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, Glutathione 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

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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-
tries in the EU, and the highest volume of sales covers Oc-
tober 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), Ger-
many (25%), and The Netherlands (11%) (PROEXPORT 
2006).

Broccoli is a fast-growing, upright, branched, annual 
plant, 60-90 cm tall whose green flower buds (inflores-
cences) are edible. Besides broccoli (B. oleracea [Italica 
Group]), the Brassicaceae family includes vegetables that 
are commonly grown and widely consumed such as Brus-
sels sprouts (B. oleracea [Geminifera group]), cabbage 
and kale (B. oleracea [Capitata group]), collards (B. olera-
cea [Acephala group]), mustard (Sinapis sp.), rape (Bras-
sica 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 
preventing the diet could prevent about 50% of all breast 
cancer cases, 75% of stomach cancer cases or 75% of co-lo-
rectal cancer cases. Eating at least five portions of vegeta-
bles and fruits each day could, in itself, reduce cancer rates 
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 vegeta-
bles are excellent dietary phytochemicals includ-
ging glucosinolates (Keck and Finley 2004; Zareba and 
Serradell 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).

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 Copen-
hagen rats and prostate adenocarcinomas, being more ef-
effective than either tomato (10%) or broccoli (10%) alone, 
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the intake of a variety of plant components (Canene-Adams 
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have not been established. Three to five servings of broccol 
aw week provides better cancer prevention than consuming 
one serving or less a week (Keck and Finley 2004). Many 
organizations, including the National Cancer Institute, re-
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vegetable consumption and cancer prevention, but the re-


"Fig. 1 Structure of glucosinolates. Glucosinolates are alkyl-N-hydroxi-
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**Glucosinolates**

The glucosinolates (GLS) belonging to organosulphur phy-
tochemicals (Fig. 1), are β-thioglucoside-N-hydroxysul-
phates (Fahey et al. 2001; Moreno et al. 2006a). The struc-
tural features common for glucosinolates are the β-d-thio-
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sinolates group, with semi-systematic names of individual 
glicosinolates 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 pre-
cursor, 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 
glicosinolates are extensively modified from these precursors, 
including glucosinolate hydrolysis products may alter the metabo-
lism 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 in-
consistent (for a review see, Higdon et al. 2007).

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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 to 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 (Pracrros et al. 1992).

Glucosinolates are also known to be the precursors 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 Piersis spp., slugs and snails, flea beetles or aphids. Plants attacked by the micromoth, Selanina 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 CO₂/O₂ (RQ ratio) (Pracrros et al. 1992). The flea beetle, Phyllorella cruciferae Goeze, and the diamondback moth, Plutella xylostella L., (both crucifer specialists) fed at equal rates on Brassica juncea and glucosinolates were not detected, 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 forlar herbivorous insects, but the effects on foliar herbivory by Piersis brassicae (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, glucobrassin, glucorucin, glucobrassicin, alkenyl glucosinolates (progoitrin, sinigrin, glucosinoleiferin, glucanapin), indole glucosinolates (glucobrassin, neoglucobrassin, 4-hydroxyglucobrassin, 4-methoxyglucobrassin) and the aryl glucosinolate glucobrassitinin. The samples with the highest glucosinolate values (40 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 epiphenionitril) have been recognized for long for their distinctive benefits to human nutrition and plant defence. This 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; Jeffrey et al. 2002). Such variation might be regulated genetically. 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 (Krinisky 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 chromanox 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 Tappl 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 (Schreier 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, Vandervlis 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. 2000).
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 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 ascorbigen, 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 ascorbigen. With regard to AA, Hmrcirik 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 hydroxycinnamyl 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/100 g f.w. edible portion. Three minor glucosides of these aglycones have been also detected, namely isoorcicerin, kaempferol 3-O-glucose and kaempferol diglucoside (Plumb et al. 1997; Price et al. 1998; Vallejo et al. 2004). The hydroxycinnamyl derivatives are from sinapic, ferulic and caffeic acids (Table 1). The predominant hydroxycinnamyl acids esters were identified as 1-sinapoyl-2-feruloylgentiobiose, 1,2-dathranoylgentiobiose, 1,2,2-trisnapanoylgentiobiose, and neochlorogenic acid (Vallejo et al. 2003d). In addition, 1,2'-disinapoyl-2-feruloylgentiobiose and 1,2-disinapoylgentiobiose, 1-sinapoyl-2,2'-diferuloylgentiobiose, isomeric form of 1,2,2'-trisnapanoylgentiobiose, 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 interme-diate colour of plant-derived foods appear ‘black’ in some commodities, or the monohydroxyphenols and orthohydroxyphenols, 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, oleano, flavanone neohesperidosides), sweet (dihydrochalcones), pungent (capsicins, curcuminoids) or astringent (procyanidins, ellagittannins) 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; Alia 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 anti-proliferative activities of common fruits has been also related to their phenolic composition (Sun et al. 2002). The structure-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 structural 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 favour cellular oxidative stress (Alia 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 aromatic ring of the flavonoids 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. 2006a).

