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Health Benefits of Broccoli. Influence of Pre- and Post-Harvest Factors on Bioactive Compounds

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ABSTRACT

Broccoli (*Brassica oleracea* L. var. *italica*) is a well recognized health-promoting vegetable due to its high beneficial compound content. Numerous epidemiological studies indicate that Brassicas, in general, and broccoli in particular, protect humans against some diseases since they are rich sources of glucosinolates as well as possessing a high content on flavonoids, vitamins and mineral nutrients. Glucosinolates are characteristic *Brassicaceae* compounds that, when the tissue is damaged, are hydrolysed by myrosinase to biologically active isothiocyanates such as sulforaphane, indole-3-carbinol and phenethyl isothiocyanate, the most responsible compounds of the anticancer activity of broccoli. Also, other phytochemicals, such as phenolic compounds and vitamin C have demonstrated antioxidant activity that protect against free radicals such as reactive oxygen species in the human body so that these have also been strongly associated with a reduced risk of chronic diseases. Accordingly, many studies have been done in order to determine the different factors that could affect these bioactive compounds. These factors could be classified into pre- and post- harvest aspects. The first group implies agronomic and environmental conditions or genetic and ontogenic factors. Thus, genotype, temperature, light radiation, fertilization, irrigation water, as well as age and harvesting time are the most important pre-harvesting factors, which may affect the bioactive composition of broccoli. The post-harvest group of factors such as packaging, storage, preservation, transport, and cooking processes have been widely reviewed and thus this review is focused on the wide bioactive content of broccoli and how the pre-harvest and post-harvest factors affect these health promoting phytochemicals.

Keywords: *Brassica oleracea* var. *italica*, glucosinolates, minerals, phenolic compounds, vitamin C

Abbreviations: AA, ascorbic acid; **CA**, controlled atmosphere; **CYP**, cytochrome; **DHA**, dehydroascorbic acid; **ESP**, epithiospecifier protein; **GLS**, glucosinolate; **GPX**, Gluthathione peroxidase; **GST**, glutathione-*S*-transferase; **ITC**, isothiocyanates; **I3C**, indole-3-carbinol; **MAP**, modified atmosphere packaging; **RGR**, relative growth rate; **RH**, relative humidity; **SFN**, sulforaphane; **SOD**, superoxide dismutase

CONTENTS

INTRODUCTION

Broccoli is a cultivated relative form of the wild cabbage (*Brassica oleracea*) belonging to the mustard family (*Brassicaceae*, syn. *Cruciferae*). This dicotyledonous plant was botanically identified by some authors as *Brassica oleracea* var. *italica* (Gray 1982; Hu and Quiros 1991; Malatesta and Davey 1996). The word ‘broccoli’ derives from the Latin root word *brachium* which means branch.

Native to the eastern Mediterranean and Asia Minor, broccoli was cultivated in Italy in ancient Roman times and was introduced into France in about the 16th century, was unknown in England until 1720, and in the United States began its tentative commercial cultivation in the 1920s, and today, broccoli is grown in nearly every state, including Alaska and Hawaii, though California remains the major producer. The U.S. per capita fresh broccoli consumption has followed a mostly increasing trend over the last two decades increasing from 0.6 kg to 2.7 kg from 1980 to 2004 (AgMRC 2005). Studies on traditional phytotherapy in central Italy showed that leaf juice was used as a cure against warts (Guarrera 2005). Nowadays, the consumption of broccoli is widespread in Europe (Eurostat). The broccoli exports from Murcia (SE Spain) are destined to other coun-

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Invited Review

tries in the EU, and the highest volume of sales covers October to March. Abstracting the results of the Sept-2005/Aug-2006 season, the total exported broccoli from Murcia was almost 59,000 MT, a 13% higher rate than the previous year, meaning a 11% of the total production of vegetables from the area (Murcia, Spain) that is mainly sent to the EU (95% of total product) with only 3 countries receiving 72% of the total: United Kingdom (36% of the total export), Germany (25%), and The Netherlands (11%) (PROEXPORT 2006).

Broccoli is a fast-growing, upright, branched, annual plant, 60-90 cm tall whose green flower buds (inflorescences) are edible. Besides broccoli (*B. oleracea* [Italica Group]), the *Brassicaceae* family includes vegetables that are commonly grown and widely consumed such as Brussels sprouts (*B. oleracea* [Gemminifera group]), cabbage and kale (*B. oleracea* [Capitata group]), collards (*B. oleracea* [Acephala group]), mustard (*Sinapis* sp.), rape (*Brassica napus*), etc.

The WCRF (World Cancer Research Fund) estimated 11 million new cancer cases occur annually around the world; also cancer causes almost seven million deaths a year, a number that is on the increase. They observed that improving the diet could prevent about 50% of all breast cancer cases, 75% of stomach cancer cases or 75% of colorectal cancer cases. Eating at least five portions of vegetables and fruits each day could, in itself, reduce cancer rates by 20%, and eating healthily, plus staying physically active and maintaining a healthy weight, could cut cancer risk by 30-40%. The foundation expert panel behind these reports estimated that 30-40% of cancers are directly linked to our diets and related factors, such as maintaining a healthy weight and staying physically active (http://www.wcrf-uk.org).

BIOACTIVE COMPOUNDS PRESENT IN BROCCOLI

Phytochemicals are biologically active compounds found in plants in small amounts that could contribute significantly in protection mechanisms against some diseases, although they are not established as essential nutrients. Many studies report a strong inverse relationship between the intake of crucifers and the risk for many cancers and the health care potential of bioactive compounds (Hooper and Cassidy 2006). In broccoli, we can find chemopreventive bioactive compounds (i.e., isothiocyanates (ITCs), hydroxycinnamic acids, etc.) against degenerative diseases over lifetime and certain types of cancer (Dreosti 2000). Cruciferous vegetables are an excellent dietary source of phytochemicals including glucosinolates (Keck and Finley 2004; Zareba and Serradelf 2004), natural antioxidants – phenolic compounds and vitamins – (Podsedek 2007), as well as dietary essential minerals (Finley *et al.* 2001). Since the content for these broccoli components varies significantly, it may not be easy to advise the general public on how much vegetable to include in their diet (McNaughton and Marks 2003).

Attention to cancer prevention by natural products is increasingly growing. The inverse relationship between active components present in cruciferous vegetables and *in vitro* and *in vivo* carcinogenesis has been associated with lower risk of lung and colorectal cancer in some epidemiological studies (Hecht 2000; Murillo and Mehta 2001; Talalay and Fahey 2001; Kristal and Lampey 2002), but there is evidence that genetic polymorphisms may influence the effect of cruciferous vegetables on human cancer risk. Although glucosinolates hydrolysis products may alter the metabolism or activity of sex hormones in ways that could inhibit the development of hormone-sensitive cancers, evidence of an inverse association between cruciferous vegetable intake and breast or prostate cancer in humans is limited and inconsistent (for a review see, Higdon *et al.* 2007).

The consumption of diets containing 5 to 10 servings of fruits and vegetables daily is the foundation of public health recommendations for cancer prevention, and tumor growth reductions were associated with reduced proliferation and increased apoptosis using a combination of tomato and broccoli (5% tomato plus 5% broccoli in the diet) in Copenhagen rats and prostate adenocarcinomas, being more effective than either tomato (10%) or broccoli (10%) alone, and supports the public health recommendations to increase the intake of a variety of plant components (Canene-Adams *et al.* 2007). Recommendations for cruciferous vegetables have not been established. Three to five servings of broccoli a week provides better cancer prevention than consuming one serving or less a week (Keck and Finley 2004). Many organizations, including the National Cancer Institute, recommend the consumption of five to nine servings (2.5-4.5 cups) of fruits and vegetables daily, but separate recommendations for cruciferous vegetables have not been established. Much remains to be learned regarding cruciferous vegetable consumption and cancer prevention, but the results of some prospective cohort studies suggest that adults should aim for at least 5 weekly servings of cruciferous vegetables (Giovannucci *et al.* 2003; Higdon *et al.* 2007).

Cruciferous vegetables and cancer risks have been the target of several comprehensive reviews. These features are not the focus for this review, but it is remarkable that *in vitro* and *in vivo* studies have reported that isothiocyanates affect many steps of cancer development including modulation of phase I and II detoxification enzymes (Bogaards *et al.* 1994; Jiao *et al.* 1996; Talalay and Fahey 2001), functioning as a direct antioxidant (Zhu *et al.* 2000a; Zhu and Loft 2001, 2003) or as an indirect antioxidant by phase II enzyme induction (Hayes and McLellan, 1999; Talalay and Fahey 2001; McWalter *et al.* 2004), modulating cell signalling (Xu and Thornalley 2001), induction of apoptosis (Yu *et al.* 1998; Chiao *et al.* 2002; Yang *et al.* 2002), control of the cell cycle (Yu *et al.* 1998; Zhang *et al.* 2003; Wang *et al.* 2004) and reduction of *Helicobacter* infections (Fahey *et al.* 2002). The most characterized glucosinolate compounds are sulforafane, phenethyl isothiocyanate, allyl isothiocyanate and indole-3-carbinol (Hecht 2000), but many other isothiocyanates that are present in lower quantities also may contribute to the anti-carcinogenic properties of crucifers (for a review, see Higdon *et al.* 2007).

Glucosinolates

The glucosinolates (GLS) belonging to organosulphur phytochemicals (**Fig. 1**), are β-thioglucoside-N-hydroxysulphates (Fahey *et al.* 2001; Moreno *et al.* 2006a). The structural features common for glucosinolates are the β-D-thioglucopyranoside group attached to C-0 as *cis* or *Z*- in the *N*-hydroximine sulphate functional group known as the glucosinolates group, with semi-systematic names of individual glucosinolates based on the name for the R- and R′-groups used as a prefix to the word glucosinolate (Bellostas *et al.* 2007). Glucosinolates are classified by their amino acid precursor, glucosinolates derived from alanine (Ala), leucine (Leu), isoleucine (Ile), methionine (Met) or valine (Val) are called aliphatic, those derived from phenylalanine (Phe) or tyrosine (Tyr) are called aromatic, and those derived from tryptophan (Trp) are called indolic. The R groups of most glucosinolates are extensively modified from these precur-

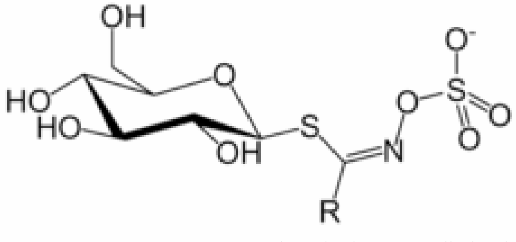

Fig. 1 Structure of glucosinolates. Glucosinolates are alkyl-*N*-hydroximine sulphate esters with a β-D-glucopyranoside group attached to the hydroximine carbon in *Z*-configuration to the sulphate group (Fahey *et al.* 2001; Bellostas *et al.* 2007).

sor amino acids, with methionine undergoing an especially wide range of transformations (Fahey *et al.* 2001). The sulphate group is strongly acidic, and these hydrophobic compounds are normally sequestered in vacuoles of most plants as potassium salts (Kelly *et al.* 1998; Grubb and Abel 2006). In addition, glucosinolates have only been localized in the phloem and in the interior of cells, which suggests that the respective concentrations in the cell wall solution are extremely low or essentially zero (Muller and Riederer 2005). The chemical structure, biosynthesis, stereo-chemistry, occurrence and metabolism in plants has been recently revised (Grubb and Abel 2006; Bellostas *et al.* 2007).

