



TITLE: On farm and fresh produce management

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## 9. On farm and fresh produce management

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### 9.1 Introduction

Over recent years a range of epidemiological and other studies have indicated that a diet rich in fruit and vegetables offers considerable health benefits [19]. These beneficial health effects have been attributed to the presence of bioactive plant secondary metabolites or “phytochemicals” found in vegetables and fruits. Phytochemicals may be defined as “non-nutrient chemicals found in plants that have biological activity against chronic diseases” [86]. In addition a number of antioxidant vitamins including carotenes,  $\alpha$ -tocopherol and ascorbate, as well as minerals such as selenium (Se), have been implicated in reduced risk of cardiovascular disease and several cancers. The World Health Organization (WHO) has identified low fruit and vegetable intake as one of the top 10 risk factors contributing to mortality. WHO recommendations set guidelines for a minimum intake of 400g of fruit and vegetables per day (excluding potatoes and other starchy tubers) for the prevention of chronic non-communicable diseases such as heart disease, cancer, diabetes and obesity as well as for the prevention and alleviation of micronutrient deficiencies especially in less developed countries [19, 46, 50]. In many countries actual consumption of fruit and vegetables is well below the recommended minimum intake. For example in the UK over 70% of adults consume less than the recommendation of 5 portions of fruit and vegetables per day [61].

Numerous peer reviewed publications support a protective role for plant phenolic compounds (especially flavonoids); isothiocyanates from Brassica species; polyacetylenes from carrot (*Daucus carota*) and flavonols and cysteine sulfoxides from onion (*Allium cepa*) in human health (reviewed in [29, 39, 89, 190]) , and these crops will form the basis for this review. Onion (*Allium cepa*) is believed to be the major source of human flavonol intake. It is one of the most important vegetable crops globally with an estimated annual production of over 72 million tonnes. Estimated world production of Brassicas in 2009 was almost 71 million tonnes, whilst carrot and turnip production was almost 28 million tonnes [45].

The Brassicaceae (Cruciferae) include a number of important cultivated crops including various members of the species *Brassica oleracea* such as broccoli (*Brassica oleracea* var. *italica*), cauliflower (*Brassica oleracea* var. *botrytis*), cabbage (*Brassica oleracea* var. *capitata*), kale (*Brassica oleracea* var. *acephala*) and Brussels sprouts (*Brassica oleracea* var. *gemmifera*). In addition the group includes turnip (*Brassica rapa* subsp. *rapa*), oriental cabbage (*Brassica rapa* subsp. *pekinensis*); as well as oil seed rape (*Brassica napus* subsp. *oleifera*, swede (*Brassica napus* subsp. *napobrassica*), radish (*Raphanus sativus*), watercress (*Nasturtium officinale*), mustards (*Brassica juncea* and *Sinapis alba*), horseradish (*Armoracia rusticana*) and rocket (*Eruca sativa*). A characteristic feature of the Brassicaceae is the production of glucosinolates, although glucosinolates are also produced in other dicot plant families. Of over 120 chemically distinct glucosinolates identified in plants, the majority occur in the Brassicaceae [44]. Glucosinolate breakdown products of the Brassicaceae have been the focus of extensive research since the discovery of potent anti-cancer activity of the isothiocyanates [138], particularly sulforaphane – the isothiocyanate breakdown product of glucoraphanin which is found at high levels in broccoli. In the intact plant cell glucosinolates and the enzyme myrosinase (thioglucosidase, EC 3.2.1.147, previously EC 3.2.3.1) are physically separated, with myrosinase sequestered in the vacuoles of specialised myrosin cells [5, 111]. Following cellular disruption the glucosinolates are released and hydrolysed by endogenous myrosinase into a range of breakdown products including isothiocyanates, nitriles, and to a lesser extent thiocyanates, epithionitriles and oxazolidines. The glucosinolate breakdown product formed is dependant on the initial glucosinolate, pH, availability of ferrous ions and the activity of epithiospecifier protein (ESP) [60]. Alkenyl and aromatic glucosinolates (Table 9.1) can form stable isothiocyanates - or oxazolidines-2-thiones if the side chain contains a hydroxyl group. At lower pH, lower temperature, and higher ferrous ion concentration the formation of nitriles rather than isothiocyanates is favoured [104, 109]. Epithiospecifier protein (ESP), a heat sensitive co-factor of myrosinase, directs glucosinolate hydrolysis towards nitrile, rather than isothiocyanate, formation [102, 103]. The indole or indolyl glucosinolates predominantly form nitrile derivatives such as indole-3-carbinol or unstable isothiocyanates which degrade to produce the corresponding alcohol and a thiocyanates ion [109]. A number of Brassicas including radish (*Raphanus sativus*), white mustard (*Sinapis alba*), horseradish (*Armoracia rusticana*) and daikon (*Raphanus sativus* var. *niger*) produce isothiocyanates only, whilst other Brassicas produce both

isothiocyanates and nitriles. Since the ratio of isothiocyanate to nitrile formation is highly heritable it is likely that this trait is related to differences in the presence and expression of ESP or an ESP like protein [103, 110]. The isothiocyanates are especially relevant in human health as they are more biologically active than nitriles [102]. Sulforaphane, the isothiocyanate breakdown product of the glucosinolate glucoraphanin (which is present at high levels in broccoli) is one of the most potent dietary anti-carcinogens described to date. Iberin, the isothiocyanate breakdown product of glucoiberin, has been reported by several authors to show anti-carcinogenic properties [110, 152, 179]. In addition some of the indolyl glucosinolates found in broccoli are broken down to form indole compounds such as indole-3-carbinol which are subsequently metabolized to putatively anti-carcinogenic compounds such as 3,3'-di-indolylmethane (DIM). Major glucosinolates described in broccoli and their corresponding isothiocyanate breakdown products are summarized in Table 9.1. Phenolic compounds found in broccoli include flavonols such as quercetin and kaempferol glycosides, hydroxycinnamoyl derivatives and chlorogenic acid. Anthocyanins are also found and accumulate to higher levels in purple broccoli [115]. Other bioactive compounds found in the Brassicaceae include the mineral selenium (Se), flavonoids such as quercetin and kaempferol, S-methyl cysteine sulfoxides and 1,2-dithiole-3-thiones and antioxidant vitamins including vitamin C, [3-carotene, lutein, and  $\alpha$ -tocopherol [49]. Other nutritional compounds found in broccoli include thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), vitamin B6, folate, calcium, iron, magnesium, phosphorus, potassium and zinc [173].

Carrot (*Daucus carota*) is a member of the Apiaceae (or Umbelliferae) family which also includes celery (*Apium graveolens* var. *dulce*), fennel (*Foeniculum vulgare*), parsnip (*Pastinaca sativa*), dill (*Anethum graveolens*), caraway (*Carum carvi*) cumin (*Cuminum cyminum*), and parsley (*Petroselinum crispum*). Uncultivated or non edible species include wild carrot / Queen Anne's lace (*Daucus carota*), hogweed (*Heracleum sphondylium*) cow parsley / wild chervil (*Anthriscus silvestris*) and poison hemlock (*Conium maculatum*). In addition to the familiar orange carrot white, yellow, purple, red and black varieties also occur. The nutritional and phytochemical content of carrots varies depending on type with different levels and profiles of carotenoids ( $\alpha$  and [3-carotene, lycopene, lutein); polyacetylenes and anthocyanins found in different coloured carrots [107, 169]. Nutritional compounds found in carrot include  $\alpha$ , and [3-carotene, vitamin A, thiamin

(vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), vitamin B6, vitamin C, calcium, iron, magnesium, phosphorus and potassium [173]. Beneficial health effects associated with carrot consumption include reduced incidence of lung and breast cancer, enhanced immune response, increased levels of serum antioxidants and improved vitamin A concentrations (reviewed in [107]). Important phytochemicals found in carrot include the polyacetylenes falcarinol, falcarindiol and falcarindiol-3-acetate; and the isocoumarin 6-methoxymellein (6-MM). Falcarinol (synonym: panaxynol, (9Z)-heptadeca-1,9-dien-4,6-dien-3-ol) is the most potent and best studied of the carrot polyacetylenes [79, 192]. Falcarindiol has recently been identified as the main compound responsible for the bitter off flavour in fresh and stored carrots. Black and purple carrot varieties accumulate 5 major cyanidin based anthocyanin pigments, three of which are acetylated (cyanadin 3 – sinapoylxylosylglucosylgalactoside, cyanadin 3 – feruloylxylosylglucosylgalactoside and cyanadin 3 -p-coumaroylxylosylglucosylgalactoside); and two of which are nonacylated (cyanadin 3-xylosylgalactoside and cyanadin 3-xylosylglucosylgalactoside). A number of anthocyanins have been reported to show anti-carcinogenic activity and in a recent study a black carrot anthocyanin-rich extract was shown to inhibit proliferation of two human cancer cell lines [122]. The acylated anthocyanins are thought to show enhanced bioactivity and sweet potato (*Ipomoea batatas*) acylated anthocyanins show strong anti-mutagenic, radical scavenging, anti-hypertensive and anti-hyperglycemic effects [101, 122].