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).

Phytochemicals 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 contain 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 formed 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 lyophilised 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 Ramdan 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 premenopausal) 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/16a-hydroxyestrone ratio (2-OHE1/16a-OHE1). 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-OHE1/16a-OHE1; 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, antiproliferative, and anti-inflammatory activities (Hamdan et al. 2003). SFN is a major potent activator of these xenobiotic enzymes (Gardiner et al. 2006). Sulforaphane glucoraphanin and 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 nutritional 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 PGI). All the parameters were determined at the beginning and after 1- and 2-month intervention. 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 (Galán 2003, 2004; Gamet-Payrástre 2006).

Certain ITCs, i.e. SFN, are potent monofunctional inducers of Phase-II enzymes although the majority of cru-
cificers 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 12.5, stems 6 and sprouts 4 (Liang et al. 2005). In edible sprouts and the biological levels of glucobrassicin (1.3-19.1 μmol/g d.w., Val-
lejo et al. 2002a), which can be hydrolyzed to indol-3-car-
binol (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 inducing different oligomeric compounds (i.e., 3,3′-bisindolylmethane). In vitro, 3,3′-diindolylmethane suppress the proliferation of different cancerous cells (breast, prostate, endometrial, col-
on 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 anti-
oxidant 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 multi-
ple 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 chemopre-
ventives 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 bene-
fit of including cruciferous vegetables as part of a balanced diet (Sram 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 cruciferous vegetables have a favorable metabolic pro-
ation 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 metabolic profile of the individual can 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 Scheme et al. (2001, 2004) and the interactions between metabolic genotypes and internal dose, biologic-
effectively effective dose and cytogenetic effects of complex and specific genotoxic exposures of human study populations, particularly on DNA-adducts derived from polycyclic aromatic hydrocarbons (PAHs). The formation of DNA add-
ducts in human hepatoma cells (HePG2 cells) and human hepatocytes exposed to PhIP (C1-compounds 2-aminomethyl-1-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 flav-

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 mea-
sured 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 ana-
lysis of data of the dietary intake 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 biomar-
ker 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 biotransforma-
tion 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 lympho-
cytes, glutathione-S-transferase (GST) activity, glutathione peroxidase (GPX), and superoxide dismutase (SOD), anti-
oxidant level (plasma iron reducing capacity, uric acid, as-
corbic 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 lympho-
cytes although the authors did not observe any induc-
tion of detoxification enzymes or effects on plasma mark-
ers for antioxidant function (Seow et al. 2005). The benefit of a modification in the metabolic profile of 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 res-
pose 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 abbreviated for the substrates most rapidly conjugated by GSTs, while this reaction is reversible, enzyme kinetics suggests that the formation of the glutathione conjugate (are low activity allele (Zhang et al. 1995). The preva-
lence 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 carcino-
genesis. The null GSTM1 and GSTT1 individuals excreted ITCs more slowly, and this may cause an accumulation of

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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 cancer research, and epidemiological studies of cruciferous vegetable/isothiocyanate 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, the evidence is clearer to summarize 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 chemo-protective 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 (Klärning 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 (Vallée et al. 2002a). Glucoraphanin levels in broccoli seed is largely determined by genotype, ranging from 5 to 100 μmol g−1 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 glucobrassican, 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% glucobrassican, 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 glucosi- nolates 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 greenhouse conditions (Vallejo et al. 2002a, 2003c). The predominant glucosinolates in all the analysed cultivars were 4-methylsulphynylbutyl glucosinolate (gluco- raphanin) and indol-3-ylmethyl glucosinolates (gluco- brassican), representing 39.5 and 53.9% respectively, while 4-OH-glucobrassican and N-methoxyglucobrassican represent 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, ecophysiologic 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
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 ratio (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 glucosinolates 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 quinone 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 glucobrassicon in leaves where higher in leaves at 12°C (44%) and 32 °C (114%) than at 22°C, with constant light of 300 μmol/m²/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 30°C 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 as 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/m²/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