When cells in the plant are damaged, glucosinolates are hydrolyzed by the plant cytosolic enzyme myrosinase (EC 3.2.3.1), a thioglucoside glucohydrolase. The breakdown products are glucose and unstable aglycone that can rearrange to form isothiocyanates, nitriles and other products (Rask *et al.* 2000). The most important glucosinolate hydrolysis products in many species are isothiocyanates. Glucosinolate breakdown products are related with the plant's mechanism of defense against both invertebrate and vertebrate herbivores and could be considered as natural pesticides but the relationship between plants and herbivore often turns out to be very complex (Pracros *et al.* 1992).

Glucosinolates are known to elicit responses from *Brassica* herbivores in laboratory studies. To study their importance in interactions with herbivores in the field, glucosinolates profiles and levels of herbivory were ascertained for wild cabbage plants growing in four neighboring populations in the UK. Glucosinolate profiles differed between plant populations, but not between different habitats withing populations. Within habitats, there was no link between individual plant glucosinolates profiles and herbivory by *Pieris* spp., slugs and snails, flea beetles or aphids. Plants attacked by the micromoth, *Selania leplastriana*, contained higher levels of 2-hydroxy-3-butenylglucosinolate and 3-indolylmethylglucosinolate than plants within the same population that were not attacked, and it was concluded that the differences in the glucosinolates profiles between the plant populations are unlikely to be due to differential selection pressures from herbivores feeding on the mature plants over the two years studies (Moyes *et al.* 2000). Glucosinolates did not reduce food assimilation or growth after 1 day of experimentation with young larvae of *Tenebrio molitor* (yellow mealworm), but they caused some inhibition of respiratory exchanges and increased CO_2/O_2 (RQ ratio) (Pracros *et al.* 1992). The flea beetle, *Phyllotreta cruciferae* Goeze, and the diamondback moth, *Plutella xylostella* L., (both crucifer specialists) fed at equal rates on *Brassica juncea* and its low-glucosinolate lines, indicating that these species are insensitive to sinigrin and suggesting that their pest status on low-glucosinolate lines of *B. juncea* will likely remain unchanged (Giamoustaris and Mithen 1995; Bodnaryk 1997). The majority of studies exploring interactions between above- and below-ground biota have been focused on the effects on the root-associated organism on foliar herbivorous insects, but the effects on foliar herbivory by *Pieris brassicacee* L. (Lepidoptera, Pieridae) on the performance of the root herbivore *Delia radicum* L. (Diptera, Anthomyiidae) and its parasitoid *Trybliographa rapae* (Westwood) (Hymenoptera, Figitidae), mediated through a shared host plant *Brassica nigra* L. (Brassicaceae) showed that foliar herbivory affected *D. radicum* and *T. rapae* decreasing significantly their survival more than 50%. The foliar herbivores can affect the development not only of root-feeding insects but also their natural enemies (Soler *et al.* 2007).

Human beings are also sensitive to the strong flavours of glucosinolate breakdown products that are important determinants of flavour in a variety of commercially important Brassicas. Harsh or bitter notes were partly associated with increased levels of total glucosinolates (Fenwick *et al.* 1983a; van Doorn *et al.* 1998; Engel *et al.* 2002). To identify a potential relationship between the bitter and pungent notes and the total glucosinolates, their concentrations were determined and reported in broccoli (Schonhof *et al.* 2004). The amount of total glucosinolates was calculated from the sum of alkyl glucosinolates (glucoraphanin, glucoiberin, glucoerucin, glucoibervein), alkenyl glucosinolates (progoitrin, sinigrin, gluconapoleiferin, gluconapin), indole glucosinolates (glucobrassicin, neoglucobrassicin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin) and the aryl glucosinolate gluconasturtiin. The samples with the highest glucosinolate values (>400 mg/100g f.w.) had the most intense pungent and bitter notes of the score plot. Low values for bitter and pungent notes were measured in the samples with total glucosinolate concentrations below 20 mg/100g f.w. (Bruckner *et al.* 2005).

As food components, glucosinolates and their degradation products (isothiocyanates, thiocyanates, nitriles and epithionitriles) have been recognized for long for their distinctive benefits to human nutrition and plant defence. The term 'functional food' describes foods that, if they are normal dietary constituents, can provide sufficient amounts of bioactive components that are valuable for health improvement. In order to acquire the full benefit of functional foods, it is necessary to know the natural variation in content of bioactive food components (Dekker and Verkerk 2003; Jeffery *et al.* 2003). Such variation might be regulated genetically or it might result from changes in the growing environment or from differences in post-harvest handling, processing, storage or food preparation (later on in this review).

Natural antioxidants

The major natural antioxidants in Brassica foods are vitamins C and E, carotenoids, and phenolic compounds, especially flavonoids (Yao *et al.* 2004; Podsedek *et al.* 2006). Vitamin E and carotenoids quench singlet oxygen (Krinsky 2001; Choe and Min 2005), and flavonoids as well as vitamin C show a protective activity to α-tocopherol in human LDL, and they can also regenerate vitamin E from the α-chromanoxy radical (Davey *et al.* 2000; Zhu *et al.* 2000b). Nutrient antioxidants may act together to reduce the level of reactive oxygen species (ROS) more effectively than single dietary antioxidants, because they can function as synergists (Wang *et al.* 1996; Eberhardt *et al.* 2000; Rossetto *et al.* 2002; Trombino *et al.* 2004). In addition, a mixture containing both water-soluble and lipid-soluble antioxidants is capable of quenching free radicals in both aqueous and lipid phases (Chen and Tappel 1996). The significant variability in the concentration of the antioxidant phytochemicals of broccoli suggested that genotypes with enhanced content of dietary antioxidants can be developed through genetic manipulation and plant breeding (Jagdish *et al.* 2006) as well as with the use of crop management strategies (Schreiner 2005).

Vitamin C, which includes ascorbic acid (AA) and its oxidation product dehydroascorbic acid (DHA), has many biological activities in the human body. The biological function of L-ascorbic acid can be defined as an enzyme cofactor, a radical scavenger, and as a donor/acceptor in electron transport at the plasma membrane (Davey *et al.* 2000; Lee and Kader 2000).

Dehydroascorbic acid (DHA), the oxidation product of AA, is unstable at physiological pH and it is spontaneously and enzymatically converted to 2,3-diketogulonic acid (Davey *et al.* 2000). According to Gokmen *et al.* (2000), DHA was the dominant form of vitamin C in cabbage accounting for 78.5% of the total vitamin C content. In contrast to this report, Vanderslice *et al.* (1990) observed that the contribution of DHA to the total vitamin C content was 14% or 8% in cauliflower and broccoli, respectively. These authors did not find DHA in fresh cabbage. Those values were in agreement with that reported for broccoli by Vallejo *et al.* (2003b), i.e. the contribution of DHA to the total vitamin C content was *ca*. 11%. The studies on antioxidants of *Brassica* vegetables have been focused mainly on broccoli florets, which are popular in Western Europe countries, USA and India (Vallejo *et al.* 2002a, 2003e; Jagdish *et al.*

Table 1 Ranges of concentrations of the natural antioxidants found in broccoli samples of different origins (mg/100 g edible portion)

Type of source	**AA**	**Vitamin C (AA+DHA)**	**β-carotene**	**Phenolic compounds**			**Total Phenolics**	**Reference**
				Flavonoids	**Caffeoyl-quinic derivatives**	**Sinapic and ferulic derivatives**		
Broccoli heads [1]	54.0-119.8		0.37-2.42					Kurilich *et al.* 1999
Broccoli [2]	34-146		0.28-1.92					Podsedek 2007
Broccoli florets [3]		25.5-82.3	0.48-1.13				44.5-82.9	Jagdish *et al.* 2006
Broccoli florets [4]		43.1-146.3		1.23-6.54	0.76-3.82	2.54-8.25		Vallejo *et al.* 2002a
Broccoli florets [5]		72.2-122.6		6.8-97.0	2.35-15.09	5.73-20.14		Vallejo *et al.* 2003e

[1] Range of value from fifty-one commercial hybrids produced in USA
[2] Data collected from different countries
[3] Six commercial hybrids/varieties of broccoli produced in India
[4] Fourteen commercial and experimental hybrids/varieties, produced in Spain
[5] Eight commercial and experimental cultivars, produced in Spain

2006). Ascorbic acid (AA) contents of *Brassica* vegetables (mg/100 g edible portion) showed variable ranges as follows: broccoli (34-146), Brussels sprouts (76-192), white cabbage (18.8-47), kale (92.6-186), and cauliflower (17.2-81) (Podsedek 2007 and references therein). Using 14 commercial and experimental lines, Vallejo *et al.* (2002a) found vitamin C concentrations ranging from 43.1 mg per 100 g f.w. ('Lord', commercial cultivar) to 146.3 mg per 100 g f.w. in 'SG-4515' (experimental cultivar), being notably higher than the average reported by other authors (~100 g mg/100 g edible portion; Lee and Kader 2000). Ranges of concentrations in broccoli of ascorbic acid are shown in **Table 1**.

In addition to AA and DHA, *Brassica* vegetables include ascorbigens, which are formed as the result of the reaction between AA and degradation products of indol-3-ylmethylglucosinolates produced in myrosinase-catalysed degradation (Buskov *et al.* 2000). According to Buskov *et al.* (2000), generally, 30-60% of the indol-3-ylmethylglucosinolates in *Brassica* plants are transformed into ascorbigens. With regard to AA, Hrncirik *et al.* (2001) suggested that the decrease of AA content, as a result of its transformation into ascorbigen, will probably not reach more than 10% during processing of *Brassica* vegetables.

More than 85% of dietary vitamin C is supplied by fruits and vegetables (Lee and Kader 2000). In general, broccoli contains high levels of AA (**Table 1**). Vitamin C levels were significantly affected by cultivar, season, fertilization (15 and 150 kg S/ha) and all interactions except for Season × Fertilisation. Therefore, no significant differences were noted in vitamin C when comparing different fertilization at early (sowing in December) or late (sowing in March) season in SE Spain. The levels were also irregular, ranging 64.1-121.7 using eight commercial and experimental cultivars of broccoli (Vallejo *et al.* 2003e).