Onions (*Allium cepa*) belong to the species *Allium* which also contains vegetables such as shallots (*Allium cepa* var. *aggregatum*), scallion / Welsh onion (*Allium fistulosum*), garlic (*Allium sativum*), wild garlic (*Allium ursinum*), leek (*Allium porrum*), and chives (*Allium schoenoprasum*). Nutritional components in onion include thiamin (vitamin B 1), riboflavin (vitamin B2), niacin (vitamin B3), vitamin B6, folate (vitamin B9), vitamin C, calcium, iron, magnesium, phosphorus, potassium and zinc [173]. Two classes of phytochemical found in onion – the sulphur containing alk(en)yl sulfoxides and the flavonols - are believed to show health promoting activity. The main sulphur containing compounds found in onion are isoalliin (S-trans-prop-1 -enyl cysteine sulfoxide, PeCSO); propiin (S-propyl- cysteine sulphoxide, PCSO); and methiin (S-methyl- cysteine sulphoxide, MCSO). The main flavonols found in onion are quercetin, quercetin 4'-glucoside, quercetin 3,4'-diglucoside, kaempferol and kaempferol glucosides. Isorhamnetin and rutin have also been identified in some cultivars [97, 161]. Quercetin has been demonstrated to inhibit growth of tumours cells containing type II

estrogen binding sites including breast, colon, ovarian, leukaemia, gastrointestinal and meningioma cancer cells (reviewed in [129]). The precise mode of action is unclear but there is evidence that quercetin can function as an antioxidant, radical scavenger and chelating agent. Antibiotic and antifungal activities of onion are thought to be primarily attributable to isoalliin (PeCSO), whilst anti-asthmatic properties have been attributed to thiosulphonate compounds. Protective effects against cardiovascular disease and stroke are thought to be due to reduced platelet aggregation and vasoconstriction mediated by quercetin and alkyl-propenylcysteine sulfoxide compounds [7, 39].

There is overwhelming evidence that the levels of bioactive compounds in a plant food crop is not fixed and can vary substantially depending on how the crop is grown. Some of these factors such as temperature, irradiation, soil type or stress treatments would be difficult or uneconomic to use as practical strategies to increase desired plant phytochemicals in field grown fruits or vegetables. However factors such as cultivar selection, fertilizer regime and post-harvest treatment could readily be incorporated into existing production practices to produce crops which are optimised in phytochemical content. A number of other treatments such as water stress, salinity and temperature although not easily applicable to field crops may find future applications in greenhouse grown crops such as lettuce or salad leaves, tomatoes and herbs where inputs are more easily controlled.

## **9.2 Pre-harvest factors affecting phytochemical content**

### **Crop and cultivar:**

The phytochemical profile of a plant is strongly dependant on genetic components and the range, type and level of individual bioactive compounds varies between different species of the same genus, between different groups of the same species or sub-species, and between different accessions or cultivars. A number of detailed studies have been carried out to evaluate the content of bioactive compounds in *Brassica oleracea* groups including broccoli, Brussels sprouts, cabbage, cauliflower and kale grown individually or under uniform cultural conditions [22, 23, 87, 126, 155-157, 160, 174, 175, 178]. In broccoli (*Brassica oleracea* var. *italica*) the predominant glucosinolates detected are glucoraphanin and glucobrassicin. In contrast in turnip (*Brassica rapa* subsp. *rapa*) gluconasturtiin and progoitrin have been reported as the predominant glucosinolates in the root,

with gluconapin predominant in the leaf (consumed as turnip greens) [20, 76, 92, 126]. In Brussels sprouts, cabbage, cauliflower and kale the predominant glucosinolates reported are sinigrin, glucoiberin and glucobrassicin [87, 178]. In a recent study examining a range of Brassica vegetables grown in a single location the level of total glucosinolates found varied from 14 to 625  $\mu\text{mol}/100\text{g}$  FW and the overall level of total glucosinolates was highest in Brussels sprouts. Levels of glucoraphanin, the precursor of sulforaphane, ranged from 0 to 141  $\mu\text{mol}/100\text{g}$  FW and were higher in broccoli (27 to 141  $\mu\text{mol}/100\text{g}$  FW). Cauliflower and kohlrabi contained relatively low levels of glucosinolates [178]. Predominant glucosinolates reported in a range of commonly consumed Brassicas are summarized in Table 9.2. Levels of myrosinase activity have been reported to be higher in broccoli, Brussels sprouts and cauliflower than in other groups such as kale and cabbage [26].

Considerable variation within the *B. oleracea* var. *italica* group has also been reported. Most studies report glucoraphanin or less commonly glucobrassicin as the predominant glucosinolate in broccoli [22, 71, 75, 83, 87, 131, 147, 155, 177]. In the study of Schonhof *et al.* (2004) glucobrassicin was the predominant glucosinolate in the purple variety Viola [155], whilst neoglucobrassicin was the predominant glucosinolate detected in 3 cultivars including cv. Marathon in the study of Vallejo *et al.* (2003) [174]. In a detailed study which evaluated the glucosinolate profile of 50 broccoli accessions grown under uniform cultural conditions using HPLC, Kushad *et al.* (1999) reported glucoraphanin as the predominant glucosinolate, with levels ranging from 0.8  $\mu\text{mol}/\text{g}$  DW in the cultivar EV6-1 to 21.7  $\mu\text{mol}/\text{g}$  DW in the cultivar Brigadier – a 27 fold difference [87]. Cultivated forms of *B. oleracea* are believed to have originated in the middle east, from where they were introduced to Italy which is regarded as the centre of diversity for *botrytis* and *italica* groups [58, 100]. Some studies have indicated that wild Brassicas contain higher levels of glucosinolates than cultivated varieties [112]. It should be noted that cultivated broccoli varieties within the *B. oleracea* var. *italica* group show considerable morphological diversity (Figure 9.1). In a study in which a green heading type (cv. Ironman), a white sprouting type (cv. TZ4039) and 3 purple sprouting types (cvs. Red Admiral, TZ5052 and TZ6002) were compared, levels of total glucosinolates ranged from 62  $\mu\text{mol}/\text{g}$  DW in the white sprouting variety to 109  $\mu\text{mol}/\text{g}$  DW in the purple sprouting variety TZ5052. Glucobrassicin was the predominant glucosinolate, except in the white sprouting variety where sinigrin was predominant. This profile is more similar to that of cauliflower and suggests the variety may be of *botrytis* x *italica* parentage [71]. Green crown type broccoli has

been reported to



have higher total glucosinolate content and higher levels of glucoraphanin than other broccoli types such as purple broccoli varieties, which contain higher levels of glucoiberin [178] or indolyl glucosinolates [156]. Levels of phenolic compounds in broccoli are likewise affected by variety although fewer studies have been carried out with a smaller number of cultivars examined under uniform cultural conditions [56, 144, 174, 175]. Kaempferol and quercetin are commonly reported as the major phenolic compounds in broccoli with lower levels of phenolic acids (Table 9.3). In a study on 12 broccoli varieties grown in Spain levels of flavonoids ranged from 12.3 mg/kg to 65.4 mg/kg and were highest in cultivars Marathon, Lord and I-9809 [175]. In a smaller study by the same authors examining three commercial varieties (Marathon, Monterrey and Vencedor), levels of phenolics were similar across these three varieties [174]. However in a Polish study which evaluated three varieties (Marathon, Lord, and Fiesta) over three years quercetin content was higher in florets of cv. Lord, whilst kaempferol was highest in florets of cv. Fiesta. Levels of total flavonols (quercetin + kaempferol) ranged from 57 to 273 mg/kg FW depending on cultivar and year [56]. In a US field trial where two varieties were cultivated, flavonoids were higher in cv. Majestic than in cv. Legacy [144].

Field studies on 113 varieties of turnip greens (*B. rapa*) [126], 36 varieties of nabicol (*B. napus* var. *pabularia*) [23, 126], 27 varieties of Chinese kale (*B. oleracea* var. *alboglabra*) [167], 28 varieties of cabbage (*B. oleracea* var. *capitata*) [76], 60 oilseed rape varieties (*B. napus* var. *oleifera*) [36] and 27 horseradish varieties (*Amoracia rusticana*) [93] likewise showed a wide range of glucosinolate levels between different accessions of these crops. Similar variation was found in an evaluation of antioxidant compounds, vitamin C,  $\beta$ -carotene, lutein,  $\alpha$ -tocopherol and total phenolics in a range of cabbage, cauliflower, Brussels sprout, Chinese cabbage and broccoli cultivars grown under uniform cultural conditions [160]. In this study higher levels of vitamin C,  $\beta$ -carotene, lutein,  $\alpha$ -tocopherol and total phenolics were found in broccoli than in the other Brassicas.

Less research has been carried out on the effect of variety on phytochemical accumulation in carrot and onion. Variation in carotenoid profile has been reported with some carrot cultivars primarily accumulating lutein whilst others primarily accumulated  $\alpha$  and  $\beta$ -carotene [157]. Levels of individual polyacetylenes differ between different carrot varieties [4, 30, 34, 62, 79, 105, 106], and between different members of the Apiaceae family [38, 192]. In an analysis of 5 members of the Apiaceae, falcarinol was found in all investigated taxa except

parsley. Falcarindiol was found in all taxa and was the main polyacetylene except in carrot, where falcarinol was the predominant polyacetylene. Levels of total polyacetylenes and of falcarinol were higher in celery (*Apium graveolens*) and parsnip (*Pastinaca sativa*) than in carrot. However as carrot is consumed more frequently it is likely to be the major source of polyacetylenes in the western diet [192]. In an earlier analysis of 12 members of the Apiaceae the highest levels of falcarindiol were found in caraway (*Carum carvi*) and hogweed (*Heracleum sphondylium*). Levels of falcarinol were extremely high in poison hemlock (*Conium maculatum*). Amongst the cultivated species levels of falcarinol were higher in chervil (*Anthriscus cerefolium*), dill (*Anethum graveolens*) and parsnip (*Pastinaca sativa*) [38]. Levels of carotenes, phenolics, and antioxidant capacity are reported as higher in purple carrot varieties than in other varieties [4, 168]. In a field study of 27 carrot varieties grown under uniform cultural conditions levels of falcarinol ranged from 0.70 to 4.06 mg/100g FW [136].

