when the meal is consumed.[218] High or low glucosinolate contents of the seed of some varieties of *B. napus* correlate positively with glucosinolate levels in the roots, at least during the early stages of in vitro plant development.[43]

Glucosinolates are also responsible for the bitter acidic flavours of *Brassicacea* species[76] and the hydrolysis by-products of glucosinolates mentioned above, such as isothiocyanates, nitriles, and thiocyanates, are responsible for the hot and pungent taste of the mustard that is often objected to by consumers.[207] Many of these degradation products are volatile[226] and also play an important role in the characteristic aroma or off-odour of Brassicacae.[84] A great deal of research has been carried out on the volatiles of these species.

Cruciferous vegetables, for example, have been reported to contain substantial quantities of isothiocyanates.[227] Volatiles and semi-volatiles from *B. oleracea* L. var. *botrytis* seeds were identified as cyanides such as 4-(methylthio) butyl-cyanide, 3-(methylthio) propyl cyanide, and isothiocyanates such as 4-(methylthio) butyl-isothiocyanate.[228] In *B. rapa* var. *perviridis*, 6 isothiocyanates were detected in the steam volatiles and identified as sec-butylisothiocyanate, 3-butenylisothiocyanate, 4-pentenylisothiocyanate, benzyl-isothiocyanate, 2-phenylethylisothiocyanate, and 5-methylthiopentylisothiocyanate. Three nitriles were also detected and identified as 2-methyl-5-hexenenitrile, 3-phenylpropiononitrile, and 6-methylthiohexanonitrile.[84] In *Brassica oleracea* var. *Botrytis* 35 volatile and semi-volatile constituents were detected.[228]

Dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, hexanal, 3-*cis*-hexen-1-ol, nonanal, ethanol,[228, 229] and hex-3(Z)-enol were identified as major constituents representing, respectively, 30.2, 24.2, and 21.7% of the volatiles.[228] Various interesting bioactivities have also been reported for hydrolysis and breakdown products of glucosinolates,[43, 230] such as strong bactericidal, antifungal properties,[218, 231, 232] and health promoting effects for plants and humans.[47, 224] Some of these glucosinolates have a potential application in the industry, for example, an aqueous extract of *B. nigra* seeds might be included in industrial biofilms as an antimicrobial agent.[233] The breakdown products of glucosinolates assist in the activity of important naturally occurring, direct- acting antioxidants such as tocopherols and also enhance the synthesis of glutathione, one of the most abundant intracellular direct antioxidants.[234] Working on rapeseed oil cake (*Brassica campestris* L. *subsp. napus*), different antioxidant compounds (indolacetonitrile, S-1-methoxy-1-(3,5-dimethoxy-4-hydroxyphenyl) ethane, 4-hydroxy-indol-acetonitrile, and 4-hydroxy-phenyl-acetonitrile) were isolated, which showed a strong antioxidant activity as evaluated by the ferric thiocyanate method.[235]

Certain glucosinolates, particularly the isothiocyanates and nitriles, have been shown to modify both xenobiotic metabolizing enzymes and induce cell cycle arrest and apoptosis. It is likely that a combination of these responses explains the chemo-preventive characteristics of *Brassica* and that a combination of different cruciferous vegetables could provide optimal protection.[225, 236] The isothiocyanate chemopreventive activity could be due to its powerful inhibition of different enzymes such as glutathione *S*-transferases (GSTs) in humans.[237, 238] Another potential cancer-blocking action, which was described for both intact and thioglucoside glucohydrolase-treated glucosinolates, as assessed by induction of GSTs activity, was found to be dependent on the nature of the side chain of the parent glucosinolate.[239]