Phenolic compounds are a large group of secondary metabolites widespread in the plant kingdom. Broccoli is a good source of flavonol and hydroxycinnamoyl derivatives (Vallejo *et al.* 2002a). The main identified flavonol glycosides present in broccoli florets are quercetin and kaempferol 3-*O*-sophoroside, representing up to 90% of the total flavonoids content (**Table 1**). The flavonoids content in 'Marathon' and 'Lord' broccoli was around 6 mg/100g f.w. edible portion. Three minor glucosides of these aglycones have been also detected, namely isoquercitrin, kaempferol 3-*O*-glucoside and kaempferol diglucoside (Plumb *et al.* 1997; Price *et al.* 1998; Vallejo *et al.* 2004). The hydroxycinnamoyl derivatives are from sinapic, ferulic and caffeic acids (**Table 1**). The predominant hydroxycinnamoyl acids esters were identified as 1-sinapoyl-2-feruloylgentiobiose, 1,2-diferuloylgentiobiose, 1,2,2′-trisinapoylgentiobiose, and neochlorogenic acid (Vallejo *et al.* 2003d). In addition, 1,2′-disinapoyl-2-feruloylgentiobiose and 1,2-disinapoylgentiobiose, 1-sinapoyl-2,2-diferuloyl gentiobiose, isomeric form of 1,2,2′-trisinapoylgentiobiose, and chlorogenic acid were also found in broccoli (Price *et al.* 1997; Vallejo *et al.* 2003e).

Phenolic compounds play an important role in the visual appearance of food, just as anthocyanin, the pigments responsible for most of the blue, purple, red and intermediate colour of plant-derived foods appear 'black' in some commodities, or the monohydroxyphenols and orthodihydroxyphenols, plant polyphenol oxidase substrates, that produce brown polymers, generally leading to a decrease in quality; they are also relevant in food taste and flavour, as they can play a role in the bitter (coumarins, oleuropein, flavanone neohesperidosides), sweet (dihydrochalcones), pungent (capsicins, curcuminoids) or astringent (procyanidins, ellagitanins) taste of some products and can also contribute to aroma, especially, simple volatile phenols (Tomas-Barberan and Espin 2001).

Some studies evidenced the possible role of phenolic compounds in health benefits due to their antioxidant and antitumoral properties (Sun *et al.* 2002; Alía *et al.* 2006a, 2006b; Khanduja *et al.* 2006), special attention has been mainly paid to caffeic acid derivatives (caffeic acid, chlorogenic acid, etc.) (Feng *et al.* 2005) and flavonols (mainly quercetin and its derivatives) (Vijayababu *et al.* 2005; Kim *et al.* 2006). The antioxidant capacities of different Brassicaceae members expressed by differences in total phenolic content and antioxidant activity assays is widely reported (Podsedek 2007; Surveswaran *et al.* 2007). As seen with seeds of *Brassica* sp. seeds (0.15-0.47 g/100 g dry weight of total phenolics correlated to ABTS, DPPH and FRAP assays (Surveswaran *et al.* 2007). The antioxidant and antiproliferative activities of common fruits has been also related to their phenolic composition (Sun *et al.* 2002). The struture-radical scavenging activity relationships of a large number of representative phenolic compounds (e.g., flavonols, phenolic acids, isoflavones, tannins, etc.) were attributed to structural differences in hydroxylation, glycosylation and methoxylation. The *ortho*-dihydroxy groups were the most important strutural feature of high activity for all tested phenolic compounds (Cai *et al.* 2004, 2006).

Dietary polyphenols like quercetin and rutin are considered beneficial because of their potential protective role in the pathogenesis of multiple diseases associated to oxidative stress such as cancer, coronary heart disease and atherosclerosis. The quercetin and rutin concentrations (0.1-100 μM) applied to HepG2 cells (human hepatoma cell lines) indicated that both natural antioxidants induce favourable changes in the antioxidant defense system of cultured HepG2 that prevent or delay conditions which favaour cellular oxidative stress (Alía *et al.* 2006a, 2006b). Quercetin and kaempferol contents in broccoli varied from 1.4 to 8.1 mg/100 g f.w. and from 3.6 to 21.3 mg/100 g f.w. respectively (Gliszczynska-Swiglo *et al.* 2007). The antioxidant activity of flavonoids – quercetin (Q) and kaempferol (K) – has been suggested to contribute to several health benefits associated with the consumption of fruits and vegetables. The presence of different numbers of –OH moieties on the B-ring of the flavonols may contribute to their antioxidant activity as well as their toxicity and may play an important role in their potency for biological action such as angiogenesis and immune-endothelial cell adhesion, which, respectively, are important processes in the development of cancer and atherosclerosis (Kim *et al.* 2006).

Chlorogenic acid, the ester of caffeic acid with quinic acid (in Broccoli, **Table 1**) could stimulate the nuclear translocation of Nrf2 (NF-E2-related factor) as well as subsequent induction of GST activity, evidencing that chlorogenic acid could protect against environmental carcinogen-induced carcinogenesis and suggesting that the chemopreventive effects of chlorogenic acid may be through its up-regulation of cellular antioxidant enzymes (Feng *et al.* 2005).

Polyphenols have been shown to induce apoptosis in a variety of tumor cells. However, their action on normal human peripheral blood mononuclear cells (PBMCs) during oxidative stress was evaluated using caffeic acid and ferulic acid (present in broccoli, **Table 1**), and they significantly inhibited DNA damage and lipid peroxidation in PBMCs (Khanduja *et al.* 2006).

Mineral nutrients

Humans require various mineral elements, some are required in large amounts (Na, Ca, K, Mg, Cl, N, P, S) and others are required in trace amounts (Fe, Zn, Cu, I, Se). All these mineral elements mainly enter the food chain through plants as soluble inorganic ions and as organic compounds or inorganic salts, in both soluble and insoluble forms (White and Broadley 2005).

Broccoli contribute high levels of minerals, however, they are likely to be affected by cultivar, environment and type of inflorescence. A wide number of plant-based foods contain calcium, but the amount of calcium, provided per 100 g or per serving, and its bioavailability vary considerably. The bioavailability of calcium from a food is influenced by the presence of a number of other compounds within a food, including fat (reduces absorption), protein and phosphorus (both increase absorption). As a result, calcium in plant foods is not generally readily absorbed, although there are exceptions such as broccoli, which contains lower concentrations of these interfering compounds (Fishbein 2004). The bioavailability of calcium from milk and milk products is in the region of 30% compared to 5% from spinach. The number of servings of broccoli needed to equal 240 g milk is 71 g (Theobald 2005, and references therein).

BIOLOGICAL PROPERTIES OF BROCCOLI PHYTOCHEMICALS

As indicated in previous section, cruciferous foods own a wealth of bioactive compounds (vitamins, carotenoids and other polyphenolics), although, the anticarcinogenic activity of the crucifers (i.e., broccoli) is attributable to their glucosinolate content. The glucosinolates are relatively inert, from a biological point of view, but they can be hydrolysed to give a wide range of bioactive compounds such as isothiocyanates (ITCs) and indoles, as a result of the action of the plant myrosinase (Juge *et al.* 2007). In the absence of plant myrosinase, the glucosinolates-to-isothiocyanate conversion is mediated by bowel microflora (Shapiro *et al.* 2001). Isothiocyanates (ITCs) are potentially anticarcinogenic phytochemicals fromed from the metabolism of glucosinolates and are found in cruciferous vegetables as well as a select number of other foods (Steck *et al.* 2007).

The carcinogenic agents/compounds may be classified as direct or indirect action agents, the latter being the most common. The activation and detoxification of carcinogens is catalyzed by Phase-I and Phase-II enzymes. Generally, Phase-I enzymes (Cytochrome p450, CYP), catalyze the activation of carcinogens of direct action, while Phase-II enzymes (glutathione-*S*-transferases, GST) catalyze the detoxification of both types of carcinogens. The induction of Phase-II enzymes is proposed as the main mechanism of action of cruciferous food bioactive substances (ITCs), to protect against the development of cancer induced by chemical agents. In rodents, 200 mg of liophylised broccoli per kg induced quinone reductase activity in the colon mucose (Wiseman 2005; Lynn *et al.* 2006).

The CYP1A family of Phase-I enzymes is responsible for the metabolism of procarcinogenic chemical agents of environmental origin and other toxins. Approximately 15% of the drugs used today are metabolized by CYP1A2. Indol-3-carbinol (I3C) is one of the major autolytic breakdown products of indole glucosinolates in *Brassica* plants. Several mechanisms have been suggested to contribute to the anticarcinogenic activities of I3C (Wu *et al.* 2005). The I3C may induce CYP1A2, as recently reported (Hakooz and Hamdan 2007), in a small study involving five Jordanian men (5) and women (5) ingesting 500 g of broccoli per day, with higher activity found in men than in the women (gender effect), something that needs to be demonstrated in more ambitious epidemiological studies.

A phase I trial of I3C in 17 women (1 postmenopausal and 16 premenopaual) from a high-risk breast cancer cohort, was carried out to supply the volunteers with 400 mg I3C daily for 4 weeks followed by a 4-week period of 800 mg I3C daily. The variables measured involved hormonal parameters, CYP1A2 induction, and the determination of the urinary 2-hydroxyestrone/16α-hydroxystrone ratio (2-OHE_1/16α-OHE_1). Comparing the results from the placebo and the 800 mg daily dose period, CYP1A2 was elevated by I3C in 94% of the subjects, with a mean increase of 4.1-fold. The apparent induction of CYP1A2 was mirrored by a 66% increase in the urinary 2-OHE_1/16α-OHE_1 in response to I3C (Reed *et al.* 2005, 2006).

One of the better known bioactives of broccoli, sulforaphane (SFN), obtained by the hydrolysis of its cognate glucosinolate, glucoraphanin, is related in most publications with the cancer-protective effects of eating broccoli and broccoli sprouts, since SFN induces Phase-II enzymes through the activation of the antioxidant response pathway involving Keap1/Nrf2. This is of interest, because SFN is capable of down-regulating the gene expression of CYP3A4 in hepatocytes (Zhou *et al.* 2007).

CYP3A4 is responsible for the metabolism of many pro-toxicants, drugs, and endogenous sterols. SFN inhibits CYP3A4 gene expression mediated by the steroid and xenobiotic receptor (SXR, also called "hPXR"). SFN is the first described natural antagonist for SXR. Because the induction of CYP3A4 may result in adverse responses to certain drugs (lack of efficacy), that may result in a public health problem. This discovery is an important step in the design and development of new approaches from diet and therapeutics to reduce the frequency of non-desirable interactions of drugs (Zhou *et al.* 2007).