Red onions are commonly reported to contain higher levels of total flavonols than yellow or white varieties. Quercetin and its derivatives, quercetin-3,4'-O-diglucoside (QDG) and quercetin-4'-O-monoglucoside (QMG), are thought to make up over 90% of the flavonoid content in onion. In red onions anthocyanins are also present. These are primarily cyanadin glucosides acylated with malonic acid or non-acylated [39, 133, 161]. Levels of flavonols vary considerably between onion cultivars with reported levels ranging from 2549 quercetin equivalents per kg fresh weight (FW) in the red onion cultivar Karmen to <1 quercetin equivalents in the white onion cultivar Contessa [161]. A study by Lombard *et al.* (2005) examined the total flavonol content in 5 onion varieties and found significantly higher concentrations in red skinned onion varieties compared to yellow varieties [37]. In a detailed study of 75 onion cultivars levels of quercetin were higher in red, pink and yellow onions (in the range 54 – 286 mg/kg FW) whilst white onions contained only trace amounts of quercetin [130]. Similar results are found in other studies [97, 133]. It has also been suggested that long day onion cultivars of Rijnsburger type from Northern Europe have higher levels of quercetin glucosides than short day onions of North American and Japanese origin [161]. In a study on 16 European onion varieties levels of quercetin glycosides in the edible parts were highest in the red onion cv. Red Baron and yellow skinned variety cv. Ailsa Craig [12]. Variation in the level of bioactivity of different onion or Allium varieties has also been demonstrated. In an analysis of ten onion and shallot varieties levels of total flavonoids and total phenolic content was strongly correlated with antioxidant activity and with inhibition of proliferation of HepG(2) and Caco-2 cells [186]. A 50-

fold variability in onion induced anti-platelet activity among cultivated and wild accessions in the genus *Allium* has been demonstrated [57].

### **Tissue type and developmental stage:**

In broccoli levels of total glucosinolates and the profile of individual glucosinolates vary in different plant tissues and at different developmental stages. Levels of glucosinolates are frequently reported to be higher in the earlier stages of plant growth: levels are higher in un-germinated broccoli seed than in seedlings, and levels in seedlings are higher than in florets. Reported levels of total glucosinolates in seed are in the region of 500 mg per 1 00g (cv. Marathon) [132].

In sprouted broccoli seedlings (cv. Marathon) total glucosinolate levels were in the range  $29.2 \pm 2.7$  to  $81.7 \pm 3.3 \mu\text{mol g}^{-1}$  DW ( $9.7 \pm 0.5 \mu\text{mol g}^{-1}$  FW and  $4.6 \pm 0.4 \mu\text{mol g}^{-1}$  FW) depending on seedling age and growth temperature. Levels of glucosinolates were higher in un-germinated seeds and levels progressively declined as the sprouts grew and developed. Levels of glucoraphanin were in the range  $17.4 \pm 1.5$  to  $49.5 \pm 1.9 \mu\text{mol g}^{-1}$  DW depending on seedling age and growth temperature [131]. A similar decline in glucosinolate content with sprout age was noted by Perez-Balibrea *et al.* (2008) and levels of total glucosinolates, total phenolics and vitamin C were observed to be highest in cotyledons than in roots or stems of the seedlings [132].

In studies on cultivated broccoli plants highest levels of glucosinolates have been reported in the floret with lower levels reported in the leaves and roots . Levels of total glucosinolates in the floret declined during development, mainly due to a decrease in the indole glucosinolates glucobrassicin and neoglucobrassicin. However levels of glucoraphanin were unchanged during head development [156]. In a similar study, levels of total glucosinolates in florets were higher in the first two developmental stages (corresponding to 42 and 49 days after transplanting) and declined as the florets matured. Recorded levels of total glucosinolates at commercial maturity were in the range 19.6 – 56.4  $\mu\text{mol g}^{-1}$  DW depending on cultivar and fertilization regime. Levels of glucoraphanin at commercial maturity were in the range 0.9 to 1.9  $\mu\text{mol g}^{-1}$  DW again depending on cultivar and fertilization regime [174]. Levels of total glucosinolates and levels of nine out of eleven individual glucosinolates measured were lower in post maturation florets in cv. Tokyodome, although slight increases in levels of hydroxyglucobrassicin and neoglucobrassicin were found [147]. In this study levels of glucosinolates

were found to differ between primary and secondary florets, with primary florets containing higher levels of glucoraphanin, glucoiberin, progoitrin, glucoalyssin, gluconapin and gluconasturtiin than secondary florets. Levels of the carotenoids 13-carotene and lutein, and of chlorophyll, are also reported to increase during development of the floret [156]. Levels of phenolic compounds in broccoli have been reported to be up to 10 times higher in the leaves than the stalks [40].

In carrot levels of phenolic compounds are reported to be higher in the outer layers of the carrot [123]. Raman spectroscopy has been used to localize the tissue distribution of polyacetylenes [9]. Highest levels of total polyacetylenes were detected in the outer part of the root in the pericyclic parenchyma and in the phloem adjacent to the secondary cambium. These data are in agreement with the observation that peeled carrots contain up to 50% less falcarindiol, a polyacetylene compound with strong anti-fungal activity associated with bitter flavour in carrots [34]. In a study which examined 16 carrot accessions, high levels of falcarindiol (31.9 – 91.5  $\mu\text{g/g}$  FW) were detected in the peel, with levels of 6.0 – 19.2  $\mu\text{g/g}$  FW in the phloem. In contrast falcarinol levels were lower and were concentrated in the phloem. Levels of falcarinol ranged from 1.3-5.3  $\mu\text{g/g}$  FW in the peel and 2.8-12.2  $\mu\text{g/g}$  FW in the phloem [123]. Similar results are reported elsewhere [30] with carrot peel containing up to 10 times more falcarindiol than the corresponding peeled roots in 6 varieties examined. In this study falcarinol was more evenly distributed across the root. Thus peeled carrots should retain the bulk of the health promoting compound falcarinol, whilst falcarindiol which has been associated with bitter taste would be largely removed. The polyacetylene falcarinol had lower anti-fungal activity than falcarindiol [123] but has been more widely reported as beneficial in human health [17]. Levels of both falcarindiol and falcarindiol-3-acetate were found to be significantly higher in small/immature (50-100g) than in large (>250g) carrot roots in an analysis of 6 Nantes type carrot varieties, however levels of falcarinol were unaffected [79]. Similarly, in a three year field study on two carrot varieties (Bolero and Kampe) harvested at different maturity stages (103-104 days, 117-118 days, 131-133 days, and 146-147 days), maturity had no effect on levels of falcarinol in fresh carrots [81].

In onion, highest levels of quercetin and kaempferol glycosides are commonly found in the outer dry skins with lower levels detected in the inner edible rings [13, 31, 128, 133], and levels are reported to decrease from the apex to the base (root part) of the bulb. In contrast anthocyanin distribution is relatively uniform [133].

Some studies report higher levels of flavonols and anthocyanins in smaller onion bulbs, however in other studies bulb size had no significant effect on quercetin glycoside content [113, 128]. In a study to evaluate the antioxidant potential of wild *Allium* species (*A. neapolitanum*, *A. roseum*, *A. subhirsutum* and *A. sativum*) growing in Italy, Nencini and colleagues report significantly higher levels of antioxidant activity as measured by FRAP test and a DPPH assay in the flowers or leaves, with lowest antioxidant capacity consistently reported for the bulbs [121].

#### **Nutrient supply – nitrogen, sulphur and selenium:**

Application of nitrogen (N), phosphorus (P), potassium (K), and sulphur (S) as fertilizer generally increases crop yield and nutritional quality. However excess N fertilizer in particular can cause undesirable effects such as increased nitrate levels in leafy vegetables, reduced quality and reduced vitamin C content and shelf life in some crops. A number of studies have shown that decreased N application results in higher accumulation of phenolic compounds and of some glucosinolates; whilst higher levels of N fertilization promote formation of carotenoids and chlorophylls in *Brassica oleraceae* species [157]. In a recent study the effect of applied N and S on phytochemical accumulation in florets of broccoli cv. Marathon was investigated [75]. Nitrogen was applied at 0, 15, 30 or 60 kg/ha and S at 50 or 100 kg/ha. In this study highest levels of flavonoids, and of the sulforaphane precursor glucoraphanin, were obtained at low N application rates. Nitrogen application at levels above 30 kg/ha caused an increase in glucobrassicin content of up to 44%, whilst levels of glucoraphanin declined by 18-34% and levels of the flavonols quercetin and kaempferol declined by 20-38%. However crop yields declined significantly (up to 40%) at N levels below 60 kg/ha. Similar effects of N fertilization are noted in other field studies on broccoli [53, 83, 92, 125] and other Brassicas [91, 92]. This suggests that there could be considerable potential to produce mini broccoli heads with enhanced levels of phenolic compounds and glucoraphanin at low N application rates.

Sulphur supplementation has been demonstrated to increase glucoraphanin content in a range of Brassica species including broccoli and to increase alliin content in onion and garlic [83, 154, 156]. In the study of Krumbein *et al.* (2001) sulphur was applied at levels up to 600mg per plant to broccoli grown in soil free media and resulted in a significant increase in glucoraphanin content [83]. However field trial based studies have

been disappointing and suggest that applying S to S-sufficient soils has only a minimal impact on glucosinolate accumulation. Vallejo *et al.* (2003) examined the effect of S application at levels of 15 and 150 kg/ha on three broccoli cultivars. Whilst significant differences were observed in glucosinolate contents of immature broccoli florets in response to S fertilization, no significant differences were observed in mature florets [174]. In a similar study S applied as gypsum at levels of 23 kg/ha resulted in a significant increase in glucoraphanin content in cv. Marathon but had no significant effect on two other cultivars [139]. In the study of Jones *et al.* (2007) S application at levels of 50 and 100 kg/ha had no significant effect of glucosinolate or flavonol accumulation [75].