Another naturally occurring isothiocyanate, sulforaphane, that is present in *Brassica* vegetables has been shown to block the formation of tumours[240] and together with 7-methylsulfinyl-heptyl isothiocyanates in broccoli (*B. oleracea* var. *italica*) extract exhibited an inhibitory effect on cancer cell invasion and matrix metallo-proteinase-9 activity in human breast cancer cells[213] and lowers the probability of acquiring colon and rectal cancers.[241] It was also proved to inhibit *Helicobacter pylori* infection, blocking gastric tumour formation. This suggests that broccoli consumption could prevent chronic atrophic gastritis induced by *H. pylori* infection and, thus, this type of stomach cancer.[242] Naturally, the wide range of glucosinolates content among different groups of *B. oleracea* would result in significant differences in their health-promoting properties.[211]

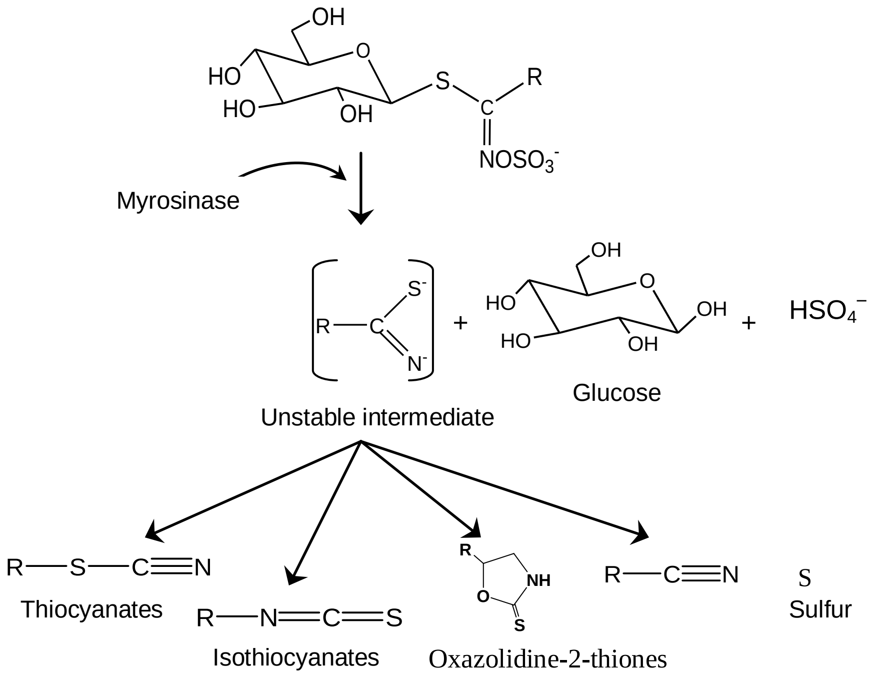

Figure 5 – Glucosinolate degradation

Indole-3-carbinol (I-3-C) is another glucosinolate breakdown product found in vegetables of the *Brassica* genus (cabbage, broccoli sprouts, Brussels sprouts, cauliflower, bok choy, and kale). Some research points to this compound as a promising anticancer agent against prostate cancer and reducing the incidence and multiplicity of mammary tumours.[243, 244] Coinciding with these studies, oral administration of I-3-C has been shown to have a possible beneficial effect on estrogen metabolism in humans and epidemiological studies support the claim that high intakes of I-3-C may have a broad chemo-preventive effect.[245] Conversely, [5,6,11,12,17,18]-hexahydrocyclonona-[1,2-b:4,5-b:7,8-b]-tri-indole (CTr), a major digestive product of indole-3-carbinol, has been proved to exhibit strong estrogenic activities increasing proliferation of estrogen-dependent breast tumour cells. Thus, the contribution of CTr to the cancer preventive or cancer-promoting effects of I-3-C remains to be established.[246] In plants, levels of secondary metabolites, such as glucosinolates are controlled by a number of factors. Although it is possible to increase levels of glucosinolates in plants by genetic manipulation, in order to enhance a particular pharmacological benefit, such a step would be premature and must await a more thorough understanding of the extremely complex interactions of these compounds and their metabolites.[50]