SFN protects cells from oxidative damage by the addition of NF-E2 p45-related factor 2 (Nrf2), mediated by antioxidant enzymes and also possesses *in vitro* antibacterial activity against *Helicobacter pylori* (stomach ulcer and cancer). Broccoli sprouts are a rich source of sulforaphane-glucosinolate glucoraphanin, converted enzymatically to SF after ingestion of fresh sprouts, demonstrating a positive effect on infected mice and human subjects treated with a high-salt diet. Forty people infected by *H. pylori* were randomly assigned to diets containing a daily dosage of 100 g of broccoli (BS) and alfalfa sprouts (AS) for 2 months. The nutritive composition was almost identical, while only difference was the phytochemical density: BS contained 250 mg SFN/100 g portion size, while AS has neither SFN nor glucoraphanin. The bacterial colonization was studied using the urea breath test (UBT) and by measuring fecal antigen (HpSA). The degree of gastritis was evaluated with serum pepsinogen I, II and I/II (PGI and PGII). All the parameters were determined at the beginning and after 1- and 2-month intervention studies, and 2 months upon finishing the intervention. The results showed that 2-month intervention with BS, but not with AS, diminished significantly the UBT, HpSA, PGI and PGII levels, but there was not 100% eradication of the bacterial infection. The values could back up to initial levels 2 months after the treatment. Therefore, the daily ingestion of BS rich in SFN suppresses the colonization of *H. pylori* in infected humans, so a diet rich in parental glucosinolate (glucoraphanin), may be useful for the chemoprevention of gastric cancer (Galan 2003, 2004; Gamet-Payrastre 2006).

Certain ITCs, i.e. SFN, are potent monofunctional inducers of Phase-II enzymes although the majority of crucifers contain a range of glucosinolates exerting a variable range of modulator effect on detoxification enzymes. For example, broccoli is the main dietary source of SFN. The sulforaphane content (µg/g, fresh weight) in various tissues of broccoli decreased as follows: Edible florets 12.9 > stalks 5.1 > leaves 1.5 (Liang *et al.* 2006). The considerable levels of glucobrassicin (1.3-19.1 µmol/g d.w., Vallejo *et al.* 2002a), which can be hydrolyzed to indol-3-carbinol (I3C) is also of interest to improve health benefits from diet (Higdon *et al.* 2007). In the presence of an acidic environment in the stomach, I3C may suffer reactions of condensation to form dimers, trimers, tetramers, and oligomers. Totally opposite to SFN, I3C and its degradation products from acid condensation induce both Phase-I and Phase-II enzymes (Aggarwal and Ichikawa 2005). Then, I3C under acidic conditions (stomach) is converted to different oligomeric compounds (i.e., 3,3′-diindolylmethane). *In vitro*, 3,3′-diindolylmethane suppress the proliferation of different cancerous cells (breast, prostate, endometrial, colon and leukemia), blocks apoptosis and the cell cycle in G1/S; besides, it inhibits cyclin-dependent kinases (CDK2, 4 and 6), and other proteins of the cell cycle and the antioxidant cell defense systems (through Nrf2, nuclear factor-E2-related factor 2). *In vivo*, I3C has been reported as a potent chemopreventive agent against hormone-dependent cancers (breast and cervical cancer). The effects of I3C are multiple and extensively reviewed by (Aggarwal and Ichikawa 2005): Induction of apoptosis; inhibition of DNA-adducts; suppression of the production of free radicals; stimulation of the 2-hydroxylation of stradiol; stimulation of angiogenesis; and hepato-protective (Wallig *et al.* 2005).

The role of cruciferous vegetables as cancer chemopreventives draws support from a large body of experimental and epidemiologic data. It is also clear that isothiocyanates, which are a major component, have biological properties that act to reduce carcinogen-induced DNA damage. On balance, epidemiologic studies demonstrate the modification of ITC effects by GST, particularly in the lung and colon, which can be predicted based on the metabolic pathway. Taken together, this evidence supports a biological role for ITC in preventing cancer, and underlines the benefit of including cruciferous vegetables as part of a balanced diet (Srám 1998; Seow *et al.* 2002, 2005).

The evidence obtained from studies with animal models indicate generally that the induction of Phase-I and Phase-II enzymes altered by the consumption of *Brassica* foods result in a favorable metabolic profile for the elimination of certain chemical carcinogens. In experimental animals cruciferous vegetables have been shown to inhibit chemically-induced colon cancer Although, it is not clear if a similar chemopreventive effect will take place in free-living human subjects, whom, unlike experimental animals, are exposed to a chronic low dosage of a wide range of carcinogenic agents. The benefit of a modification in the metabolizing enzyme equilibrium (Phase I and II) can not be predicted, and it will depend on the exposure of an individual to a given carcinogen (Lynn *et al.* 2006). Phase I and Phase II xenobiotic-metabolising enzyme families are involved in the metabolic activation and detoxification of various classes of environmental carcinogens. Particular genetic polymorphisms of these enzymes have been shown to influence individual cancer risk. A brief overview was presented by Schoket *et al.* (2001) of the relationship between metabolic genotypes and internal dose, biologically effective dose and cytogenetic effects of complex and specific genotoxic exposures of human study populations, particularly on DNA-adducts derived from polycycle aromatic hydroxicarbons (PAHs). The formation of DNA adducts in human hepaptoma cells (HepG2 cells) and human hepatocytes exposed to PhIP (C^{14}-labeled 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) was examined using co-treatments with SFN (1-10 µmol), or the flavonoids, quercetin (5-20 µmol). The dietary isothiocyanates and flavonoids modulate Phase I and Phase II enzyme expression, hence increasing the rate of detoxification of the dietary carcinogen PhIP in human HepG2 cells but do not affect the rate of PhIP-DNA adduct repair. The formation of PhIP-DNA adducts in human hepatocytes was also dose-dependent with PhIP-concentration and the levels of protection by SFN or quercetin were up to 60% after 10 nmol PhIP treatment, but showed large inter-individual variation with no observed protection in some individuals (Bacon *et al.* 2003).

Epidemiological evidence of an inverse relationship between broccoli ingestion and cancer

There are substantial epidemiological evidences of an inverse association between the consumption of cruciferous foods and the risk of cancer, more consistent in the case of lung, stomach and colorectal cancers. Early studies measured the exposure to isothiocyanates (ITCs) inducing a higher frequency in the ingestion of the individual isolated ITC from a plant-based food, or the mix of different ITCs in a given food. Recent studies use a more exhaustive analysis of data of the ingestion of ITCs from different food sources incorporating known amounts of ITCs with the data of questionnaires of diet control. The development and validation of the total levels of ITCs in urine as a biomarker of exposure is supposed to be a useful tool to clarify the association between ITCs and disease in epidemiological studies (Seow *et al.* 2005).

Currently, it is widely accepted that ITCs exert their chemopreventive function by modulating biotransformation enzymatic pathways; firstly because of the induction of Phase-II enzymes, and because of that, they improve the clearance of activated carcinogens. Mixed sprouts (broccoli, radish, clover and alfalfa) (Gill *et al.* 2004) administered to HT29 cells, and in parallel, to healthy volunteers, males and females, during 14 days (113 g/day of the mixed sprouts) to measure the effects on DNA damage in lymphocytes, glutathione-*S*-transferase (GST) activity, glutathione peroxidase (GPX), and superoxide dismutase (SOD), antioxidant level (plasma iron reducing capacity, uric acid, ascorbic acid and α-tocopherol), blood lipids, lutein and lycopene. A significant antigenotoxic effect was observed against cell DNA-damage caused by oxidative stress in HT29 colorectal cancer cell model and *in vitro* human lymphocytes although the authors did not observe any induction of detoxification enzymes or effects on plasma markers (*in vivo*). These results support the theory that cruciferous food consumption is related with a reduction in the risk of cancer through the diminished damage to cell DNA (Gill *et al.* 2004).

The GST activity in humans, via the antioxidant response element (ARE), through a pathway which involves the Nrf2 factor and increases synthesis of GST, catalyzes the conjugation of glutathione to ITCs, and ITCs are among the substrates most rapidly conjugated by GSTs, while this reaction is reversible, enzyme kinetics suggests that the equilibrium favours the conjugation (Lampe and Peterson 2002; Seow *et al.* 2005). This pathway goes to the formation of N-acetyl-serine conjugates (dithiocarbamates) which are excreted in urine. In humans, genes coding for the major GST isoenzymes have been identified, and polymorphisms at these loci characterized. The homozygous deletion of GSTM1 gives rise to the null phenotype in which there is no expression of the GSTM1 protein, whereas in other cases (e.g., GSTP1) the polymorphism may result in a low activity allele (Zhang *et al.* 1995). The prevalence of the null genotype varies between ethnic groups, 27 to 53% for GSTM1 and 20 to 47% for GSTT1. The genetic polymorphisms for GST are also relevant because a high GST is beneficial for the inhibition of chemical carcinogenesis. The null GSTM1 and GSTT1 individuals excreted ITCs more slowly, and this may cause an accumulation of

ITCs in target organs and activate other Phase-II enzymes, or exert other anticarcinogenic effects blocking cell cycle, or inducing apoptosis in cells with damaged DNA (Seow *et al.* 2002). It is worth considering that the null-GST individuals will be less prepared to conjugate and excrete these compounds, so they will have higher levels of ITCs in their tissues that could probably improve the protective effect. The study and research work on this path, design and carrying out a careful epidemiological study comparing the protective effect on non-null against null individuals for GSTs will help to explain these issues and will reinforce the role of ITCs as major chemopreventive bioactive compounds of *Brassica* foods, and improving our knowledge of the factors influencing the biological properties of these compounds at the cellular and metabolic level. Seow *et al.* (2005), summarized epidemiologic studies of cruciferous vegetable/isothiocyante intake, GST genotype and cancer risk. Using urinary ITC as maker of exposure, there are 4 studies on lung cancer. The use of dietary cruciferous vegetable and ITC intake (high *versus* low) there are also 2 studies on colon cancer. Broccoli intake and urinary ITC in breast cancer are also reported (2 studies) as well as the effect of cruciferous vegetable intake on colorectal adenomas and squamous cell cancers of the head and neck. In general, results are clearer to support the relationship between GST and ITC for lung cancer, with more limited evidence for colorectal cancer. Nevertheless, in the case of lung cancer, evidence shows that the relationship is more marked in smokers than in ex-smokers or non-smokers. To date, the epidemiological data did not clarify the chemoprotective effects of ITCs on human subjects compared to the results of the animal assays. This is the biggest problem for the current and future research: the validation of the results obtained from experimental animal models to design clear and concise human trials to be carried out correctly (Lynn *et al.* 2006).