Given the high content of glucoraphanin found in broccoli sprouted seed some authors have investigated the effect of N and S application during growth of broccoli and other Brassica sprouts [3, 78]. In the study of Aires *et al.* (2006) broccoli cv. Marathon seeds were grown in Petri dishes on rockwool disks and watered with pure water supplemented with different combinations of potassium nitrate ( $\text{KNO}_3$ ) and potassium sulphate ( $\text{K}_2\text{SO}_4$ ) from 6 days after sowing. Sprouts were harvested and analysed 11 days after sowing. However, in this study fertilization was found to have a significant detrimental effect on accumulation of aliphatic glucosinolates including glucoraphanin. This may have been due to salt stress at the concentrations used [3]. In the study of Kestwal *et al.* (2010) broccoli, radish and cabbage seeds were sprouted in soil supplemented with S as sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) at S concentrations equivalent to 20 to 60 kg/ha. This range was selected following an initial experiment to determine the optimal treatment range where sprout growth was not significantly adversely affected. Sprouts were harvested for analysis at 12 days after sowing. In this study levels of total glucosinolates including glucoraphanin were increased in S supplemented radish, broccoli and cabbage sprouts. Levels of total phenolics were higher in S supplemented radish, but not broccoli or cabbage sprouts, and antioxidant activity was higher in S supplemented radish and broccoli but not cabbage [78].

In most plant species selenium (Se) can be toxic to the plant, however Brassica and Allium species are able to utilize Se and are referred to as seleniferous plants or “selenium accumulators” [70]. In most soils worldwide Se is deficient and selenium enriched Brassica and Allium crops can be grown by supplementing the soil with Se. Given the potential health benefits of a “super broccoli” containing higher levels of both sulforaphane and Se, attempts have been made to increase levels of both sulforaphane and Se in broccoli. However, these efforts have been frustrated since there appears to be an inverse relationship between Se and

glucoraphanin accumulation. Broccoli and other crucifers typically contain relatively low amounts of Se (0.1-0.3  $\mu\text{g/g DW}$ ) [144]. In supplementation experiments where sodium selenate solution was added to broccoli plants from 1 week prior to floret development onwards, accumulation of Se to as much as 950  $\mu\text{g/g DW}$  was achieved. Little effect was observed on total glucosinolate levels but a significant decrease in levels of sulforaphane and some phenolic, particularly cinnamic, acids was observed [51, 144]. In studies where both Se and S were applied to hydroponically grown *Brassica oleracea* plants an interaction between S and Se metabolism was observed. Plants exposed to increased levels of S (as sulphate, 37ppm) showed increased accumulation of glucosinolates with levels of glucoiberin and glucoraphanin 11% and 16% higher than controls. Plants exposed to Se (as selenate, at 0.5, 0.75, 1.0 and 1.5 ppm) showed reduced accumulation of glucoiberin, glucoraphanin and other glucosinolates with increasing Se. At 1.5 ppm Se levels of glucoiberin and glucoraphanin were reduced by 58% and 68% respectively compared to controls. In combined Se/S treatments, levels of Se in leaf tissue were 178  $\mu\text{g g}^{-1}$  and levels of glucoraphanin were only moderately reduced compared to controls. Thus the authors conclude that it may be feasible to produce selenium enriched Brassica crops that maintain adequate levels of glucoraphanin by selenate fertilization [170].

There have been fewer field studies on the impact of fertilizer application on bioactive content in onions and carrots. Levels of quercetin in onion were shown to be unaffected by either the type or amount of nitrogen fertilization [113]. Sulphur application can increase yield and bulb size, and as might be expected, led to increased levels of the S containing alk(en)yl sulfoxides and increased pungency (measured as pyruvate content in macerated tissue) [52]. Selenium enriched garlic has been produced and has been shown to have higher bioactivity when grown in Se rich soil. Increased activation of phase II enzymes and enhanced production of Se-methyl-selenocysteine (an inhibitor of tumourigenesis) in the Se enriched plants have been demonstrated [8, 70]. Field studies with carrot have indicated that levels of total phenolics were increased in response to increasing N fertilization [162].

### **Seasonal effects – light and temperature:**

A number of studies carried out on broccoli and cauliflower cultivars [26, 82, 154-156] have indicated that increasing irradiation combined with lower daily temperatures, led to increased levels of glucoraphanin and

glucoiberin. In purple broccoli varieties the glucosinolate content was unaffected. In addition low daily mean temperatures promoted synthesis of lutein,  $\beta$ -carotene and ascorbic acid in broccoli [156, 157]. In a study on greenhouse grown broccoli (cv. Marathon), Schonhof *et al.* (2001) found that levels of alkenyl glucosinolates such as gluconapoeiferin and progoitrin were unaffected by temperature or irradiation. In contrast alkyl glucosinolates such as glucoiberin and glucoraphanin showed increased accumulation at lower temperature ( $<12^{\circ}\text{C}$ ). Of the indole glucosinolates glucobrassicin was increased by high temperature ( $>18^{\circ}\text{C}$ ) and low radiation [82, 154]. The authors found a significant correlation between alkyl glucosinolates in broccoli florets and levels of the stress indicator proline in leaves and postulate that stress responses may play a role in glucosinolate accumulation. In greenhouse grown broccoli Charron and Sams (2004) found higher levels of glucosinolates in broccoli leaves from plants grown at  $12^{\circ}\text{C}$  and  $32^{\circ}\text{C}$  as compared to those grown at  $22^{\circ}\text{C}$  suggesting that temperature stress may be responsible for increased glucosinolate content [26]. In other *Brassica oleracea* crops including cabbage and kale field based trials have indicated that there is a significantly higher total glucosinolate content in spring sown crops and variations in the level of individual phytochemicals [24]. Levels of myrosinase activity (measured as activity/FW and specific activity) in a range of Brassicas showed a response to temperature and photosynthetic photon flux (PPF) [27]. Activity FW was generally higher where daily mean temperatures and PPF in the 2 weeks prior to harvest were lower. The authors suggest that light may affect myrosinase activity indirectly via modulation of ascorbic acid – since myrosinase is inhibited by high concentrations of ascorbic acid and the accumulation of ascorbate is itself increased by light [184].

Levels of glucosinolates in broccoli sprouts are also temperature responsive. For commercial production sprouted seeds are commonly grown at  $20\text{--}28^{\circ}\text{C}$ . Sprouted broccoli (cv. Marathon) seedlings grown under a  $30/15^{\circ}\text{C}$  (day/night) temperature regime showed significantly higher total glucosinolate levels, specific increases in glucoraphanin content and corresponding increased induction of phase II enzymes than sprouted seed grown at  $22/15$  and  $18/12^{\circ}\text{C}$  temperature regimes [131]. Mean recorded glucoraphanin levels in experimental sprouts on the sixth day after sowing were  $49.5\ \mu\text{mol g}^{-1}\text{ DW}$  and glucoraphanin made up 61.3% of total glucosinolate content. When sprouted seed was grown at either supra- or sub-optimal constant temperatures of either  $33.1^{\circ}\text{C}$  or  $11.3^{\circ}\text{C}$  glucoraphanin and total glucosinolate contents were also increased although sprout growth was negatively affected by non-optimal temperature. In addition the authors raised the concern that seeds



sprouted at higher temperature or longer time would be more susceptible to microbial contamination and thus such practices may not be suitable for commercial production. In an additional study it was reported that phytochemical content of sprouted broccoli seeds (cv. Marathon) was light responsive, with sprouted seed grown under a 16 h light/8 h dark photoperiod showing enhanced levels of glucosinolates, phenolic compounds and vitamin C than dark grown sprouts [132]. Levels of total glucosinolates, total phenolics and vitamin C were 33%, 61% and 83% higher respectively.

The induction of enzymes for flavonoid synthesis by light is well known [99]. Vegetables grown in full sun have been reported to contain higher levels of flavonoids and exposure to sunlight is known to enhance production of flavonols in onion bulbs [129, 146, 157]. In a five year study which examined the effect of climatic conditions on flavonoid content in two Portuguese landrace onion varieties, total and individual flavonoid levels varied significantly between years, with highest levels observed in hot, dry years [146]. In a three year field study which examined levels of the flavonols kaempferol and quercetin in three broccoli varieties (Marathon, Lord and Fiesta) the level of total solar radiation over the growing period had a significant effect on both flavonols with higher levels under increased radiation [56]. In some instances the interplay between climatic factors may result in complex regulation of different phytochemicals. For example although high light can increase flavonoid synthesis, high temperature has been reported to decrease anthocyanin synthesis [116]. In carrot levels of polyacetylenes were significantly different in different harvest years indicating a seasonal effect on falcarinol and falcarindiol [81], however no meteorological data is presented and the underlying mechanism is unclear.

### **Biotic and abiotic stress:**

Many phenolic compounds, the polyacetylenes and the glucosinolates are considered as defensive compounds within the plant and numerous studies show their regulation in response to abiotic and biotic stresses [105, 120, 123, 149, 150]. Abiotic stresses include water stress, salinity, and temperature stress. Biotic stresses include wounding, pathogenesis, insect or animal herbivory and treatment with elicitors which mimic these responses, as well as competition with neighbouring plants. Phenylalanine ammonia lyase (PAL) the key entry point enzyme for synthesis of phenolic compounds is well known to be up-regulated in response to biotic and

abiotic stresses including UV light, low temperature, nutrient deficiency, wounding and pest or pathogen attack [120].

