Potential Health Benefits of Broccoli- A Chemico-Biological Overview

Hannah R. Vasanthi[1,2], Subhendu Mukherjee[1] and Dipak K. Das[1,*]

[1]*Cardiovascular Research Center, University of Connecticut School of Medicine, Farmington, Connecticut, USA;* [2]*Department of Biochemistry, Sri Ramachandra Medical College & Research Institute, Sri Ramachandra University, India*

Abstract: The concept that bioactive components in functional foods are efficacious for the improvement of health, has recently gained much importance. The cruciferous vegetables which include broccoli, cabbage and cauliflower are excellent source of phytochemicals including glucosinolates and their byproducts, phenolics and antioxidant vitamins as well as dietary minerals. Broccoli consumption mediates a variety of functions including providing antioxidants, regulating enzymes and controlling apoptosis and cell cycle. The organosulfur chemicals namely glucosinolates and the S-methyl cysteine sulphoxide found in broccoli in concert with other constituents such as vitamins E, C, K and the minerals such as iron, zinc, selenium and the polyphenols namely kaempferol, quercetin glucosides and isorhamnetin are presumably responsible for various health benefits of broccoli.

There exists no comprehensive review on the health promoting effects of phytochemical compounds present in broccoli so far. This review compiles the evidence for the beneficial role of glucosinolates in conjugation with the other phytoconstituents for human health. It also gives an overview on the chemical and biological characterization of potential bioactive compounds of broccoli including the interaction of phytoconstituents on its bioactivity. Further, the molecular basis of the biological activities of the chemicals present in broccoli potentially responsible for health promotion, from chemoprevention to cardio protection, are outlined based on *in vitro* and *in vivo* studies with a note on the structure activity relationship of sulforaphane and a few other isothiocyanates.

Key Words: Broccoli, glucosinolates, sulforaphane, vitamins, minerals, chemoprevention, cardio protection, antiulcer.

1. INTRODUCTION

The commercial success of functional foods has led to intense interest in the discovery and the characterization of plant based bioactive compounds. In the post-genomic era, it remains true that the goal of the pharmaceutical industry is not simply to find novel drug targets, but to find small molecule compounds that modulate their biological activity. A corollary, of course, is that small molecules can also be exploited to discover novel targets. Hence, pharmaceutical and nutraceutical potential of a plant based material is of great value in the present days. Consumption of Brassica vegetables such as broccoli, cabbage, brussel sprouts and cauliflower, belonging to crucifers provide modest support for the hypothesis that their high intakes reduce the risk of degenerative disorders such as cancer of all types [1-5] and cardiovascular diseases [6]. Broccoli is known as the "Crown Jewel of Nutrition" since it posses all the nutrients namely vitamins, minerals, secondary metabolites and fiber proclaiming its exceptional health benefits. The breakdown products of the sulfur containing glucosinolates, isothiocyanates are the active principles in exhibiting the anticancer property at every stage. The medicinal properties of broccoli consumption are most likely mediated through these bioactive compounds by inducing a variety of functions including acting as antioxidants, regulating enzymes and controlling apoptosis and cell cycle. The purpose of this review is to provide evidence how the phytoconstituents of broccoli are helpful to use it as a preventive medicinal food for the maintenance of health and the mechanism behind the same.

*Address correspondence to this author at the Cardiovascular Research Center, Department of Surgery, University of Connecticut School of Medicine, Farmington, CT 06030-1110, USA; Tel: (860) 679-3687; Fax: (860) 679-4606; E-mail: DDAS@NEURON.UCHC.EDU

2. HEALTH PROMOTING CONSTITUENTS OF BROCCOLI

Broccoli as well as all the cruciferous vegetables are dietary sources of glucosinolates and their bioactive degradation products (isothiocyanates). Crucifers also contain other bioactive components including flavonoids (e.g. quercetin), minerals (e.g. selenium) and vitamins (e.g. Vitamin C) [7, 8].

A. Organo Sulfur Compounds

Sulphur-containing phytochemicals of two different kinds are present in all *B. oleracea* (Cruciferae) vegetables. These are glucosinolates (GLSs, previously called thioglucosides) and *S*-methyl cysteine sulphoxide (SMCSO). The two types of organosulphur phytochemicals found in all *B. oleracea* vegetables, GLS and SMCSO, or, more specifically, many of their metabolites, show anticarcinogenic action that could be useful as cancer chemopreventive agents in humans. There are more than 120 GLS characterized in edible plants; although their function in the plant is unclear, their potent odour and taste suggest a role in herbivore and microbial defence [9, 10]. Glucosinolates are chemically defined compounds; all characterized GLS share a similar basic structure consisting of a -d-thioglucose group, a sulphonated oxime group and a side chain derived from methionine, phenylalanine, tryptophan or branched-chain amino acids.