DETERMINING FACTORS OF BIOACTIVE COMPOUNDS PRESENT IN BROCCOLI

In broccoli, the content of glucosinolates, minerals, antioxidant vitamins and flavonoids varies with genotype, environment and processing (Fenwick *et al.* 1983b; Jeffery *et al.* 2003). The main factors that influence the levels of these phytochemicals in vegetables are cultivation, storage and processing conditions (Dekker *et al.* 2000; Lee and Kader 2000; Oerlemans *et al.* 2006). Understanding the mechanism by which environmental, post-harvest and processing affect the production of bioactive components can lead to genetic control of these factors, and the development of a high health-promoting activity product (Jeffery *et al.* 2003). Thus, all these factors could be classified in two general groups: pre-harvest and post-harvest conditions.

Influence of preharvest strategies and conditions

The cause for the reported variation in different bioactive compounds across a number of broccoli experimental and commercial cultivars and lines might be related to differences in genotype, or might include effects of the environment, changing with the farm soil, season or harvest conditions (Jeffery *et al.* 2003; Abercrombie *et al.* 2005; Charron *et al.* 2005).

A model was developed to predict the effects of environmental conditions on growth, yield and quality characteristics of broccoli. As an example of a quality characteristic, the content of the GLS glucoraphanin was estimated. Carbohydrates produced by photosynthesis are transformed into different substances of the biomass at different carbohydrate use efficiencies depending on the ratio of available carbohydrates to potential crop growth, or related state variables. Parameters were estimated and the model was tested based on experiments in greenhouses at different levels of temperature and irradiance. The model explained a large part of the variation in total plant dry matter (94%), yield (72%), dry matter content (73%) and glucoraphanin content (79%). However, the model still needs the validation under field conditions, and the addition of further quality characteristics and environmental effects (Kläring *et al.* 2001).

The diversity in levels of potential bioactive compounds reported in broccoli suggested that its health-promoting properties are significantly dependent on the cultivar selected (Vallejo *et al.* 2002a). Glucoraphanin level in broccoli seed is largely determined by genotype, ranging from 5 to 100 µmol/g seed, regardless of the environment in which seed was produced. Although significant environmental and genotype × environment effects were observed for glucoraphanin and a significant genotype × environment effect was observed also for glucoiberin, these effects were small compared to the genotype effects (Farnham *et al.* 2005).

Genetic factors have a direct influence on all compounds of vegetables. Moreover, the broccoli types differed in their content of glucosinolates (in % of major glucosinolates). The alkyl glucosinolates were mainly found in green broccoli type (broccoli, 47% glucoraphanin; white cauliflower, 22% glucoiberin, green pyramidal cauliflower (romanesco type), 21% glucoraphanin) (Schreiner 2005). The GLS profiles could be different over multiple environments and the contribution of genotype, environment and genotype by environment interactions on total phenotypic GLS variability among lines is central to cultivar recommendation for cancer chemoprotection and are important considerations for plant breeders (Abercrombie *et al.* 2005). Of particular concern are qualitative changes in GLS profiles that could result in production of compounds that lack chemoprotective activity or could reduce palatability of broccoli. Evaluation of a subset of 10 accessions grown over 4 years (environments) allowed to determine the extent to which glucosinolate content varies with genotype and environment (**Table 2**). Synthesis of aliphatic glucosinolates was clearly regulated by genotype (60%), with environmental and environment × genotype components exerting smaller effects (5% and 10%, respectively). In contrast, the effects of genotype (12%), environment (33%) and environment × genotype (21%) on the content of indolyl glucosinolates appeared reversed, with regulation being primarily due to non-genetic causes. The results emphasize the importance of using multiple environments for genotype evaluation and cultivar development (Brown *et al.* 2002).

Intact glucosinolates in broccoli were evaluated in order to determine variations in amount and type across cultivars grown under similar conditions (Vallejo *et al.* 2002a, 2003c). The predominant glucosinolates in all the analysed cultivars were 4-methylsulphinylbutyl glucosinolate (glucoraphanin) and indol-3-ylmethyl glucosinolates (glucobrassicin), representing 39.5 and 53.9% respectively, while 4-OH-glucobrassicin and *N*-methoxyglucobrassicin represented only 3.8 and 2.8% respectively. The diversity in levels reported (**Table 2**) suggests that the potential health benefits from cruciferous vegetables are greatly dependent on the cultivar selected. Moreover, previous studies have suggested that enhancing the levels of glucosinolates in cruciferous vegetables through conventional breeding or genetic engineering can be expected to improve the chemopreventive properties of these vegetables (Fenwick *et al.* 1983a, 1983b; Fahey *et al.* 2001).

In addition to the genetic influence, ecophysiological factors such as the climate parameters of irradiation, temperature, and water and nutrition supply, have a strong influence on the phytochemical composition of vegetables. All factors are responsible for the wide variation in the formation and content level of phytochemicals at pre-harvest and varying phytochemical contents at harvest (Schreiner 2005).

The effect of the increase in plant density on broccoli commercial characteristics is marked by a decreased commercial spear (inflorescence plus a portion of stem 10 cm

Table 2 Ranges of concentrations of glucosinolates found in broccoli of different origins

Broccoli source	Organ	Cultivation practices	Glucoraphanin	Total Aliphatic-GLSs	Total Indole-GLSs	Total GLS	Units	Reference
Ten accessions [1]	Edible portion	Field, spring and autumn crop	1.5-22.9	1.3-26.3	0.7-5.9		mmol/g dry weight	Brown *et al.* 2002
Fourteen cultivars [2]	Florets	Field, spring crop	1.3-8.3			3.0-28.3	µmol/g dry weight	Vallejo *et al.* 2002a
Three broccoli types [3]	Heads	Field, autumn crop	6.6-39.7	10.3-42.4	9.9-15.2		mg/100g fresh weight	Schonhof *et al.* 2004
Three cultivars [4]	Edible parts	Field, spring and autumn crops	0.3-14.9	5.3-13.8	6.7-14.9	18.9-25.2	µmol/g dry weight	Charron *et al.* 2005

[1] Two broccoli F5 inbreds (Ev6-1(F6) and Eu8-1(F6), two doubled haploids, Su003 and VI-158, five commercial hydrid cultivars, 'Baccus', 'Brigadier', 'High Sierra', 'Majestic' and 'Pirate', and one landrace, 'Broccolette Neri E Cespuglio' (USA)

[2] Commercial cultivars 'Marathon', 'Lord', 'Monterey', 'Pentahtlon', 'Vencedor', 'Furia', and experimental lines 'Z-2724', 'SG-4515', 'SG-4514', 'I-9905','I-9904','I-9903','I-9809' (Spain)

[3] Green spear, crown type and purple broccoli ('Emperor', 'Shogun', 'Marathon', 'Viola', Chinese broccoli) (Germany)

[4] Commercial cultivars 'Brigadier', 'Emperor', 'Bubbles' (USA)

long) weight (but it was due to the stem portion of the spear and not to the edible portion). As plant density increased, and consequently the erectness of the upper leaves and stem length increased, the degree of shading increased, the net assimilation rate (NAR) decreased and the leaf area ration (LAR) increased. This compensatory change between NAR and LAR, kept the relative growth rate (RGR) for individual plants almost constant (Francescangeli *et al.* 2006).

Glucoraphanin is one of the most abundant glucosenolates present in broccoli and its cognate ITC is SFN, a potent inducer of mammalian detoxification (Phase II) enzyme activity and anti-cancer agent, as already shown previously in the review. There were significant environmental and genotype-by-environment effects on levels of glucoraphanin and quinine reductase induction (Phase II enzyme) potential of broccoli heads; however, the effect of genotype was greater than that of the environmental factors. The glucoraphanin concentration and quinone reductase induction potential were positively and significantly correlated with one another and also with days from transplant to harvest. The development of a broccoli phenotype with a dense head and a high concentration of glucoraphanin to deliver maximum chemoprotective potential (high enzyme induction potential/glucoraphanin content) is a feasible goal (Farnham *et al.* 2004). Because glucoraphanin and other GLSs in cruciferous crops are important for cancer chemoprotection, climatic conditions should be considered when planning planting dates or when making breeding selections for GLS concentration (Abercrombie *et al.* 2005; Charron *et al.* 2005).

The climate effect of irradiation was also observed for the indole glucosinolates on broccoli (**Table 2**). The result have implications for quality-oriented production and crop management strategies in warmed up autumn periods aiming to optimize health-promoting substance content in broccoli under low radiation conditions. The alkyl glucosinolates glucoraphanin and glucoiberin increased 8-fold and ascorbic acid 0.5-fold, whereas the main indole glucosinolate glucobrassicin was reduced (Schonhof *et al.* 2007a).

Temperature and irradiation (photosynthetic photon flux), with lower influence of the photoperiod, affected glucosinolates and myrosinase in leaves, stems and roots of rapid-cycling base populations (RCBP) of short life cycle used as a model system of *Brassica oleracea* (Charron and Sams 2004). Aliphatic and total glucosinolates in leaves where higher in leaves at 12°C (44%) and 32 °C (114%) than at 22°C, with constant light of 300 µmol/m^2/s. An inverse relationship of total glucosinolates in leaves and in roots was found at different temperatures, so it could be that the glucosinolates were mobilized from roots to shoots at stress-inducing environmental conditions that influence the glucosinolates-myrosinase system (Charron *et al.* 2005).

Irradiation intensity has a definite influence on flavonoids metabolism. The effect of solar radiation on the quercetin and kaempferol contents in the inflorescence of three broccoli cultivars ('Lord', 'Marathon', and 'Fiesta') were highly positively correlated with total solar radiation in the period of planting to harvest of broccoli inflorescences. Quercetin and kaempferol contents varied from 14.3 to 81 mg/kg f.w. and from 35.9 to 213 mg/kg f.w. respectively, of broccoli grown in seasons with different solar radiation (Gliszczynska-Swiglo *et al.* 2007).

In broccoli sprouts, total and individual glucosinolates increased at 30ºC whereas at lower temperature regimes decreased (Pereira *et al.* 2002). Also, in high air temperatures during the head development resulted in an increase of glucosinolate concentration (Radovich *et al.* 2005). Schonhof *et al.* (2004) showed significant differences amongst different broccoli cultivars for total and individual glucosinolates (**Table 2**), over three years of field cultivation in autumn conditions of NE Europe climate. The distinct differences in the individual glucosinolate proportions among the groups were not significantly affected by the weather. Thus, the differences in the glucosinolates pattern among the investigated groups are mainly genetically determined and nearly unaffected by the climate conditions.

The effect of climatic factors may also be the result of different biosynthetic pathways for the numerous glucosinolates groups. The glucosinolates groups derive from different amino acids and have various aglucon structures, which might lead to a diverse sensitivity to temperature and irradiation between the glucosinolates groups (Schreiner 2005).