Some reports have indicated that water stress can increase accumulation of certain phytochemicals with a doubling of glucosinolate content in broccoli with reduced water supply [154]. In carrot changes in polyacetylene profile and the content of individual polyacetylenes have also shown a response to water stress, although results are contradictory. In a greenhouse pot trial three novel polyacetylene compounds were found only in stressed carrots subjected to drought or waterlogged conditions. Levels of eight other polyacetylenes including falcarinol, falcarindiol and falcarindiol-3-acetate were lower in control samples, although an earlier field study by the same authors showed higher levels of polyacetylenes in field grown drought stressed carrots [95]. Both drought and salt stress cause production of ROS within the plant and result in increased levels of secondary metabolites, including phytochemicals. Some of these plant secondary compounds can function as free radical scavengers and osmo-protectants (reviewed in [159]) Studies on tomato (*Solanum lycopersicum*) have indicated that moderate salt stress can increase levels of bioactive compounds such as lycopene by up to 85% depending on cultivar [41, 85]. Commonly however the biomass of drought or salt stressed plants is considerably reduced. Four recent studies have shown that salt stress can increase levels of glucosinolates and phenolic compounds in Brassicas. Total glucosinolate content and total phenolic content were significantly increased and myrosinase activity was inhibited in radish sprouts germinated under a 100mM NaCl treatment [188]. In hydroponically grown Pak-choi levels of total glucosinolates were increased significantly by 50mM NaCl, however under 100mM NaCl the content of indole glucosinolates increased whilst aromatic glucosinolates decreased [68]. In a greenhouse study on the effect of salt stress (80mM NaCl) on three broccoli varieties (cvs. Marathon, Nubia and Viola) the salt stress treatment significantly increased levels of glucosinolates in leaf and stalk tissue of the purple variety Viola but not the green broccoli varieties Marathon or Nubi a [40]. In this study salt treatment significantly affected levels of phenolic compounds in some tissues but not others and in some varieties but not others indicating a significant variety x salt stress and tissue type x salt stress interaction on phenolic accumulation. Salt stress (40mM and 80mM NaCl) caused a significant increase in levels of total glucosinolates in floret tissue of cv. Marathon [94]. Floret vitamin C content was unaffected. Phenolic compounds in the floret showed a complex response with some such as sinapic acid derivatives increased at

40mM but not 80mM NaCl and flavonoids decreased at 80mM NaCl. Temperature stress has been reported to increase glucosinolate content in broccoli [26]. In carrot temperature stress (35°C) resulted in a reduction in net photosynthesis and a reduction in root biomass, levels of total phenolics in foliage were unchanged [69].

Carrot polyacetylenes were originally of research interest due to their role in defence and pathogenesis responses. Both falcarinol and falcarindiol are implicated in variability of resistance to carrot root fly (*Psila rosae*) amongst different carrot cultivars and act together with other compounds such as the phenolic compound methyl-isoeugenol as oviposition stimulants [38]. The carrot polyacetylenes, falcarindiol in particular, are implicated in resistance to storage pathogens [105, 123]. Increased accumulation of carrot phenolic compounds and increased expression of PAL in response to mechanical wounding, ethylene and methyl jasmonates treatment and elicitor treatment have been reported [64, 72, 158]. Schonhof *et al.* (1999) report that the synthesis of glucosinolates in broccoli could be induced by mechanical stress such as leaf damage [156] however attempts to induce glucosinolates in other Brassica crops were unsuccessful [111]. The glucosinolates in Brassica species can be induced in response to pathogen attack, herbivory, and in response to elicitors or plant hormones involved in defence responses including salicylic acid, jasmonic acid and methyl jasmonate [2, 84, 149]. There is a complex relationship between glucosinolates and pests, and it is currently understood that whilst glucosinolate breakdown products may repel generalist pests, some glucosinolates in particular aliphatic glucosinolates, may act as attractants towards specialized pests [111, 176]. Spacing effects with other plants are also apparent. Schonhof *et al.* (1999) found that high plant density in broccoli cultivation (97,000 plants per ha) could increase glucoraphanin content by up to 37% in comparison with lower density planting, indole glucosinolates were not affected [156]. In onion and other Allium plants a high level of arbuscular mycorrhizal colonization is common and this association can result in increases in yield especially in low nutrient soils. Quercetin mono- and di-glucoside concentrations in onion bulb can be significantly increased by application of arbuscular mycorrhizal fungal inocula due to induction of plant defence responses [135].

### **Means of production – organic and conventional agriculture:**

European Union Council Regulation No. 2092/91 [43] defines a number of parameters for a plant product to be considered organic including: a ban on synthetic pesticides, herbicides and mineral fertilizers; a

ban on genetically modified cultivars and lower nitrogen levels than conventional agriculture (a maximum limit for manure application of 170 kg N ha<sup>-1</sup> year<sup>-1</sup> [142]. Within the EU the directive is interpreted by national certification bodies such as the Soil Association in the UK, or the Irish Organic Trust and IOFGA (Irish Organic Farmers and Growers Association) in Ireland. Certification standards of these bodies can be more stringent than regulation 2092/91, but may not be less stringent. Three main types of studies – market purchase studies, paired farm surveys, and field trials - have been used to compare nutritional or less frequently, phytochemical content, between organic and conventionally grown fruits and vegetables. Market purchase studies require multiple sampling over extended time to compensate for variation due to seasonal, annual, handling and variety effects. Paired farm surveys can give information on varieties and treatments used in crop production but are reliant on a sufficient number of paired matched farm systems, whilst field trial studies can be difficult to design in such a way that they give statistically reliable data. A number of long term field studies of organic agriculture have been set up (for review see [141]). A limited number of research studies have compared nutritional content in organic and conventionally grown vegetables rather than fruit, with very few examining phytochemical content (reviewed in [35, 191]). In general the evidence suggests little difference in the nutritional content of organically cultivated crops with the exception that levels of nitrates are lower and levels of vitamin C and dry matter content may higher than in conventionally grown crops [18, 142, 181, 183]. Some reports suggest increased levels of phytochemicals in organically grown crops [187, 191] and some authors have suggested that phytochemicals which can be considered as defence related secondary metabolites could be considerably higher in organic vegetables [18]. Several studies have evaluated antioxidant levels rather than measuring individual phytochemicals (reviewed in [14]). It is unclear to what extent reported differences may be due to factors such as low nitrogen, use of disease resistant cultivar types or increased pest damage in organic systems. In addition crops cultivated using organic production methods typically have significantly lower yield than conventional counterparts, with average yield reductions of up to 20% [142]. In an investigation of polyphenolic content, antioxidant activity and anti-mutagenic activity of five green vegetables – Chinese cabbage (*Brassica rapa* subsp. *pekinensis*), spinach (*Spinacia oleracea*), Welsh onion (*A. fistulosum*), green pepper (*Capiscum annuum* var. *annuum*) and the Japanese vegetable “qing-gen-cai” the antioxidant activity, anti-mutagenic activity, and composition of flavonoids including quercetin were higher in the organically cultivated vegetables [143]. In a

study by Young *et al.* (2005) leaf lettuce (*Lactuca sativa*), collard greens (*Brassica oleracea* var. *viridis* cv. Top Bunch) and Pak-choi (*Brassica rapa* var. *chinensis* cv. Mei Qing) were cultivated on adjacent plots, and levels of individual and total phenolics were quantified. In this study levels of kaempferol-3-O-glucoside were significantly higher in organically cultivated collard greens, but levels of other phenolics and total phenolic content were not significantly different in collards or leaf lettuce. In the case of Pak-choi, levels of total phenolics were significantly higher in organically cultivated plants, however the authors attribute this to a greater damage to the organic plants by flea beetle [187]. A market purchase study by Meyer and Adam (2008) found significant differences in glucosinolate content between organic and conventional broccoli and red cabbage, with higher levels of glucobrassicin and neoglucobrassicin in organic samples. No significant difference in glucoraphanin content were found, however gluconapin was present at lower levels in organic red cabbage [108]. In an analysis of polyacetylenes in carrot (cv. Bolero) grown under one conventional and two organic treatments as part of the Danish VegQure rotation experiment no difference in levels of falcarinol were found over a 2 year field trial. In this study levels of applied nutrients were 120, 18 and 58 kg/ha of N, P and K for the conventional treatment and either green manure or 54, 4, and 20 kg/ha of N, P and K for the organic treatments [163]. The recent meta-analysis of organic foods by Dangour *et al.* [35] found that organically produced crops had a significantly higher content of phosphorus and higher titratable acidity, whilst conventionally cultivated crops had a significantly higher content of nitrogen. No differences were found in levels of vitamin C, soluble solids, magnesium, potassium, zinc, copper, calcium, or in levels of phenolic compounds. Five rejection criteria were used in this meta-analysis: provision of a definition of organic production methods used including the name of the certification body; specification of the crop variety or livestock breed; a statement of the nutritionally relevant substance analysed; description of analytical methods used; and statement of methods used for statistical analysis. The meta-analysis includes studies on phytochemical content in only two vegetable crops – the study of Young *et al.* [187] and Meyer *et al.* [108] described above.

#### **Other factors:**

An influence of soil type on phytochemical accumulation including glucosinolates and phenolic compounds is commonly mentioned anecdotally in the literature [24, 56, 75, 81, 129, 156] and in a study by

Jones *et al.* (2007) higher levels of glucosinolates were found in broccoli florets of the cultivar Marathon grown in light clay soils as compared to those grown in sandy loam type soils [75]. However such observations are complicated to interpret as crops grown in different areas will also experience different climatic and other agronomic conditions. Application of amino acid precursors of glucosinolates have been studied in Brassicas. Foliar fertilization or leafstalk infusion of methionine (the precursor of alkenyl glucosinolates) resulted in an increase in total and individual glucosinolate content [84, 153].

### **9.3 Harvest and post-harvest management practices:**

The effect of harvesting and on-farm post-harvest management practices on phytochemical content will depend on the crop and the impact of factors such as the degree of mechanical injury caused during harvest and transport, water loss and oxygen stress at wound sites, temperature at harvest and during storage, and on how these factors affect the synthesis, retention or breakdown of individual phytochemicals. Mechanical injury results in cellular disruption and can allow enzymes such as myrosinase (EC 3.2.1.147) , peroxidases (EC 1.11.1.7) and polyphenol oxidase (EC 1.10.3.1) to come into contact with their substrates. Water loss and oxygen entry can trigger stress and defence responses including modulation of the phenylpropanoid pathway leading to altered expression of phenolic compounds. Lower temperatures would be expected to reduce enzyme activity as well as inhibit the growth of spoilage organisms. Harvest and post-harvest treatments commonly rely on reducing injury, water loss, and temperature and have been largely designed to maintain visual appearance - for example preventing yellowing of green produce due to chlorophyll breakdown, preventing browning due to oxidation of phenolic compounds by polyphenol oxidases and preventing loss of turgor [74, 185]. The impact of harvest and storage techniques on phytochemicals has only recently begun to be explored. In general phenolic compounds are considered to be relatively stable at cool temperature storage. Anthocyanins especially in fruit can increase at temperatures above 1 °C but may be lost at high temperature which may be associated with water loss [117]. Low temperature can reduce loss of organo-sulphur compounds such as glucosinolates in Brassicas and cysteine sulfoxides in onion [74].