1389-5575/09 $55.00+.00

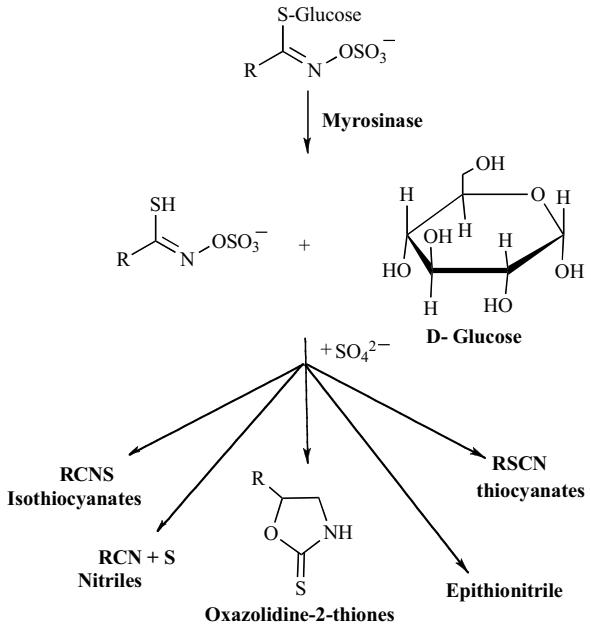

Fig. (1). Hydrolysis of Glucosinolates.

The sulphate group of a GLS molecule is strongly acidic and plants accumulate GSL by sequestering them as potassium salts in plant vacuoles [11]. GLSs are not bioactive in the animal that consumes them until they have been enzymatically hydrolyzed to an associated isothiocyanate [12] by the endogenous myrosinase enzyme that is released by disruption of the plant cell through harvesting, processing, or mastication [9] (Fig. **1**). The most characterized GLS compounds in broccoli are sulphoraphane, phenethyl isothiocyanate, allyl isothiocyanate and indole-3-carbinol (Fig. **2**), but many other isothiocyanates that are present in lower quantities may also contribute to the anti-carcinogenic properties of crucifers [13, 14]. Some researchers have concluded that the evidence of health benefits from sulphoraphane is strong enough to warrant product development [15, 16]. Indole-3-carbinol, benzyl isothiocyanate and phenethyl isothiocyanate, natural bioactives in broccoli, and GLS breakdown products, may be responsible for selective induction of apoptosis in cancer cells, supporting the potential preventive and/or therapeutic benefit of the GLS hydrolysis products against different type of cancers [17, 18].

The largest single group of glucosinolates contains a sulphur atom in various states of oxidation. Another small group of benzyl glucosinolates has an additional sugar moiety, rhamnose or arabinose, in glycosidic linkage to the aromatic ring [13, 14, 19]. The efficient isolation, identification and quantification of glucosinolates in *Brassicas* and more specifically in broccoli has been methodically developed from preliminary chromatography to sophisticated analytical techniques such as NMR, LC (MS) or supercritical fluid chromatography with light scattering detection [20-23]. The effect of cooking on glucosinolates has received a relatively large amount of attention. Cooking reduces glucosinolates levels by approximately 30–60%, depending on the method (e.g. conventional, microwave, high pressure), cooking intensity (e.g. temperature, time), and on the type of compound [24]. Also thermal degradation and washout occur, leading to large losses of intact glucosinolates [25].

B. Polyphenols

Broccoli is a good source of health promoting compounds since it also contains polyphenolics. The occurrence of at least two main flavonol glycosides (quercetin 3-O-sophoroside and kaempferol 3-O sophoroside) in broccoli florets has been reported [26]. Later, the flavonoid composition of broccoli inflorescences has been studied by liquid chromatography-UV diode-array detection-electron spray ionization mass spectrometry, and a large number of hy-