Crop management strategies of the model crops broccoli, cauliflower and radish demonstrate the possibility to enhance the content of phytochemicals through targeted usage of the ecophysiological factors temperature and irradiation. Thus, the planning of the cultivation period in the annual course combined with the selection of types and cultivars as well at the developmental stage at harvest are the primary means of ensuring consumer-oriented quality production. For the production of glucosinolates-enriched raw plant material for functional foods or supplements, the cultivation of the green coloured broccoli (e.g., 'Marathon' or 'Shogun'), in the spring season marked by relatively low daily mean temperatures (about 14°C) combined with rising daily mean irradiation up to 450 µmol/m^2/s of the photosynthetic photon flux density is recommended (Schreiner 2005).

Broccoli could be produced as a fresh market product characterized by a large anti-oxidative potential due to the high carotenoid content as well as being enriched with the anti-oxidatively effective ascorbic acid (**Table 1**) by selecting the correct time of planting and harvesting. As found for glucosinolates, low daily mean temperatures promoted the synthesis of lutein and β-carotene in broccoli. This temperature effect is also observed for ascorbic acid formation (Schonhof *et al.* 1999). To produce broccoli as a fresh vegetable with a high anti-oxidative potential, fully developed heads originated from spring and autumn cultivation sets should be harvested. Cultivation in summer with daily mean temperatures above 20°C led to a diminution of these anti-oxidatively effective compounds, and therefore should be avoided (Schreiner 2005).

Plant age is one of the major factors affecting the composition of health-promoting compounds. In broccoli, flavonoids increased with the development of the inflorescence, from initial stages of the inflorescence (head initiation) to commercial fresh-cut stage (40-100 mm diameter), maturity (~130 mm diameter), and over-mature heads (>160 mm diameter). Thus, in the first development stage, the highest value in cv. 'Monterrey' was 42 mg/kg f.w. (representing only a 4% of the total flavonoids accumulated at the end of development) and increased until 1043 mg/kg at the last one. At the commercial maturity stage, 713 mg/kg were reached. Although the individual values showed that the content was higher in rich than in poor fertilization, as a general rule, there were no significant differences between poor and rich sulfur fertilization (Vallejo *et al.* 2003a).

The sprouts of cruciferous vegetables are excellent sources of glucosinolates (Fahey *et al.* 1997). The total content of glucosinolates in broccoli (2 µmol/g fresh weight) is much lower than in the sprouts (4 µmol/g fresh weight) (Tian *et al.* 2005). Very few studies have reported the phytochemical composition of edible sprouts (Moreno *et al.* 2006b). The aliphatic glucosinolates profile of broccoli sprouts includes glucoraphanin, glucoerucin, and glucoiberin, and the indole GLSs (glucobrassicin, neoglucobrassicin, 4-methoxyglucobrassicin) are in much less amount than glucoraphanin (>1 µmol/g fresh weight) (Tian *et al.* 2005). A higher concentration of glucoraphanin was detected in the green broccoli heads and flower heads than in other reproductive tissues. However, the highest content of glucoraphanin occurred at the green head stage and then declined as flowering was initiated. The highest concentration of glucoraphanin occurred in young broccoli seedlings and seeds. This information should be useful for the development of those compounds as nutraceuticals (Rangkadilok *et al.* 2002a). An accumulation of sulforaphane and vitamin C from early head initiation through commercial maturity was also observed (Omary *et al.* 2003). The different behaviors among glucosinolates, phenolic compounds, and vitamin C during developmental stages could be an interesting tool to determine the optimum harvest stage, depending on the desired compound.

Mineral nitrogen nutrition is considered as the most important growing factor, determining yield and quality of broccoli (Babik and Elkner 2002). These authors studied the effect of nitrogen fertilization (rates of 100, 200, 400 and 600 kg N per ha) and irrigation (either natural precipitation or irrigation when soil moisture dropped to the level at which soil suction exceeded 30 kPa) on broccoli quality and they found more attractive green colour but incidence of hollow stem with higher N rates and irrigation. The contents of nitrates in broccoli heads increased too, when high nitrogen rates were applied. Irrigation lowered the contents of nitrates, whereas the level of sugars, ascorbic acid and β-carotene did not change as compared to broccoli from non irrigated treatments.

To obtain information to generate recommendations for fertilizing broccoli with potassium, greenhouse experiments with increasing levels of K (0, 70, 140, 210 mg/kg soil; 9 kg soil per pot) were carried out using broccoli under fertigation. The mean yields of broccoli under fertigation were 33.5% higher than conventional management. There was a greater absorption of K under fertigation because of more adequate supply of water, which helped to economize water and fertilizer (Vidal-Martínez *et al.* 2006).

The source of nutrition available to plants can affect yield and quality, and reduce input costs, as in the case of using organic fertilizers with broccoli (cow, poultry, pig and rabbit manures), without reducing dry matter yield (11.1-12.1 g/100 g) or ascorbic acid contents (34.4-53.2 mg/100 g fresh weight). The use of organic materials may provide opportunities for broccoli production (Sanwal *et al.* 2006).

Fertilization practices could greatly influence the content of bioactive compounds in broccoli plants even if some controversy exists about its effects. Vallejo *et al.* (2003c, 2003e) showed that, in general, there were significantly more total glucosinolates and phenolic compounds under sulphur-rich fertilization (150 Kg ha^{-1} Ca_2SO_4 (13% S)) than under sulphur-poor fertilization (15 Kg ha^{-1} Ca_2SO_4 (13% S)), whereas vitamin C was not affected by sulphur fertilization. A similar experiment but with higher sulphur (S) concentration (S was applied as gypsum (anhydrous calcium sulphate, 23% S) at rates of 50 Kg ha^{-1} for low and 200 Kg ha^{-1} for high S treatment), showed that there were significant genotypic differences for the content of both S and glucoraphanin in all plant organs at different growth stages with gypsum applications. Sulphur present in glucoraphanin accounted for only 4-10% of total S content in broccoli heads. However, S present in glucoraphanin in mature seeds accounted for 40-46% of the total S in the seeds of moderate and high glucoraphanin cultivars ('Marathon' and TB-234). Differences in S uptake, S distribution between organs, and partitioning of S into glucoraphanin largely explained the differences in glucoraphanin content in the green heads and mature seeds for the cultivars of broccoli and the S treatments (Rangkadilok *et al.* 2004).

Moreover, it has been demonstrated that in broccoli, N supply should always be considered in combination with the application of S, so that an optimal N supply could only be beneficial when sufficient S is available to allow the synthesis of S-containing substances such as glucosinolates. The N:S ratios between 7:1 and 10:1 promoted plant yield and enhanced overall appearance, and the total glucosinolate concentrations were high at insufficient N supply, independent of the S level, and low at insufficient S supply in combination with an optimal N supply. This was mainly due to the presence of the alkyl GLSs glucoraphanin and glucoiberin. Furthermore, with S concentrations above 6 g/kg d.w. and an N:S ratio lower than 10:1, the GLS concentrations were on average around 0.33g/kg fresh weight and differed significantly from those plants characterized by an S concentration below 6g/kg dry weight and a N:S ratio above 10:1 (Schonhof *et al.* 2007b).

Broccoli sprouts fertilized with S (0, 14.6, 29.2 mg/l) or N (0, 45.5, 91.0 mg/l) showed detrimental effects of mineral supply on the levels of aliphatic GLSs (aerial part, 11-28 µmol glucoraphanin/g d.w.; roots 1-4 µmol glucoraphanin/g dry weight) whereas the opposite was noted for indole and aromatic glucosinolates, for some of the fertilization combinations tested. Overall, the results indicate that broccoli sprouts did not benefit from fertilization (Aires *et al.* 2006).

Zinc fertilization could also influence changes in individual glucosinolates, while gluconapin decreased, glucobrassicin and 4-methoxyglucosbrassicin increased with increasing Zinc levels (Coolong *et al.* 2004).

Selenium (Se) and Se-enriched foods have been investigated rigorously, but the enrichment of foods with Se has been done without consideration of interactions with other nutritive and/or non-nutritive components (Finley *et al.* 2001). However, reports of a novel interaction between Se and glucosinolates in broccoli provide an example of an unintended consequence of manipulation of a single bioactive compound. In an effort to determine how variety, stress, and production conditions affect the production of secondary plant compounds that have bioactivity (glucosinolates and phenolics acids), broccoli was grown in the greenhouse with and without selenium (Se) fertilization, and in the field under conventional or organic farming procedures and with or without water stress. The HPLC-MS analysis aided to separate and identify 12 primary phenolics compounds. Variety had a major effect: there was a preponderance of flavonoids in the 'Majestic' variety, but hydroxycinnamic esters were relatively more abundant in the 'Legacy' variety. Organic farming and water stress decreased the overall production of phenolics. Se fertilization increased glucosinolates in general, and sulforaphane in particular, up to a point; above that Se fertilization decreased glucosinolate production, and changed the profile and decreased the total amount of polyphenols. The selection of one bioactive component (Se) may decrease the content of other bioactive components such as phenolics and glucosinolates (Robbins *et al.* 2005; Finley 2005), and produced mixed responses in terms of amino acid content (Lee *et al.* 2005).

In light of the above, Farnham *et al.* (2007), studied the differences in Se concentration per head and total Se head content for a collection of broccoli hybrids (20) and inbreds (15) grown in field environments, without supplemental Se fertilization, to assess the relative importance of genotype vs. environment in affecting Se levels and to determine if Se content is associated with other important horticultural traits. When analysed over three environments, there was a significant genotype effect for Se head concentration with hybrids, but not inbreds, but the environmental effect was about 10 times larger than that for genotype. Total Se content (ng/head) varied significantly among hybrids and inbreds, but as with concentration, environmental effects were also much larger for this trait. Head Se concentrations for hybrids ranged from 52.7 to 84.7 ng/g and total Se accumulation ranged from 563 to 885 ng/head. The same respective traits ranged from 49.3 to 80.0 ng/g and 678 to 876 ng/head for inbreds. There was no correlation between Se head concentration and head dry mass or days from transplant to maturity for either hybrids or inbreds. There was no evidence that Se might be diluted in broccoli heads as mass increases with cultivars that produce dense heads. It should be feasible to combine relative high Se concentration or content with high head dry matter, a phenotype that broccoli breeders might strive to achieve (Farnham *et al.* 2007).