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

<table>
<thead>
<tr>
<th>Broccoli source</th>
<th>Organ</th>
<th>Cultivation practices</th>
<th>Glucoraphanin</th>
<th>Total Aliphatic-GLSs</th>
<th>Total Indole-GLSs</th>
<th>Total GLS</th>
<th>Units</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fourteen cultivars</td>
<td>Edible portion</td>
<td>Field, spring and autumn crop</td>
<td>1.5-22.9</td>
<td>1.3-26.3</td>
<td>0.7-5.9</td>
<td>3.0-28.3</td>
<td>μmol/g dry weight</td>
<td>Brown et al. 2002</td>
</tr>
<tr>
<td>Three broccoli heads</td>
<td>Field, autumn crop</td>
<td>1.3-8.3</td>
<td>10.3-42.4</td>
<td>9.9-15.2</td>
<td>18.9-25.2</td>
<td>μmol/g dry weight</td>
<td>Vallejo et al. 2002a</td>
<td></td>
</tr>
<tr>
<td>Three broccoli types</td>
<td>Edible parts</td>
<td>6.6-39.7</td>
<td>5.3-13.8</td>
<td>6.7-14.9</td>
<td>14.9-25.2</td>
<td>mg/100g fresh weight</td>
<td>Schonhof et al. 2004</td>
<td></td>
</tr>
</tbody>
</table>

---

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), mature heads (110-160 mm diameter), and 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, glucoraphanin, 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 (Rangkadi-klok et al. 2002a). An accumulation of sulfuraphane 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/ha) and irrigation (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 fertilization. The mean yields of broccoli under fertilization were 33.5% higher than conventional management. There was no change in absorption of K under fertilization because of more adequate supply of water, which helped to economize water and fertilizer (Vidal-Martinez 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 Ca₂SO₄ (13% S)) than under sulphur-poor fertilization (15 Kg ha⁻¹ Ca₂SO₄ (3%) S), whereas vitamin C was not affected by sulphur fertilization. A similar experiment but with higher sulphur 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 (Rangkadi-klok 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 6 g/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 glucoraphanin decreased, glucobrassicin and 4-methoxyglucobrassicin 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 de-
increased 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 produces 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 dry heads 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 cyclic glucosinolate glucoiberin, while glucosinolates other than the cyclic glucosinolate glycoalkylolin were 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 H2O) 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 to double (Paschold et al. 2000). Bishara 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) Cu/sulphate 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 the quantity to almost six fold, 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). 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, flavonoids and carotenoids. There are many ways in which produce is produced. All factors that affect the quality of 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**

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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, showed a significant loss of glucosinolates 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 refrigerater temperatures (4-8°C) glucosinolate content decreased, and at -85°C a significant loss of glucosinolates was observed due to freeze-thaw damage 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 (Galagano 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% O2 contained significantly higher glucoraphanin levels than heads stored in air at the same temperature (Rangkadioklok et al. 2002b). Optimum broccoli quality was maintained when held in a controlled atmosphere of 10% CO2 and 5% O2 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% O2 and 5-10% CO2 (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 in MAP using 11 um 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% O2; 2% CO2 after 7 days at 1°C, indicating only minor atmospheric modification.

Ordinary packaging films such as LDPE, PP (polypropylene), OPP (oriented PP), and PVC (polyvinylidene chloride) can generate suitable in-pack gaseous environments 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 can generate suitable in-pack gaseous environments for this purpose. These films contain a large number of micro-perforations for enhancement of gaseous diffusion of O2 and CO2 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 action of myrosinase with myrosinase, but this depends on the type of glucosinolate (Barillari et al. 2002). After choping 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-methoxyglucobrassinin, 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 brocoli 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 (general 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 (10-20 mm 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 (Song et al. 2007). Sliced broccoli 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 antioxidant properties all justified by the presence of ascorbic acid, tocopherol, β-carotene, and polyphenols (Kurilich et al., 1999; Tomas-Barberan and Espín 2001). Ready to eat-prepacked 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°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 revealed better sensory quality than ones stored at -20°C (Gebczynski and Lisiewska 2006).

The content of as, P, K, Ca, Mg, Na, Fe, Zn, Mn, Cu, Cr and Ni was determined in four species of brassicas: Brassica rapa, B. oleracea, and B. nigra, and cabbage flowers. 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 observed in the weight 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). Matusheshki et al. (2004) found that the epithepsiospecific 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. SFN had a far more potent effect on Phase I and II enzymes that SFN-nitrite (Matusheshki 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-nitrite 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 (Matusheshki 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-nitrite 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 (Matusheshki 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-nitrite 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 (Matusheshki 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-nitrite 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 (Matusheshki 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-nitrite 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 (Matusheshki 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-nitrite 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 (Matusheshki 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-nitrite 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 (Matusheshki 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-nitrite 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 (Matusheshki 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-nitrite 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 (Matusheshki 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-nitrite produc...
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 development.

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 need 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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