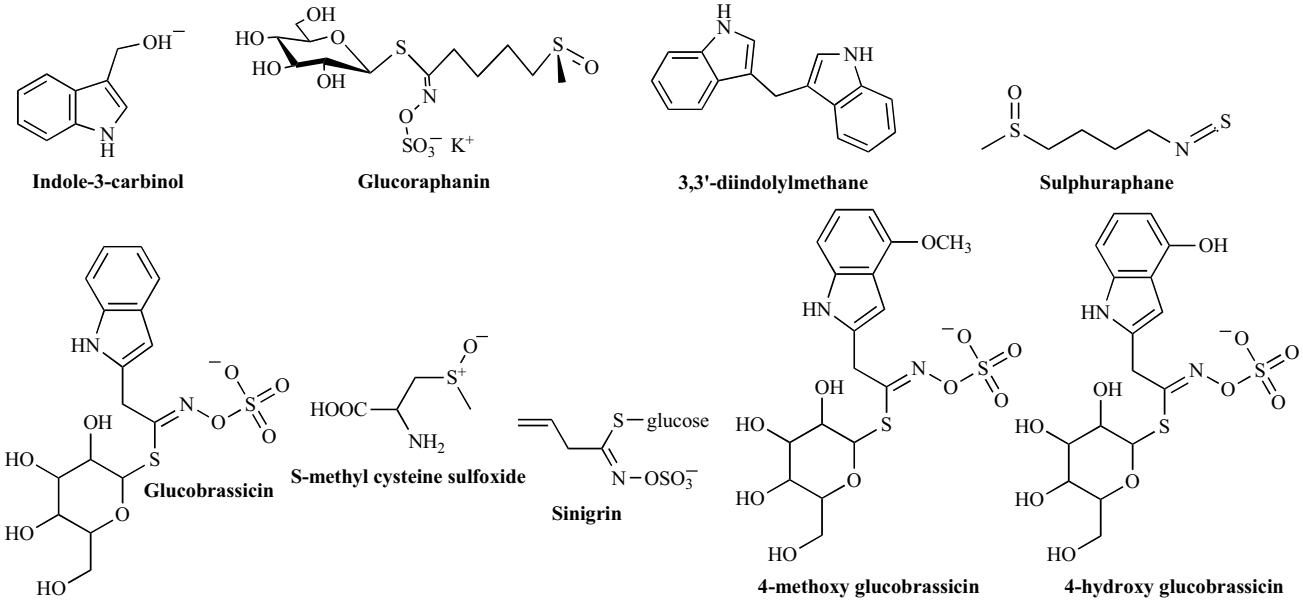

Fig. (2). Common Glucosinolates in Broccoli.

droxycinnamic acid esters of kaempferol and quercetin glucosides were characterised and the structures of the flavonoid glycosides were analysed after alkaline hydrolysis, and identified as 3-sophoroside/sophorotrioside-7-glucoside/ sophoroside of kaempferol, quercetin and traces of isorhamnetin [27] (Fig. **3**). Vallejo and coworkers [28] analysed the phenolic content of broccoli florets from 14 cultivars in Spain using HPLC and found out that flavonoids (as rutin), caffeic acid derivatives (as chlorogenic acid) and sinapic acid derivatives (as sinapic acid) varied in concentration in most cultivars. However, in all the analysed cultivars, kaempferol 3-O-sophoroside represented up to 90% of the total flavonoid content. The contribution of dietary flavonols to health improvement has been shown to be related to their high antioxidant activity [29]. The antioxidant activity and total phenolic content of broccoli extracts have been evaluated by using a model system consisting of β-carotene and linoleic acid [30]. Broccoli is found to have a high antioxidant activity correlated significantly and positively with total phenolics. Recently, other authors [31] have employed the phytochemical content of 22 broccoli genotypes to determine correlations among chemical composition (carotenoids, tocopherol and polyphenolics), chemical antioxidant activity (ORAC) and measures of cellular antioxidation (prevention of DNA oxidative damage and of oxidation of dichlorofluorescein in HepG2 cells) using hydrophilic and lipophilic extracts of broccoli. Moreover, broccoli flavonols are now studied to indicate the type and magnitude of effects among humans *in vivo*, on the basis of short-term changes in biomarkers [32]. Quercetin was found to be the main representative of the flavonol class, found at high concentration in broccoli influencing some carcinogenesis markers *in vivo* [33].

C. Vitamins

Cruciferae seeds and ready-to-eat sprouts are a good source of Vitamin K, B_1 and B_2, [34]. Vitamin K (phylloquinone) is a fat-soluble vitamin that functions as a coenzyme and is involved in blood clotting and bone metabolism, and broccoli contain >100 μg phylloquinone/100 g vegetable, either raw or cooked [35]. Apart from these vitamins, broccoli also contains good quantities of antioxidant vitamins namely tocopherols and ascorbic acid and carotenoids (luetin and zeaxanthin) [36, 37] (Fig. **4**). Vitamin C is abundant in broccoli and is used as a biomarker to identify the nutritional quality of organic, conventional, and seasonally grown broccoli. However, the variation in the levels of the different vitamins varied in the 50 varieties of broccoli tested [38]. This variation is probably due to various factors such as with genotype [23, 39], environmental stress [40], growth conditions [41-43] storage and food processing [7, 44-47].