To date, there are almost no reports on the effect of methionine fertilization on the glucosinolate content in vegetable crops. Since the metabolite methionine is a precursor of alkyl and alkenyl glucosinolate synthesis in broccoli, Scheuner *et al.* (2005a, 2005b) hypothesized that fertilization with methionine a Sulphur-containing amino acid, will increase the content of glucosinolates. Methionine was applied in five different concentrations (10, 30, 60, 90, 150 mg/plant) at the developmental stage of head formation. The percentage of the alkyl glucosinolates on total glucosinolates were on average 80%. The alkyl glucosinolate concentration increased in the highest methionine treatment by 16% due to significant increases of glucoraphanin and the cultivar specific glucosinolate glucoiberin, while glucoalyssin and glucoerucin remained unaffected. Twenty percent of the detected glucosinolates were indole glucosinolates, which were not changed by the methionine treatment (Scheuner *et al.* 2005a). In a different experiment, broccoli plants were sprayed with DL-methionine (895 mg dissolved in 30 ml distilled H_2O) at head formation, and head sizes of 4-5 cm and 5.5-7.5 cm, and the methionine foliar fertilization increased the glucosinolate content in broccoli heads. The best effects were obtained when methionine was applied at the time of head formation (Scheuner *et al.* 2005b).

A reduced water supply could lead to increased contents of phytochemicals. For instance, in the case of broccoli, less irrigation caused the glucosinolates content to double (Paschold *et al.* 2000).

Bañuelos *et al.* (2003) studied the tolerance and accumulation of Se and Chlorine (Cl) in different varieties ('Emerald City', 'Samurai', 'Greenbelt', 'Marathon') of broccoli (*B. oleracea* L.) irrigated with water of the following different qualities: (1) non-saline [electrical conductivity (EC) of <1 dS/m; (2) Cl/sulfate salinity of similar to 5 dS/m, 250 μg Se/l, and 5 mg B/l; and (3) non-saline and 250 μg Se/l. One hundred and ten days after transplanting, plants were harvested and dry weight yields and plant accumulation of Se, B, and Cl was evaluated in floret, leaf, and stem. Irrespective of treatments floret yields from var. Samurai were the lowest among all varieties, while floret yields from var. Marathon was the only variety to exhibit some sensitivity to treatments. For all varieties, plant Se concentrations were greatest in the floret (up to 51 mg/kg dry weight) irrespective of treatment, and B and Cl concentrations were greatest in the leaves; 110 mg B/kg and 5.4% Cl-, respectively. At post harvest, treatment 2 (with salinity, B, and Se) increased soil salinity to almost 6 dS/m, total Se concentrations to a high of 0.64 mg/kg dry weight soil, and water soluble B concentrations to a high, of 2.3 mg B/l; soluble Se concentrations were insignificant. The results indicate that var. Emerald City, Greenbelt, and Marathon should be considered as recipients, of moderately saline irrigation enriched with Se and B under field conditions.

In coastal regions of Mediterranean areas, summer crops are often irrigated with saline water. As a consequence, salts may accumulate in the root zone, damaging the following winter crops if the rainfall is insufficient to leach them. Residual salts from the summer irrigations and salt-induced permanent modifications of the soil physical–chemical properties may both affect yield and mineral composition of non-irrigated winter crops such as cauliflower and broccoli (de Pascale *et al.* 2005).

The evidence is increasing that the consumption of bioactive compounds in broccoli and Brassicas reduces the risk of cancer. The possibilities of designing foods that will help reduce the risks of specific cancers have been a great impetus to the 'functional food' industry (Finley 2005). However, there are still questions to answer on the factors influencing the phytochemical quality of plant-based foods from the farm to the table.

Influence of postharvest practices for quality and composition of broccoli

In this group of conditions, we could include factors that influence plant-based food quality after harvesting, such as storage, packaging or cooking (Tomas-Barberan and Espin 2001; Rosa *et al.* 2002; Vallejo *et al.* 2003b). Recently, the influence of postharvest procedures on quality and glucosinolate content in broccoli has been reviewed by Jones *et al.* (2006) who described the effects of commonly used postharvest handling procedures of temperature, relative humidity, storage under controlled atmosphere (CA) or modified atmosphere packaging (MAP) and processing including cooking, on glucosinolate content in broccoli heads. Fresh broccoli contains a wide range of phytochemicals including glucosinolates, flavonols and carotenoids. There are many ways in which produce is treated after harvest, and it is not yet known whether the best postharvest practices for maintaining quality will result in the optimum retention of phytochemicals. Cooking is often neglected as an important process in the postharvest chain stretching from harvest to ingestion, and recent research has shown that cooking method has perhaps the most profound effect on glucosinolate on broccoli. To date, a generally agreed recommendation on cooking method that retains phytochemical content and bioefficacy has not been widely circulated (Jones *et al.* 2006).

Fresh broccoli is highly perishable after harvest with visual quality best maintained by rapid cooling to temperatures <4°C and placing heads in packaging designed to preserve high relative humidity >90% (Song and Thornalley 2007). Glucosinolate levels mirror visual quality in broccoli as they generally decrease during postharvest handling, with low temperatures (<4°C) clearly slowing the loss of both quality and glucosinolates. Glucoraphanin content in broccoli florets declined by 82% after 5 days at 20°C, but by only 31% at 4°C (Rodrigues and Rosa 1999). Similarly, Rangkadilok *et al.* (2002b) reported a 50% decrease in glucoraphanin in 'Marathon' heads after 7 days at 20°C, but no decrease after 7 days at 4°C. A high relative humidity (RH) of 98-100% is recommended to maintain postharvest quality in broccoli (Toivonen and Forney 2004). It appears, therefore, that if broccoli is kept cold (i.e., less than 4°C) there may be no benefit in maintaining 100% humidity, but if broccoli is kept at 20°C it is necessary to maintain high RH with packaging to retain both visual and glucosinolate content (Jones *et al.* 2006).

Broccoli heads stored at temperatures of 1 or 4°C, 99% RH, for 2, 7, 14 or 28 days to simulate domestic and export transport conditions, after the removal from cool storage, heads were then placed at 8, 15 or 20°C, with 99, 90 or 70% RH, respectively, for 3 days to simulate marketing conditions. At the end of both phases, visual quality declined significantly with increasing temperature and length of storage, caused primarily by increasing yellowing and loss of turgor. Glucoraphanin, quercetin and kaempferol contents were not significantly affected by storage and marketing temperature and time. Therefore, current transport and marketing practices are not likely to have a deleterious effect on the levels of aliphatic glucosinolates and flavonols in broccoli (Winkler *et al.* 2007).

Broccoli was stored for 7 days at different temperatures (12-22°C), did not show a significant decrease in glucosinolate content although the visual appearance started to decay; at domestic refrigerator temperatures (4-8°C) glucosinolate content decreased, and at −85°C a significant loss of glucosinolates was observed due to freeze–thaw fracture of plant cells and accessibility of myrosinase to glucosinolates. Similar losses of vitamin C and sulforaphane were observed when broccoli was chilled at 6°C and 95% R.H. for 35 d and stored at -18°C for 60 d, mainly due to the blanching step (Galgano *et al.* 2007).

Controlled atmosphere (CA) storage is very effective in maintaining broccoli quality, and can double postharvest life (Toivonen and Forney 2004). The effect of CA storage on GLS content in broccoli, however, remains unclear. 'Marathon' broccoli heads stored for 25 days at 4°C, under a CA atmosphere of 1.5% O_2 contained significantly higher glucoraphanin levels than heads stored in air at the same temperature (Rangkadilok *et al.* 2002b). Optimum broccoli quality was maintained when held in a controlled atmosphere of 10% CO_2 and 5% O_2 more than held in air (Eason *et al.* 2007).

It is often difficult to maintain low temperatures throughout the broccoli distribution and marketing phase, and in fluctuating temperatures, modified atmosphere packaging (MAP) can help extend shelf life of broccoli, when atmospheres within MAP reached 1-2% O_2 and 5-10% CO_2 (Jacobson *et al.* 2004). In comparison with the glucosinolate content of freshly harvested broccoli, glucoraphanin content of 'Marathon' broccoli heads stored for 7 days at 1°C under MAP using 11 µm low-density polyethylene (LDPE) bags decreased by approximately 48% (Vallejo *et al.* 2003b). A further 17% was lost after 3 days at 15°C. Atmospheres within the MA packs reached 17% O_2; 2% CO_2 after 7 days at 1°C, indicating only minor atmosphere modification.

Ordinary packaging films such as LDPE, PP (polypropylene), OPP (orientated PP), and PVC (polyvinylidene chloride) can generate suitable in-pack gaseous environment for fresh produce with low and medium rates of respiration. However, for highly respiring produce such as mushroom, broccoli, asparagus and sprouts, packaging in these film packages results in anaerobic conditions within a short period. Therefore, their quality and quantity cannot be preserved using ordinary polymeric film packages. Micro-perforated films using the press-perforated rollers during the manufacturing process have been developed for this purpose. These films contain a large number of micro perforations for enhancement of gaseous diffusion of O_2 and CO_2 across the film packages, which avoid anaerobic respiration of the packaged produce. Several studies have proved that these films have great potential for packaging of highly respiring produce. However, at present their use is limited as they are quite expensive (Rai and Paul 2007).

Therefore, that both CA storage and MAP appear to be useful tools in maintaining glucosinolate content after harvest, in that the atmospheres reached and/or RH achieved may have prevented membrane degradation and subsequent mixing of glucosinolates with myrosinase. However, far more work is necessary to confirm this view and more clearly elucidate the atmospheres that may best maintain glucosinolate content (Jones *et al.* 2006).

Any processing step that causes a disruption of cellular integrity may result in a loss of glucosinolates, due to the mixing of glucosinolates with myrosinase, but this is dependent on the type of glucosinolate (Barrilari *et al.* 2002). After chopping and storage of both broccoli and cabbage at room temperature (approximately 20°C) there were significant reductions in aliphatic glucosinolates (e.g., glucoraphanin), but an increase in some indole glucosinolates (Verkerk *et al.* 1997, 2001). Total glucosinolates and the indole 4-methoxyglucobrassicin, in particular, were also found to increase in whole (unchopped) broccoli heads during storage at 20°C in air by Hansen *et al.* (1995, 1997).

Fresh broccoli is highly perishable, but fresh-cut broccoli, after processing, has a shorter shelf-life than that of the whole broccoli. This has resulted in a great economic loss in the production of the vegetables every year over the world. Thus, a great concern, sometimes an emergency for producers and distributors, is to find a way to prolong the shelf-life of fresh-cut broccoli. The exposure of intact broccoli to 6 ml/kg ethanol for 5 h during storage at 10°C, was effective in inhibiting the senescence of fresh-cut broccoli florets. There had been higher activities of peroxidase (POD), SOD, and catalase (CAT) in ethanol-treated broccoli, during the early stages at 10°C (generally a consequence of the system ability to delay senescence), but the fresh-cut broccoli treated with ethanol maintained better quality during the storage. The mechanisms of delaying senescence of ethanol vapor in broccoli need to be investigated further in cellular and molecular fields. Ethanol vapor would be commercially a good candidate for extending the shelf-life of fresh-cut broccoli florets and reducing the loss in postharvest (Han *et al.* 2006).