10 Conclusion

Brassica vegetables represent a major part of the human diet all over the world providing nutritionally significant constituents, such as phenolic compounds, vitamins, fibres, soluble sugars, minerals, fat, and carotenoids. Cruciferous vegetables are a source of some very promising chemopreventive dietary constituents, which may protect against free radical damage and LDL oxidation implicated in the pathogenesis of cardiovascular diseases, as well as DNA damage and cancer. This might be useful information from the point of view of identifying appropriate raw materials, rich in these protective components, for the development of safe food products and additives with appropriate antioxidant properties. As mentioned above, *Brassica* plants are rich in many metals including calcium and iron-containing compounds. However, there is substantial variation both within and between subspecies, which suggest, a difference in potential health benefits depending on genotype, as well as on the growth conditions and environment. This review provides a massive body of evidence supporting the nutritional value of *Brassica* vegetables and should ultimately lead the population to better food choices.

11 Future Perspectives

Many anti-cancer agents are of plant origin, but their actual function or the mechanism behind the role they play in the plant has not yet been fully elucidated. For example, plant-derived molecules with known roles in plant cell death may be novel candidates for use in clinical oncology. But a better understanding of the molecular and cellular mechanism of action of such compounds and their structure-activity relationships is necessary for the development of new derivatives of these molecules with more favourable chemopreventive activities. Different classes of anti-cancer compounds merit continued research at a basic and pharmacological level in order to yield novel chemotherapeutic agents. However, in order to correctly evaluate the effect of such compounds in food, it is necessary to bear in mind that some constituents such as phenolic acids, tannins, and other anti-nutritional compounds may form complexes with nutritionally important compounds, reducing their bioavailability and thus lowering the nutritional value of *Brassica* products. Additional studies are needed to determine the amount of isothiocyanates or their metabolites that reach target tissues, and the concentration needed to exert biological effects.[225] Further elucidation of the protective mechanisms of food and the identification of active constituents is needed.

Enhancing the phytonutrient content of plant foods through selective breeding or genetic improvement is a powerful tool for dietary disease prevention. However, most, if not all, of these bioactive compounds confer a bitter, acid, or astringent taste to the food which is rejected by most consumers. Moreover, in the past, some of these compounds have even viewed as plant-based toxins and, as a result, the food industry routinely removes these compounds from plant foods through selective breeding and a variety of debittering processes. This poses a dilemma for the designers of functional foods because increasing the content of bitter phytonutrients for health may clash with consumer choices. Studies on phytonutrients and health, taking sensory factors and food preferences into account, constitute an important area of research.

Another aspect of these valuable *Brassica* vegetables that deserves full attention is the edaphic conditions in which they are grown.

These plants can be biofortified by growing them in a high mineral-containing medium, attaining high levels of nutritionally important minerals that can be used to produce dietary supplements. But this advantage which is due to their metal tolerance (and allows their use for phytoremediation as previously explained) can be negative as observed in crops that are irrigated with polluting metals. The excessive heavy metals (macro or micro nutrients in excess) and plant and human pathogenic microbes concentrated in the soil from this water cause stress conditions for plants. Quality parameters of *Brassicacea* vegetables are very susceptible to great changes with these stress conditions which produce different effects on the levels of *Brassica* vegetables metabolites, affecting their flavour and leading to the changes in nutritional value. Studies are needed to clarify the route of exposure, mechanisms of sensitization, and clinical importance of these phenomena.

Another question about cruciferous vegetables is their flavonoid content. Epidemiological data indicate that the present rate of consumption of these vegetables is beneficial. However, earlier studies also raised the question on the advantages of recommending an increased consumption of *Brassica* vegetables and/or phytochemical supplements. One of the reasons for this lies in the flavonoid content of these vegetables which, as explained above, is quite high in some of the species. Unfortunately, the potentially toxic effects of excessive flavonoid intake are still largely ignored. It is known that at high doses, flavonoids may act as mutagens, that is, pro-oxidants that generate free radicals, so that their adverse effects may well outweigh their beneficial ones. It is imperative that further research be conducted to learn more about the toxicological properties of flavonoids, apart from other putative health promoting compounds in *Brassica* vegetables, thus clarifying the balance of potential adverse and beneficial effects included in their mechanisms of action.