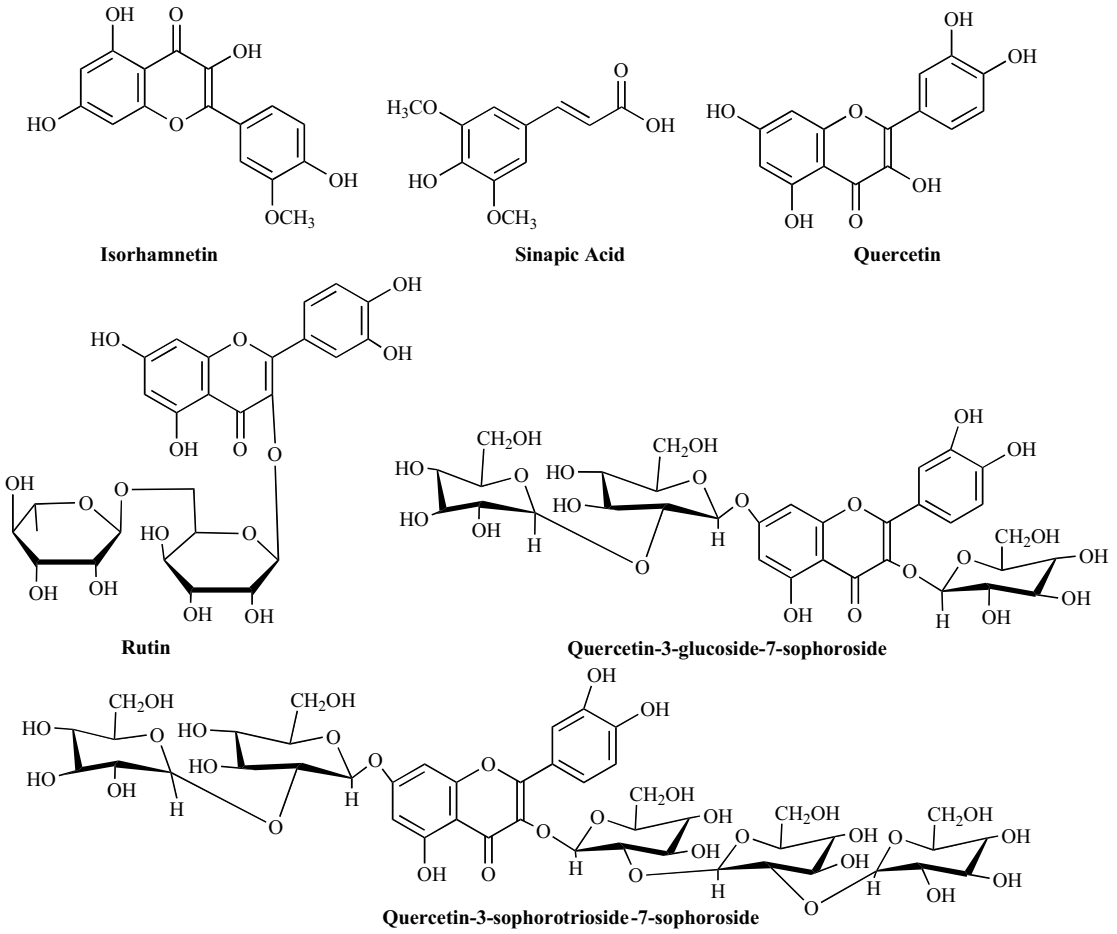

Fig. (3). Polyphenolic Compounds in Broccoli.

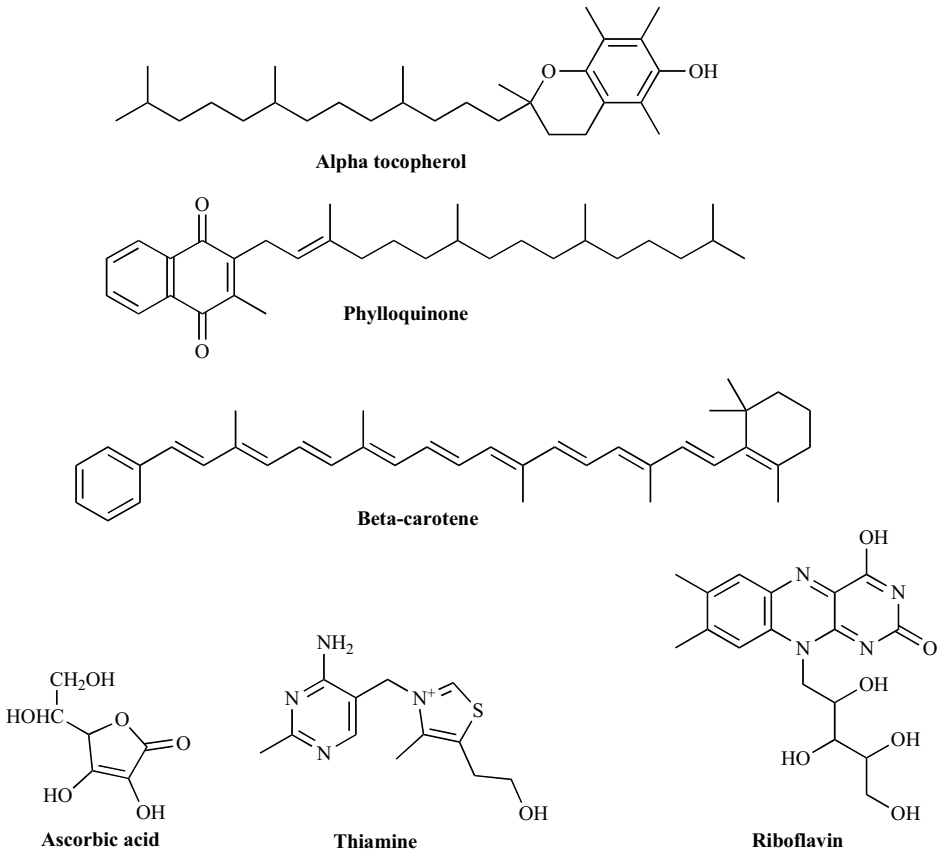

Fig. (4). Vitamins in Broccoli.