When *Brassica* vegetables were diced (to 5 mm cubes), up to 75% of the glucosinolate content was lost during the subsequent 6 h at ambient temperature. The extent of glucosinolate loss increased with post-shredding time. The effect of shredding varied for the vegetables studied: green cabbage lost *ca.* 60% of total glucosinolate analyte content, whereas broccoli, Brussel sprouts and cauliflower lost 75% of total glucosinolate analyte content over 6 h (Song and Thornalley 2007). Some shredded "ready-to-cook" vegetables are also purchased from retail stores but the shredding is usually very coarse. When vegetables were shredded into large pieces of coarse shredding (10-20 g florets, quartering of Brussel sprouts and 4 × 4 cm cabbage leaf sectors), losses of total glucosinolate analyte content were less than 10%. When shredded vegetables were analysed for corresponding ITCs, these analytes were indeed detected and represent approximately 30-50% of the total loss of glucosinolates; no amine degradation products were detected. This indicates that other hydrolysis products are produced during the auto-hydrolysis process. Some ITCs – those with high volatility (e.g., allyl isothiocyanate) – may suffer loss by evaporation (Jones *et al.* 2006; Song and Thornalley 2007).

One of the most important merits of fruit and vegetables is their antioxidantive properties all justified by the presence of ascorbic acid, tocopherol, β-carotene, and polyphenols (Kurilich *et al.*, 1999; Tomas-Barberan and Espin 2001). Ready to eat vegetable products are more and more popular among individual consumers and catering services. The investigation concerned frozen broccoli produced using a traditional method, i.e. from the raw material blanched before freezing, and a modified method of freezing cooked broccoli (Gebczynski and Lisiewska 2006). In frozen products stored for 0, 4, 8 and 12 months at -20 or -30°C and then cooked, a steady decrease was observed in the content of all the constituents. Compared with the raw material cooked broccoli stored for 12 months contained

29-33% of vitamin C, 54-66% of polyphenols, 80-97% of carotenoids, 69-80% of β-carotene and showed a 29-35% decrease in the antioxidative activity. Products at -30°C retained more antioxidants and revelaed better sensory quality than ones stored at -20°C (Gebczynski and Lisiewska 2006).

The content of ash, P, K, Ca, Mg, Na, Fe, Zn, Mn, Cu, Cr and Ni was determined in four species of brassicas: Brussels sprouts, broccoli, and green and white cauliflowers. The investigation covered the raw material, the material blanched or cooked before freezing and frozen products after 12 months of refrigerated storage and prepared for consumption. Frozen products were obtained by the traditional method of freezing the blanched material or by the modified method of freezing the cooked material. The processing of vegetables before freezing (washing, grinding, blanching or cooking) caused statistically significant decreases in most constituents analysed. Blanching did not basically change the content of Na and Ca; or that of Cr in both types of cauliflower; Cu and Ni in white cauliflower; and Ni and P in Brussels sprouts. Cooking in brine, however, caused increases in the content of ash, Na and Ca in white cauliflower, decreases in the content of K and Fe and, in some species, of the remaining constituents. However, no significant differences were noted in the level of Cr in all the samples; in the level of Ca in broccoli and green cauliflower; of Ni in broccoli; of Ni, Cu and Zn in white cauliflower; and of Cu in green cauliflower (Kmiecik *et al*. 2007).

Drying of intact broccoli at 50-65°C maintained glucosinolates and myrosinase activity and it is only when the product was re-hydrated that glucosinolates were hydrolysed (Rosa *et al*. 1997). Matusheski *et al*. (2004) found that the epithiospecifier protein (ESP) favoured nitrile production over ITCs in broccoli under certain conditions and indicated that heating broccoli may result in a more bioactive product. Heating broccoli at 60°C for 5 min, or more, inactivated ESP, resulting in more SFN being produced, providing myrosinase had not been inactivated. Myrosinase was inactivated at 100°C for 5-15 min, so any heat treatment of 60-70°C for 5-10 min would inactivate ESP, but not myrosinase and result in higher SFN production. As SFN had a far more potent effect on Phase I and II enzymes that SFN-nitrile (Matusheski and Jeffery 2001), the health effects of predominant SFN production could be significant. Other conditions, such as pH, also had a significant effect on SFN:SFN-nitrile production. A neutral or alkaline pH resulted in predominately SFN production, whereas an acidic pH (3.5, typical of salad dressings), resulted in more SFN-nitrile (Matushski and Jeffery 2001).

The postharvest process that has arguably the most effect on glucosinolate and other phytochemical content is cooking (Jones *et al*. 2006; Song and Thornalley 2007). Many nutrients, vitamins and phytochemicals are known to significantly decline during cooking. Ascorbic acid, carotenoids and phenolics in broccoli all decreased significantly with time during boiling or microwave cooking (Zhang and Hamauzu 2004; Lin and Chang 2005; Turkmen *et al*. 2005, 2006).

The type and time of cooking used can also dramatically affect the glucosinolate content in broccoli at the time of consumption, with a five to 10-fold difference in the level of glucosinolates available resulting from differences in cooking (Dekker *et al*. 2000). Microwaving and boiling resulted in the largest losses in glucosinolates in broccoli (Vallejo *et al*. 2002b). Steaming, on the other hand, appeared to minimize the loss of glucosinolates (Rosa *et al*. 1997), although the degree of loss varied. Some individual glucosinolates were more thermolabile than others: glucoraphanin and neoglucobrassicin were more tolerant of cooking than other glucosinolates (Vallejo *et al*. 2002b). There is also a significant variety-dependent variation in the decline of glucosinolates after cooking in broccoli, as well as other brassicas (Rosa *et al*. 1997; Jones *et al*. 2006).

The effects of common household practices such as chilling, freezing, and cooking on vitamin C retention in broccoli (cv. 'Marathon'), as well as their influence on the release of SFN upon enzymatic hydrolysis of glucoraphanin by the endogenous enzyme myrosinase, when broccoli was chilled at 6°C and 95% RH for 35 d, showing a vitamin C and SFN loss of about 39% and 29%, respectively, while storage at -18°C for 60 d resulted in similar losses, but mainly due to the blanching step (Galgano *et al*. 2007). Cooking methods that retain some of the endogenous myrosinase activity may also be beneficial by increasing the conversion of GLSs to ITCs during chewing (Song and Thornalley 2007).

The main influence on the production of ITCs *in vivo* is the way in which *Brassica* vegetables are cooked, rather than the effect of the meal matrix (Rungapamestry *et al*. 2007). The intake of glucosinolates was not significantly different between lightly cooked (microwaved 2.0 min) broccoli and fully cooked (microwaved 5.5 min) broccoli. The total sulforaphane-mercapturic acid (SFMA) output was approximately 3 and 10 μmol, corresponding to 5 and 20% of glucoraphanin intake, after consumption of fully cooked and lightly cooked broccoli, respectively. Then, the estimated yield of SFMA (biomarker of production of ITCs) was about 3-fold higher after consumption of lightly cooked broccoli than fully cooked broccoli (Rungapamestry *et al*. 2007).

Lately, it was shown that during stir-frying with different edible vegetable oils, both phenolic compounds and vitamin C were more affected than glucosinolates and minerals in broccoli (Moreno *et al*. 2007). The total intact glucosinolate contents in stir-fried broccoli florets were significantly reduced when using refined olive oil (49% loss) and sunflower oil (37% loss) with respect to the uncooked controls (43.22 mg/100 g wet basis), whereas the concentration of these beneficial components remained almost unaltered when using extra virgin olive, soybean, peanut, or safflower oils. The cooking method used in the present experiment, stir-frying, gave data on reduction of the flavonoids and sinapic acid derivatives of broccoli depending on the oil used. Nonetheless, the effect was completely independent of the cooking temperature but not of the type of oil, since edible oils from olive origin demonstrated a negative effect on flavonoids and sinapic acid derivatives. Vitamin C (AA+DHA) losses were significant and considerably high for peanut oil, safflower oil, and soybean oil (40% to 48% loss) or refined olive oil (80% loss). None of the cooked samples showed significantly lower concentrations of minerals than the uncooked controls.

Induction boiling, a new cooking method widely used in the food-service industry and in some households, appeared to be as good or better than conventional boiling or microwave steaming for preparation of vegetables, due to the cooking yields and retention values for the No differences in the retentions of -carotene, -carotene and lutein/ zeaxanthin were observed by the cooking method, with the exception of b-carotene retention in broccoli where retentions were higher for those that were induction boiled (90.3%) than those that were microwave steamed (62.2%). A trained panel judged the color scores of three vegetables by the cooking method as similar. The mean flavor scores (1 = extremely bland; 9 = extremely intense) for broccoli conventional (4.7–5.4) and induction (5.3–5.5) boiled were lower than those that were microwave steamed (5.9–7.0). The mean texture scores (1 = extremely mushy/tender; 9 = extremely firm/tough) for induction-boiled (5.0–6.0) broccoli were higher than the conventionally boiled (3.4–5.2) and lower than the microwave steamed (5.1–6.6) broccoli (Nunn *et al*. 2006).

In general, we could observe that the nutritional quality of broccoli inflorescences were preserved better in steaming process (without contact with water) than cooking when broccoli is in contact with water (Vallejo *et al*. 2002b, 2003e; Gliszczynska-Swiglo *et al*. 2006; Jones *et al*. 2006).

Further analysis should be carried out in order to improve our knowledge about the potential for improving the density of phytochemicals (glucosinolates and phenolics) and micronutrients (vitamins and minerals) in processed food matrices, and their respective absorption (bioavailability) to have an effect on the human metabolism once ingested and digested (Moreno *et al*. 2006a, 2007).

CONCLUSION AND PERSPECTIVES

This review of the literature regarding pre- and post-harvest factors influencing the phytochemical composition of broccoli finds many gaps in the current knowledge of how, *Brassica* in general and broccoli in particular, can be enriched in such wealth of compounds to develop marketable healthy food as well as sources of raw ingredients for future applications in functional food and nutraceutical developments.

The available evidence indicates that both genetic background and crop management strategies are involved in the postharvest quality, bioactivity and bioavailability of the bioactive compounds in broccoli and cruciferous foods, and the recent literature suggest that the potential of chemoprevention by dietary interventions will needs the establishment of dietary recommendations including three or more servings of cruciferous foods per week, besides the current recommended three-to-five a day portions of fruits and vegetables, based in scientific and epidemiological evidences of the influence of bioactive compounds from broccoli and cruciferous-based foods on the different stages of degenerative diseases and cancer.

Consequently, the gaps and raised questions in the current knowledge and state-of-the-art of the phytochemicals present in broccoli and cruciferous foods for health, should encourage scientists for the plant and the human nutrition areas to join efforts and develop strategies and synergistic collaborative work for the improvement of health through foods, especially with a nature's marvel, broccoli.

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