### Harvest and post-harvest management of onion:

Bulb formation in long day onions grown in Northern Europe is initiated as the day length begins to shorten in mid summer and mature bulbs are mechanically harvested at 50-100% leaf fall-down. In the UK and Ireland commercially grown onion bulbs are generally mechanically harvested in late August to mid September from a March sowing and are cured by forced air drying at 25-28 °C and 65-75 % RH for ten days to six weeks. Commonly grown varieties include the yellow (brown) variety Hyskin and the red variety Red Baron. Curing seals the neck of the onion and forms a dry outer skin, which reduces moisture loss and disease. Forced air curing reduces the incidence of neck rot caused by *Botrytis allii* and bacterial soft rots caused by *Erwinia* and *Pseudomonas* species. In addition the dried outer skins can be easily removed by mechanical cleaning after curing, resulting in a cleaner and darker skin finish demanded by consumers [28, 42]. Subsequently, onions may be kept in cold storage at around 1 - 4°C in the dark to induce dormancy and prevent sprouting, however sprouting commonly initiates within one to three weeks after removal from cold storage [165]. Maleic hydrazide (Fazor) can be used to prevent sprouting in bulb onions by prolonging natural dormancy and is applied to field onions a week before harvest. Traditionally and in hot dry climates, onions can be left to cure in the field in windrows or mesh bags and this has been reported to increase quercetin content [114, 124, 129]. The effect of curing temperature on flavonols and anthocyanin content in brown (yellow) and red onion skin has been investigated [42]. Two brown (yellow) (cvs. Wellington and Sherpa) and a red onion (cv. Red Baron) were cured at 20°C, 24°C or 28°C for six weeks followed by cold storage at 1 °C for seven months. Samples were analysed immediately after curing and at seven months after storage. In this study quercetin levels in the skin were not affected by curing temperature but levels of quercetin 4-glucoside, quercetin 3,4-diglucoside and anthocyanins were significantly higher in cv. Red Baron cured at 20°C. In a study of different storage methods on onion. bulbs stored at 5 °C, 24°C and 30°C for up to five months showed an initial rise in total quercetin levels followed by a decline. Changes were most pronounced under the 24 °C treatment. Onion bulbs stored under controlled atmosphere did not show significant changes in quercetin content over the 5 month storage period [130]. In a study on two onion varieties (Red Baron and Crossbow) cured at 28 °C for 10 days and stored for 6 months at  $\leq 4$  °C an initial drop in the level of quercetin monoglucosides (which the authors attribute to removal of the outer dry skin) occurred and thereafter there was little change in levels of quercetin monoglucoside or quercetin 3,4'-O-

diglucoside [137]. Total anthocyanins are reported to decrease in red onion (cv. Tropea) during storage, with higher levels of loss of anthocyanins at higher temperature [55]. During onion storage the enzyme alliinase (S-alk(en)yl-L-cysteine sulfoxide lyase, E.C.4.4. 1.4) catalyses the breakdown of cysteine sulfoxides into the flavour compounds pyruvate, ammonia and volatile sulphur compounds. Levels of pyruvate, allinase activity and cysteine sulfoxides in onion bulbs (cv. Hysam) increased during storage at 0.5 °C over nine weeks under normal atmosphere conditions [172]. A slight decline in phenolic content in onion at the end of cold storage has been noted in some studies [15, 137] and there is an inverse relationship between total phenolic content and sprout development [15]. The effect of a post curing heat treatment (36 °C for 24 or 96 hours) on onion flavonols has recently been investigated as a method of increasing shelf life [124]. Three onion varieties Recorra, Hyred and Red Baron were cured at RT or in the field for two weeks and then heat treated prior to cold storage at 2°C for up to 8 months. Neither storage nor heat treatment had a significant effect on total flavonoid content, however levels of quercetin 3,4-diglucoside increased in the 24 hour heat treated cvs. Red Baron and Hyred. A lower content of total flavonols was found in all varieties after eight months of cold storage following the 96 hour heat treatment and the authors suggest this may be due to negative effects of heat treatment on onion metabolism. UV Irradiation is currently used as a post harvest treatment in several products for sterilization, and to inhibit sprouting and delay maturity. A number of studies have indicated that post harvest UV irradiation can increase the levels of atocopherol and flavonoids in several fruits and vegetables including onion [66, 127, 145]. In onion, short wave UV irradiation was shown to significantly increase levels of both free and total quercetin, and could reduce incidence of spoilage moulds including *Penicillium allii* and survival of human pathogens such as *Escherichiae coli* [65, 127, 145].

### **Harvest and post-harvest management of broccoli:**

In Ireland and the UK commercially grown green broccoli is normally produced using modulator transplants which can be sown from mid February to June and transplanted in the field from mid April to late July. Florets are harvested by hand when the florets are 250-600g with tight unopened flowers. To increase shelf life the crop is cooled to below 6 °C within 12 hours and kept at holding temperatures of 3-5 °C and high humidity. Commonly grown varieties in Ireland are cvs. Ironman, Steel, Parthenon, Manaco, and Monterey. The



variety Marathon was widely grown in several countries including Ireland but has largely been superseded by newer varieties.

Broccoli is normally harvested in the early morning to allow time for processing and packing on the same day, however a recent study has indicated that evening harvest could better maintain quality and may affect phytochemical content. In this study broccoli florets (cv. Iron) were harvested at 8am, 1pm and 6pm and quality parameters as well as levels of total phenolics and antioxidant capacity were measured over 5 days at 20 °C storage. Chlorophyll loss was significantly accelerated in florets harvested at 8am. Levels of total phenolics and antioxidant capacity was significantly lower in 8am harvested florets on day 3 of storage, but differences were not significant on other days [63]. Several studies [67, 74, 90, 140, 147, 182] have examined the effect of post-harvest handling and storage conditions on glucosinolate and/or phenolic compounds in broccoli. Levels of glucoraphanin, quercetin and kaempferol in broccoli cv. Marathon were not significantly affected by post-harvest storage treatments designed to simulate commercial storage and marketing. Florets were stored at 1- 4 °C at 99% relative humidity (RH) for 2 to 28 days to simulate initial storage and transport conditions, and were then kept at 8 –20 °C and 70–99% RH in order to simulate marketing conditions [182]. Storage of both primary and secondary broccoli florets (cv. Tokyodome) at either room temperature (~20°C) or at 4 °C for 5 days showed that higher temperature storage led to a significant reduction in total and individual glucosinolates although levels of hydroxyglucobrassicin and gluconasturtiin increased [147]. Under refrigerated (4 °C) storage the decrease in total glucosinolates was considerably lower at 16% and 4% for primary and secondary inflorescences respectively. Levels of glucoraphanin declined by 82% and 89% in primary and secondary inflorescences under the RT storage treatment and by 31% and 10% under the refrigeration treatment [147]. In a similar study broccoli florets (cv. Marathon) were stored at either 4°C or 20°C in open boxes or in plastic bags. Storage at 20 °C in both systems caused a significant decrease in glucoraphanin by day 7, although the decline was more rapid in the open box system. In broccoli stored in open boxes a 55% loss of glucoraphanin was observed by day three, in broccoli stored in bags a 56% loss was observed by day seven. At 4 °C little decrease in glucoraphanin content was observed for either system [140]. Levels of sulforaphane in florets of broccoli (cv. Arcadia) have been determined over a 21 day storage period at 4 °C [67]. Levels of sulforaphane measured in fresh broccoli samples were 36.7 to 49.4 mg/100g depending on year of harvest. After five days storage at 4 °C levels of

sulforaphane had declined by 33% and by 21 days storage levels had declined by 49- 55%. Levels of total phenolic compounds, flavonoids and antioxidant capacity have been reported to increase during storage of broccoli florets under both 5 °C and 20°C storage, with changes in ROS scavenging enzymes also reported [90]. Research to date indicates that storage factors currently used to maintain visual appearance and nutritional quality in broccoli i.e. low temperature and/or high RH can maintain reasonable levels of glucosinolates and other bioactive compounds such as phenolic compounds. In a review of post-harvest treatments on glucosinolate content in broccoli the authors suggest that if broccoli is stored at 4 °C there is little benefit in maintaining high RH, however where broccoli is stored at room temperature high RH should be maintained by use of packaging in order to maintain both glucosinolates levels and visual appearance [74].

### **Harvest and post-harvest management of carrot:**

Commercial harvesting practices for carrot commonly include mechanical lifting, topping to remove the leaves, and grading followed by brushing, tumble-washing and hydro-cooling. Storability is improved at low temperature and high RH. Main-crop carrots in the UK and Ireland are generally harvested in October and November when the carrot roots are fully mature. The variety cv. Nairobi is widely grown in the UK and Ireland. Mechanical harvesters may be either “top lifters” which lift the crop by the foliage, or “share lifters” which run in the soil lifting the crop which is then separated from the soil by mechanical shaking and sieving. The mechanical force of harvesting and transport operations and severing of the foliage would be expected to induce plant wound and stress responses and consequent increases in phenolic compounds.