D. Minerals

Broccoli is a good vegetable source of major mineral elements such as Na, K, Ca, Mg, Cl, P and S and trace elements such as Fe, Zn, Cu, Mn, and Se for human nutrition [48] (Fig. **5**) and studies have shown that broccoli is an important alternative source of Ca in segments of the population that consume limited amounts of dairy products [49]. Broccoli is known for its ability to accumulate high levels of Se with the majority of the selenoamino acids in the form of Se-methylselenocysteine [50]. The presence of the inorganic elements is substantiated with the high ash content of 11.3 g/kg of the raw florets of broccoli. However, freezing the samples exhibited reduced ash content [51]. It is interesting to note that broccoli has high micronutrient content due to its bioaccumulation capacity. In a study on the use of composted sewage sludge as horticultural growth media, it was identified that broccoli roots have good micronutrient extraction efficiency from composted sewage sludge rich in Fe, Cu, Mn, and Zn. However, heavy metal accumulation such as Cd and Pb were low in relation to micronutrients [52]. Chromium a micronutrient exhibiting lot of health benefits [53] is present in broccoli to the level of 12 µg/100gm [54]. It is recently projected that chromium picolinate improves glycemic control in overweight to obese individuals with type 2 diabetes as an adjuvant to current antidiabetic medications [55]. Hence, presence of mineral micronutrients such as zinc, chromium and selenium, in broccoli is lucrative to be a potential antidiabetic and antiobese functional food apart from its chemoprotective and cardioprotective role.

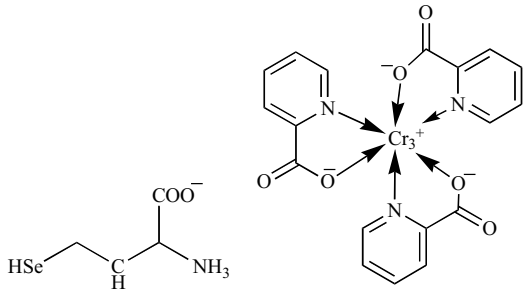

Fig. (5). Bioactive Organometallic Compounds in Broccoli.

E. Other Phytoconstituents

Apart from the compounds described above, the health benefits of broccoli is also enhanced due to the presence of proteins containing the essential amino acids isoleucine, leucine, lysine phenylalanine, tryptophan, methionine, valine and threonine and the beneficial fatty acids [56]. However, the percentage of the aminoacid content varied in the raw florets and the stem compared to the industrial processing of

blanching and freezing. The total protein content varied from 29.5 to 32.5 g/kg and 47.2g/kg in frozen and raw broccoli florets respectively. Similarly, the total lipid content varied between 5.9 to 9.5 g/kg for raw, frozen and canned florets. The fatty acids identified were: lauric acid ($C_{12:0}$), myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), hexadecanoic acid ($C_{16:1}$), hexadecadienoic acid ($C_{16:2}$), hexadecatrienoic acid ($C_{16:3}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) [52]. The presence of the essential aminoacids and the essential fatty acids is an added potential for the nutritive value of broccoli.

3. INTERACTIONS OF HEALTH PROMOTING CONSTITUENTS OF BROCCOLI: EFFECTS ON BIOACTIVITY

Synergism between bioactive components of a plant may result in unexpected metabolic outcomes within the plant and within an animal that consumes it. As mentioned earlier, broccoli contains sulforaphane, phenolics and selenium and a wide range of vitamins. In a series of studies, it was identified that the enrichment of selenium in the florets of broccoli greatly decreased the content of specific phenolic acids such as caeffic, ferulic and sinapic acid. It was also confirmed that selenium influenced a modest decrease in the indole, aliphatic and total glucosinates content in the broccoli extracts [57]. A similar picture is seen with the synergisitic inhibitory effects of polyphenols and antioxidant vitamins on lipid peroxidation and co-oxidation of dietary antioxidants. In simulated stomach fluid, it was demonstrated that phytochemicals can prevent the build-up of oxidized lipid products (lipid hydroperoxides and malondialdehyde) and destruction of vitamin E and β-carotene (and vitamin C to a lesser extent) [58]. In the gastric fluid, vitamin C could enhance the activity of polyphenols through a synergistic antioxidant effect. In another study, broccoli fed rats with sulforaphane exhibited an elevated thioredoxin reductase activity an important selenoprotein that reduces thioredoxin and has antioxidant activity. A subsequent study demonstrated that the thioredoxin reductase activity was synergetically increased by simultaneous addition of selenium and suforaphane [59]. However, the mechanism of action of both these bioactive compounds is different in mitigating cancer [60]. These results probably suggest that functional foods could be developed considering these interactions and synergestic activities.

Another interesting observation on the synergistic effect of the phyto-constitutents is that they are dose dependant in various experimental models. In human colon cancer cells, combinations of sulforaphane and 3, 3'-diindolylmethane showed antagonistic effects on cell proliferation, cell cycle progression and apoptosis at physiologically low concentrations (2.5 μM), an effect that gradually turned into a positive synergistic interaction at the highest combined concentration of 40 μM [61]. The combined effect of two other bioactive compounds from broccoli, indolo-3-carbinol and crambene, was studied in a rat model. The high dose experimental groups were protected against aflatoxin B1 induced toxicity, showing synergistic effects, whereas no effect was observed in the low dose groups [62]. These findings underline the need to elucidate mechanistic interactions in order to better predict beneficial health effects of bioactive food ingredients.

4. MOLECULAR MECHANISM AND SAR OF HEALTH PROMOTING CONSTITUENTS OF BROCCOLI

The biological properties of broccoli consumption are most likely mediated through the "bioactive compounds" that induce a variety of physiological responses including acting as direct or indirect antioxidants, regulating enzymes and controlling apoptosis and the cell cycle. The probable biological action is through the chemical structure of the phenolics, vitamins, glucosinates and their metabolic products. Small changes to the side chain structures can have significant effects.

A. Anticancer

The consumption of cruciferous vegetables has been associated to the prevention of lung, pancreas, bladder, prostate, thyroid, skin, stomach and colon cancer [63]. A study suggested that intake of cruciferous vegetables have an inverse effect on bladder cancer [3]. Like other cruciferous vegetables, broccoli also has anticancer properties due to the presence of constituents like sulforaphane, indoles, polyphenols, vitamins and minerals. Understanding the mechanisms of the chemoprotective effects of isothiocyanates is of great importance because isothiocyanates and their glucosinolate precursors are widespread in plants consumed by humans. Research on anticarcinogen functional foods has focused on broccoli and its bioactive constituents [6, 10, 64]. A review by Verhoeven and coworkers [63] on brassica consumption and cancer risk found that 67% of all studies reported an inverse association between consumption of total *Brassica* vegetable intake and risk of cancer at various sites; cohort studies found the greatest inverse associations between the consumption of broccoli and risk of several cancers including lung and stomach cancer [65]. Sulforaphane has been found to have protective effects against carcinogen-induced tumorogenesis in rodents. Both broccoli extracts, and sulforaphane alone can reduce the incidence, multiplicity and rate of development of mammary tumors in dimethylbenz anthracene (DMBA)-treated rats [66,67] and also can block forestomach tumors evoked by benzo [a] pyrene (BaP) in case of ICR mice [68]. Sulforaphane can reduce the formation of colonic aberrant crypt foci in azoxymethane (AOM) treated rats [69] and suppressed the growth of intestinal polyps in the APC^{min} mouse [70]. A small clinical trial found that the consumption of 250 gm/day (9 oz/day) of broccoli sprouts significantly increased the urinary excretion of a potential carcinogen found in well-done meat, namely 2-amino-1-methyl-6-phenylimidazo [4, 5-*b*] pyridine (PhIP) [71]. Experiments on broccoli suggested that it can protect against human breast cancer [5,6]. Examples of the protective effect of broccoli against certain other degenarative disease have also been reported. It can reduce the risk of cancer by blocking DNA damage [2]. Broccoli can also regulate the growth and death of intestinal cell in colon cancer [4]. Broccoli juices (from leaves) can also prevent skin diseases [72]. Literature shows the protective effects of isothiocyanate against cellular oxidative stress [73] and high cholesterol [32]. In addition, recent studies have indicated that broccoli sprouts can induce carcinogen-detoxifying enzymes [74].

Indol 3 carbinol, the hydrolytic rearrangement product of glucobrassicin (indolylmethyl glucosinolate) in broccoli is readily converted both *in vitro* and *in vivo*, under mildly acid conditions to polymeric indoles that bind with very high affinity to the Ah receptor, and thereby enhance transcription of cytochrome P450IA1 [75]. In contrast to the complex effects of isothiocyanates on phase I enzymes, the effect of these agents on phase II enzymes of rodent tissues is quite straightforward. Isothiocyanates are potent electrophiles like most other inducers of phase II enzymes [76]. Hence, in common with many chemically unrelated chemoprotective compounds, the administration of isothiocyanates to rodents evokes a generalized "electrophile counterattack" response, characterized by the induction of phase II enzymes and increases in tissue GSH levels [77, 78]. Therefore, the isothiocyantes present in broccoli may mediate its anticarcinogenic potential by inducing the phase II enzymes.