A detailed study on the effect of hand or machine harvesting and simulated transport on 5 carrot varieties (cvs. Bolero, Panter, Yukon, Napa and Newburg), showed increased mechanical stress led to increased respiration and increased ethylene synthesis. Levels of the phytoalexin phenolic compound 6-methoxymellein were increased in response to ethylene, as were other phenolic compounds such as chlorogenic and isochlorogenic acid, whilst sugars decreased ([64] and references therein). In this study machine harvesting did not induce significant changes compared to hand harvesting, however the severity of post-harvest handling had a significant effect [64]. Increased respiration and increased levels of phenolic compound accumulation in carrots related to the severity of post-harvest handling, storage and processing treatments have been widely reported

[10, 11, 77, 151], however such responses can be slowed by low temperature storage [59]. Increases in phenolic compounds are associated with increased antioxidant potential, however oxidation of phenolic compounds can result in undesirable browning during storage in carrot and other crops; and accumulation of certain phenolic compounds such as isocoumarins can result in bitter flavour [59, 64, 151]. In a recent study the effect of wounding intensity, methyl jasmonate and ethylene treatment on accumulation of total and individual phenolic compounds, antioxidant capacity and PAL enzyme activity in carrot (cv. Chocataw) were examined [64]. The relative proportions of chlorogenic acid, dicaffeoyl-quinic acid, ferulic acid, isocoumarins and antioxidant capacity differed under different stress combinations and the authors suggest that environmental modification could be used to enhance the phenolic profile in stored and processed carrots.

Few studies have been carried out to evaluate the effect of storage on carrot polyacetylenes [62, 79, 81]. In a study in which raw carrots (cvs. Bolero, Rodelika and Fancy) were stored at 1°C and 98% RH falcarinol content was initially in the range 22.3 - 24.8 mg/kg. Levels were largely unchanged during the first month but subsequently declined by nearly 35% after 120 days storage, which the authors attribute to a change in the balance between biosynthesis and degradation [62]. However in a study in which polyacetylene content in carrot roots of six Nantes cultivars (cvs. Bolero, Fancy, Duke, Express, Line 1 and Cortez) stored at 1°C for 4 months was evaluated levels of falcarinol, falcarindiol and falcarindiol-3-acetate increased significantly during storage. [79]. In the most recent study [81] levels of falcarinol, falcarindiol and falcarindiol-3-acetate in roots of two carrot varieties (Kampe and Bolero) were reported to stabilize during storage with an increase noted in samples that were initially low and a decrease in samples initially high in polyacetylenes [81].

## **9.4 Future prospects**

### **Novel uses for crop wastes:**

Plant tissues that are currently discarded during harvest or processing such as broccoli leaves, carrot foliage and onion outer leaves may represent a significant source of phytochemicals. For many Brassica crops including broccoli, cauliflower and Brussels sprouts less than 50% of the biomass is used for human consumption with the remainder either discarded, re-incorporated into the soil or used for fodder [150]. One

potential use of Brassica wastes based on the antimicrobial properties of glucosinolates and their isothiocyanate breakdown products has been as a biofumigation agent for control of soil borne pathogens as an alternative to methyl bromide soil fumigation. Approaches using both dried and fresh material, and in the use of *Brassica* species as green manures have been investigated in a number of studies (reviewed in [32]). A commercial green manure BQ Mulch<sup>TM</sup> consisting of a mixture of Brassica species has been developed and marketed in New Zealand for control of soil nematodes and soil pathogens such as *Phytophthora* and *Pythium* [98, 166] and there are approaches to develop Brassica derived biocidal dried plant pellets for biofumigation [88]. Brassica products meals have also been investigated for their herbicidal and insecticidal activity [118]. Given the health promoting and antioxidant properties of onion, broccoli, carrot and other vegetable wastes numerous studies have investigated potential for production of functional ingredients [39, 40, 96, 118, 148, 180].

#### **Plant breeding for bio-fortified crops:**

Given the wide within group variation in phytochemical profiles between different varieties there is considerable potential to increase levels of key bioactive compounds using plant breeding approaches which may exploit the genetic diversity of older varieties and seed bank accessions. Breeding approaches have been used to increase levels of flavonoids in onion and carotenes and anthocyanins in carrot [33, 80, 119]. Although precise comparisons between wild and cultivated broccoli species is complicated by differences in floret morphology some authors have estimated that florets of cultivated broccoli lines contain 3 – 10  $\mu\text{mol g}^{-1}$  DW of glucosinolates whilst wild species contain between 50 – 100  $\mu\text{mol g}^{-1}$  DW glucosinolates [110]. The development of hybrid broccoli varieties with higher levels of glucoraphanin, the precursor of the anti-carcinogenic isothiocyanate sulforaphane, by introgression of the wild broccoli ancestor *Brassica villosa* has been described [47, 110, 152]. Hybrid broccoli lines have been licensed to a commercial company for commercialisation, which is expected to occur in the next couple of years (R.Mithen personal communication). Following mild cooking the high glucosinolate broccoli lines produced 3-fold higher levels of sulforaphane than conventional varieties. Extracts from hybrid broccoli lines were tested for their ability to induce quinone reductase in murine hepa1c17 cell lines as a marker for induction of phase II detoxification enzymes in

mammalian cells. Interestingly the level of induction was higher than expected from the glucosinolate content, and it was proposed that this may have arisen from a greater conversion of glucosinolate to sulforaphane, although the mechanism resulting in increased sulforaphane production was not clear [110]. In a recent human trial the effect of consumption of standard broccoli (cv. Marathon) and the high glucosinolate broccoli variety on gene expression in human gastric mucosa was examined using Affymatrix whole genome microarrays. Gastric mucosal biopsy samples from volunteers who consumed the high glucosinolate broccoli showed significant up-regulation of a number of phase II detoxification enzymes, whilst only one such gene was significantly up-regulated by consumption of standard broccoli [54].

As noted earlier the epithiospecifier protein (ESP), together with ferrous iron, plays an important role in directing hydrolysis of glucosinolates towards nitrile rather than isothiocyanate formation. In a study in *Arabidopsis thaliana*, enhanced sulforaphane nitrile production was observed in transgenic plants over-expressing ESP compared to wild type plants [189]. The ESP gene from broccoli has been cloned and the recombinant protein expressed in *E. coli* [103]. A polyclonal antibody to the recombinant protein was used to examine ESP expression in members of the Brassicaceae. Reactive bands indicating ESP activity were found in broccoli and cabbage, but not in daikon or horseradish. Since daikon and horseradish do not produce nitriles as breakdown products of glucosinolates the clear implication is that the presence of ESP is required for the formation of nitriles rather than isothiocyanates. In addition the authors examined ESP activity in floret tissue of 20 commercial broccoli varieties using an assay based on hydrolysis of epi-progoitrin. There was a considerable variation across varieties with levels of ESP ranging from 17.1 to 46 (expressed as mole percentage of epithionitrile formed from epiprogoitrin). Glucoraphanin content and fractional sulforaphane formation were also determined and a significant negative correlation between ESP activity and sulforaphane formation was reported [103]. The wide variability in ESP activity levels in broccoli varieties suggests that it may be possible to develop broccoli lines with reduced ESP activity and thus enhanced potential for sulforaphane production by traditional breeding approaches.

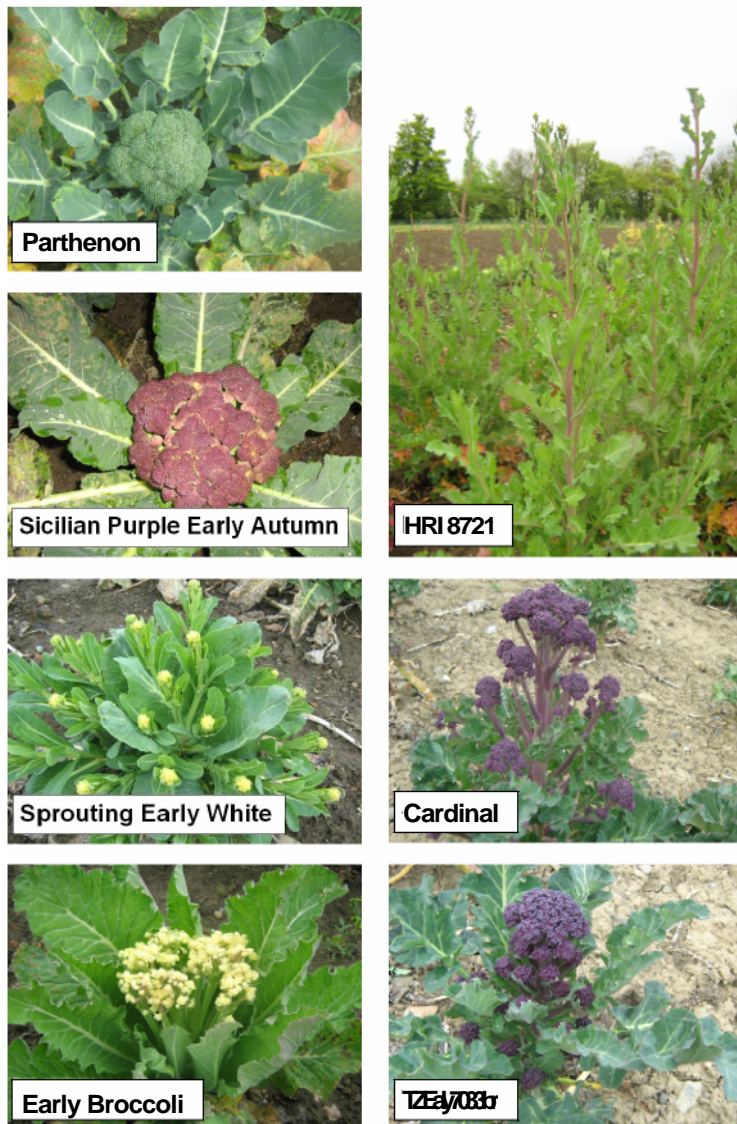
### **Functional fruits and vegetables:**

A number of nutritional and clinical studies based on a high dose intake of single phytochemicals identified as having a health promoting effect from epidemiological and *in vitro* studies have been carried out, however results have largely indicated that dietary supplements were not as effective as increased consumption of fruit and vegetables that a reduced risk of cancer is more closely related to a diet rich in multiple phytochemicals rather than with high levels of a single phytochemical [6, 73]. Some antioxidants such as epigallocatechin gallate have been shown to have pro-oxidant activity and exhibit potential carcinogenicity under certain conditions and this area requires further study. Antioxidant phytochemicals may in fact exert anti-proliferative effects on cancer cell lines via pro-oxidant activity e.g. generation of hydrogen peroxide and other reactive oxygen species (ROS), which *in vivo* could have a harmful effect on healthy cells. However, pro-oxidant activity may also function in some instances to produce hydrogen peroxide as a signaling molecule, resulting in the up-regulation of antioxidant enzymes such as catalase, superoxide dismutase and peroxidases. These data indicate that phytochemicals may show complex interactions or have synergistic effects with other components (reviewed in [89, 171]). For example co- administration of quercetin and isorhamnetin has been found to increase uptake of both in rats and in Caco-2 cells [124].