In vitro and *in vivo* studies have demonstrated that sulforaphane affects different steps of cancer development. It can modulate early stages of carcinogenetic process (initiation) or promotion and progression phases. It also can modulate events, such as apoptosis, cell proliferation, and angiogenesis. Sulforaphane modulates initiation phase of carcinogenetic process by blocking or by competition reactions with xenobiotic metabolizing enzymes (phase I and phase II enzymes), trapping electrophiles and scavenging free radicals. Several studies demonstrated that sulforaphane can reduce the effect of carcinogens *via* inhibition of phase I enzymes or *via* induction of phase II enzymes. After entering into human body all the dietary and environmental carcinogens are subjected to metabolism through oxidation, reduction and hydrolysis to form more hydrophilic molecule and bind to macromolecules like DNA, RNA and protein and damage them. This event is known as phase I metabolism, which is primarily catalyzed by cytochrome P450 enzymes (CYPs). A large body of data shows that sulforaphane can inhibit DNA-adduct and chemical carcinogenesis through the alteration of certain CYP isoform level in rodents [79, 80], it can inhibit the activity of CYPs1A1, 2B1/2 isoforms in rat hepatocytes [81] and can inhibit CYP2E1 in microsomes from acetone treated rat liver [82].

Induction of phase II enzymes leads to protection of cells/tissues against carcinogenic intermediates by converting them into inactive metabolites, which are readily excreted from the body and thus prevent them to react with macromolecules. For several decades, sulforaphane has drawn the attention as a natural inducer of phase II enzymes in both human and animals [83, 84]. Sulforaphane can induce quinone reductase (NQO1), glutathione S-reductase (GST) and UDP-glucuronosyltransferase (UGT) in *in-vitro* assays. For example, sulforaphane can increase both UGT1A1 and UGTA1 mRNA levels in hepG2 and HT29 cells [85]. Twenty- four hour sulforaphane exposure can increase the NQO1 level three fold over the control at 2.5 µM in Hepa1c1c7 cells [86]. Sulforaphane has also been found to be an inducer of phase II enzyme *in vivo.* It is shown that rat and mice treated with high dose of sulforaphane can produce higher levels of phase II enzyme in liver, lung, mammary gland, pancreas, stomach [87,88]. Researchers have started to understand the molecular mechanism of induction of phase II antioxidant detoxifying enzymes by sulforaphane from broccoli. Two mechanisms, i.e., disruption of Nrf2-Keap 1 interactions and mitogen-activated protein kinase (MAPK) activation, acting synergistically or seperately, have been proposed.

Sulforaphane isolated from broccoli is chiral, possessing the R configuration, but both R-SF and the synthetic (R, S)-SF show identical inducer potency. Change of the oxidation state of the sulfur atom in the methylthiol group from sulfoxide to sulfone reduced inducer activity 4-fold, and the sulfide analog was more than 10-fold less active. Moreover, if the sulfoxide group was replaced with the methylene group, the inducer activity was reduced 75-fold. However, the sulfoxide group could be replaced with a carbonyl group without losing any inducer activity. A change in the number of methylene units from 4 to 5 or 3 did not significantly affect inducer activity, nor did the rigidity of the methylene bridge have much effect on inducer activity [89]. Structure–activity studies have examined the effects of altering the oxidation state of the methylthio group (S, S=O, SO_2) and the length of the methylene chain (n=3, 4, or 5) separating the methylthio- and the −N=C=S groups of sulforaphane on the inducer potency for quinine reductase in murine hepatoma cells. [89]. A series of synthetic acetylnorbornyl isothiocyanates varied in inducer potencies depending on positions and steric relations of the acetyl and isothiocyanate groups. Some of these analogues were potent inducers, but in this series, none was more potent than sulforaphane [88]. Remarkably, replacement of the S=O by C=O produced an analogue that was equally potent to sulforaphane [88].

Apart from the role of isothiocyanates like sulforaphane in exhibiting the antocarcinogenic effect of broccoli, the presence of selenium also adds to the bioactivity. Selenium is now proved to posseses good chemoprotective effect [90]. Methylated amino acids such as Se-methylselenocysteine (SeMSC) bio-accumulated in broccoli are metabolised primarily in the excretory pathway, and limited data suggest that methyl selenol generated in this pathway is the metabolite, which is most responsible for preventing cancer [91, 92]. Moreover, the suggested mechanisms for cancer prevention by selenium include its effects upon programmed cell death, DNA repair, carcinogen metabolism, immune system and its role in selenoenzymes. Selenium also acts as an anti-angiogenic agent and its specific inhibition of tumour cell growth by certain selenium metabolites.