Fruits and vegetables are already “functional foods”, however there is considerable potential to increase their health promoting effects by promoting consumption of plant groups and plant tissues known to be rich in important phytochemicals, and by nudging plant metabolism towards increased synthesis and retention of particular phytochemicals during cultivation and storage. Edible sprouts represent an excellent opportunity to develop phytochemically enriched foods. In the USA a number of products based on glucoraphanin and/or sulforaphane enhanced broccoli sprouts have been developed and patented including BroccoSprouts<sup>R</sup>, Brassic<sup>R</sup> tea with SGS <sup>TM</sup> (“sulforaphane glucosinolate”), and a supplement Xymogen Oncoplex SGS [16]. Similar products are now available in Europe. In Australia and New Zealand a “Vital Vegetable” project has developed optimized varieties, cultivation and storage procedures for enhanced phytochemical content in vegetable crops and the first product, a high sulforaphane broccoli called Booster<sup>TM</sup> is now available commercially in Australia and New Zealand [1].

**Figure 9.1 Diversity of broccoli (*B.oleracea* L. var. *italica*).**

Anti-clockwise from top: Green heading type (cv. Parthenon), Purple heading type (cv. Sicilian Purple Early Autumn HRI accession 8626), White sprouting types (cv. Sprouting Early White HRI accession 3545 and Early Broccoli HRI accession 3612), Purple sprouting types (cv. TZ 7033 and Cardinal), Traditional landrace (HRI accession 8721).



**Table 9.1** Major Glucosinolates described in broccoli (*Brassica oleracea* L. var. *italica*).

Glucosinolate	Systematic name	Proportion of total glucosinolates (%)	Major hydrolysis product (isothiocyanate, nitrile or oxazolidine)
<i>Aliphatic and Alkenyl Glucosinolates – methionine precursor</i>			
Gluoraphanin	4-methylsulfinylbutyl glucosinolate	10 - 55%	Sulforaphane (isothiocyanate)
Glucoalysin	5-methylsulphinylpenyl glucosinolate	0- 1.6%	5-methylsulphinylpenyl isothiocyanate
Glucoiberin	3-methylsulfinylpropyl glucosinolate	0.7 - 14.8 %	Iberin (isothiocyanate)
Gluconapin	3-butenyl glucosinolate	0 - 7.8%	3-butenyl isothiocyanate
Glucobrassicinapin	4-Pentenyl glucosinolate	2.3%	4-Pentenyl isothiocyanate
Sinigrin	2-Propenyl glucosinolate	0– 1.5%	Allyl isothiocyanate
Glucoerucin	4-methylthiobutylglucosinolate	0– 1.2%	Erucin (isothiocyanate)
Napoleiferin	2-Hydroxy-4-pentenyl glucosinolate	5.5%	Oxazolodine-2-thiones
Progoitrin	2-Hydroxy-3-butenyl glucosinolate	0.1 - 15.8%	5-Vinyloxazolidine-2-thione
<i>Aromatic Glucosinolates –phenylalanine or tyrosine precursor</i>			
Gluconasturtiin	2-Phenylethyl glucosinolate	3.1%	Phenethyl isothiocyanate (PEITC)
<i>Indole Glucosinolates – tryptophan precursor</i>			
Glucobrassicin	Indol-3-ylmethyl glucosinolate	8.6 – 57.5%	Indole-3-carbinol
Hydroxyglucobrassicin	4-Hydroxyindol-3-ylmethyl glucosinolate	0.4 - 1.6%	Indole-3-carbinol
Methoxyglucobrassicin	4-Methoxy-3-indolylmethyl glucosinolate	1.4 - 3.9%	Indole-3-carbinol
Neoglucobrassicin	N-Methoxy-3-indolylmethyl glucosinolate	1.6–42.6%	Indole-3-carbinol



**Table 9.2** Predominant glucosinolates in commonly consumed members of the Brassicaceae.

Plant name	Binomial name	Diploid chromosome number	Predominant glucosinolate	Reference
Broccoli	<i>B. oleracea</i> var. <i>italica</i>	2n = 18	Glucoraphanin Glucobrassicin	[22, 71, 87, 112, 175, 178]
Cauliflower	<i>B. oleracea</i> var. <i>botrytis</i>	2n = 18	Glucobrassicin Sinigrin Glucoiberin	[22, 87, 112, 178]
Brussels sprouts	<i>B. oleracea</i> var. <i>gemmifera</i>	2n = 18	Glucobrassicin Sinigrin	[22, 87, 164, 178]
Cabbage	<i>B. oleracea</i> var. <i>capitata</i>	2n = 18	Sinigrin Glucobrassicin Glucoiberin	[24, 76, 87, 112, 178]
Savoy cabbage	<i>B. oleracea</i> var. <i>sabauda</i>	2n = 18	Glucoiberin	[76]
Kale	<i>B. oleracea</i> var. <i>acephala</i>	2n = 18	Sinigrin Glucobrassicin Glucoiberin	[22, 24, 87, 176, 178]
Kohlrabi	<i>B. oleracea</i> var. <i>gongylodes</i>	2n = 18	Glucoerucin	[22]
Collard	<i>B. oleracea</i> var. <i>viridis</i>	2n = 18	Glucobrassicin	[22]
Chinese kale	<i>B. oleracea</i> var. <i>alboglabra</i>	2n = 18	Gluconapin	[112, 167]
Turnip	<i>B. rapa</i> subsp. <i>rapa</i>	2n = 20	Progoitrin Gluconasturtiin	[20, 76, 92]
Pak-choi	<i>B. rapa</i> subsp. <i>chinensis</i>	2n = 20		
Oriental cabbage	<i>B. rapa</i> subsp. <i>pekinensis</i>	2n = 20	Progoitrin	[112]
Oil seed rape	<i>B. napus</i> subsp. <i>oleifera</i>	2n = 38	Progoitrin	[164]
Leaf rape	<i>B. napus</i> subsp. <i>pabularia</i>	2n = 38	Glucobrassicinapin	[23]
Swede	<i>B. napus</i> subsp. <i>napobrassica</i>	2n = 38	Progoitrin	[76]
Mustard	<i>B. juncea</i>	2n = 36	Sinigrin	[22]
Radish	<i>Raphanus sativus</i>	2n = 18	Glucoerucin	[21]
Watercress	<i>Nasturtium officinale</i>	2n = 32	Gluconasturtiin	[48]
Rocket	<i>Eruca sativa</i>	2n = 22	Glucoerucin	[25]
White mustard	<i>Sinapis alba</i>	2n = 24	Sinigrin	[164]

Horseradish

*Armoracia rusticana*

$2n = 32$

Sinigrin

[93]

**Table 9.3** Major phenolic compounds reported in carrot, broccoli and onion compiled using the Phenol Explorer database [134], ‘nr’ indicates the compound has not been reported in references used to compile the database.

Polyphenol class		Sub-class	Carrot (mg/100g FW)	Broccoli - Green (mg/100g FW)	Onion - Yellow (mg/100g FW)	Onion- Red (mg/100g FW)
<b>Total Phenolics:</b>			8.21 - 156.00	25.02-337.00	16.80- 180.84	81.50- 126.00
<b>Flavonoids</b>	<b>Flavonols</b>	Kaempferol	0.00-0.60	3.08 -7.20	0.00- 1.98	0.00-4.50
		Myricetin	0.00-0.40		0.00 - 4.13	0.00- 3.20
		Quercetin	0.00- 1.50	1.50 -13.70	2.81 -46.32	5.55- 45.00
	<b>Flavones</b>	Luteolin	0.00-0.80	n.r.	n.r.	0.00- 1.10
		Apigenin	n.r.	n.r.	n.r.	0.00-2.10
	<b>Anthocyanins</b>	Cyanidin 3-O-(6"- malonyl-3"-glucosyl- glucoside)	n.r.	n.r.	n.r.	1.00- 1.00
		Cyanidin 3-O-(6"- malonyl-glucoside)	n.r.	n.r.	n.r.	1.50- 1.50
		Delphinidin 3-O- glucosyl-glucoside	n.r.	n.r.	n.r.	6.50-6.50
		4-Hydroxybenzoic acid	5.5–5.5	n.r.	n.r.	n.r.
		Protocatechuic acid	0.13 - 0.13	n.r.	1.00- 1.00	2.00- 2.00
<b>Phenolic acids</b>	<b>Hydroxybenzoic acids</b>	Syringic acid	1.00e-02 - 1.00e-02	n.r.	n.r.	n.r.
		Vanillic acid	0.89 - 0.89	n.r.	n.r.	n.r.
		Caffeic acid	14.00- 14.00	n.r.	n.r.	n.r.
		p-Coumaric acid	0.14- 0.14	n.r.	n.r.	n.r.
	<b>Hydroxycinnamic acids</b>					

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