B. Cardioprotection

Cardiovascular disease which includes, ischemic heart disease, atherosclerosis, and hypertension is a serious problem all over the world. Dietary intervention with cardioprotective agents is of immense necessity. A study on the intake of broccoli sprouts high in glucoraphanin, whose metabolite sulforaphane is a potent phase II protein inducer, decreased oxidative stress and inflammation in kidneys and the cardiovascular system explaining the reduction of the risk of developing cardiovascular problems of hypertension and atherosclerosis in spontaneously hypertensive stroke prone rats [93]. The promotion of cardiovascular function was similar to that seen with long-term consumption of pharmacological antioxidants. A recent clinical study with twelve healthy

subjects has suggested that consumption of fresh broccoli sprouts [100 gm/day] for a week reduced LDL and total cholesterol and increased HDL cholesterol [94]. Another related prospective study of 34,492 postmenopausal women in Iowa showed that broccoli was strongly associated with reduced risk of coronary heart disease [95].

Our recent study has demonstrated that broccoli consumption can prevent the reduction of mRNA level and protein level of thioredoxin super family members due to ischemic reperfusion injury [96]. This probably is due to the presence of sulforaphane present in broccoli which can induce Trx, a cardioprotective protein through the antioxidant-responsive element [97]. Ischemia/reperfusion causes cardiomyocyte death by activating death signaling pathway and/or inhibiting survival signaling pathway [98]. But broccoli treatment can induce the components of survival pathway [96]. Our recent study also demonstrated that broccoli treatment can improve cardiac function, reduce myocardial infraction and cardiomyocyte appoptosis after ischemic reperfusion injury [96]. Taken together, broccoli can protect mammalian heart from ischemic reperfusion injury by boosting thioredoxin level. The cardioprotective effects of broccoli probably involves multiple interlinked mechanisms, such as: (a) inhibition of phase I enzymes and DNA adducts; (b) induction of phase II antioxidant detoxifying enzyme; (c) antioxidant function; (d) induction of cell cycle arrest; (e) inhibition of angiogenesis; and (f) anti-inflammatory properties. In order to study the bioactive compound mediating cardioprotection in broccoli two active principles namely sulphoraphane and oltipraz treated rats are being subjected to ischemic reperfusion injury and the study is underway. However, structure activity relationship studies in this line are warranted.

Recently UK researchers reported that eating brocolli could reverse the damage done to heart blood vessels by diabetes. They confirmed the reversal of the biochemical dysfunction of endothelial cells in hyperglycemia by sulforaphane by activation of Nrf2 and related ARE-linked gene expression and explain that it is a novel strategy to suppress endothelial cell dysfunction and possibly also the development of vascular disease in diabetes [99].

C. Antioxidant Activity

Broccoli has been a favorite test vegetable for several researchers because of its potential antioxidant properties [100-103]. As mentioned earlier broccoli comprises of a mixture of antioxidants including ascorbic acid, carotenoids, tocopherol and phenolics. However, among 50 different varieties, the bioactive components varied in concentration [36]. Broccoli stem have been found to exhibit strong antioxidant properties than flowers and leaves [104]. A recent study on the different broccoli cultivars in India explains that the Aishwarya, Packman and Punjabi broccoli are potential cultivars to be used in germplasm improvement programs, since they exhibit good antioxidant activity due to the highest ascorbic acid and carotenoid content [37].

Broccoli is a significant source of flavonoids and phenolics such as caffeic and sinapic acids [105]. A large number of hydroxycinnamic acid esters of kaempferol and quercetin glucosides has been characterized in broccoli inflorescence [26]. The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations [106,107]. The structure of phenolic compounds is a key determinant of their radical scavenging and metal chelating activity, and this is referred to as structure–activity relationships (SAR). In the case of phenolic acids, the antioxidant activity depends on the numbers and positions of the hydroxyl groups in relation to the carboxyl functional group [108,109]

The SAR of flavonoids is generally more complicated than that of hydroxybenzoic and hydroxycinnamic acids due to the relative complexity of the flavonoid molecules. The degree of hydroxylation and the positions of the –OH groups in the B ring, in particular an *ortho*-dihydroxyl structure of ring B (catechol group) in the flavonoid results in higher activity as it confers higher stability to the aroxyl radical by electron delocalisation [110], or acts as the preferred binding site for trace metals [111]. The trace element selenium probably interacts with the flavonoid structure in broccoli and thus exhibits potent antioxidant activity. A double bond between C-2 and C-3, combined with a 3-OH, in ring C, also enhances the active radical scavenging capacity of flavonoids, as seen in the case of kaempferol [110], Substitution of the 3-OH, results in increase in torsion angle and loss of coplanarity, and subsequently reduced antioxidant activity [112]. Another possible mechanism by which broccoli exhibits its health benefits might be through the above structure activity mechanism.

Apart from the vitamins and phenolics, the isothiocyanate (Sulforaphane) present in broccoli also enhances antioxidant capacities. Sulforaphane is not a direct-acting antioxidant or pro oxidant, since it is very unlikely that the isothio-cyanate group can participate in oxidation or reduction reactions under physiological conditions [113]. There is however, substantial and growing evidence that sulforaphane administration acts indirectly to increase the antioxidant capacity of animal cells, and their abilities to cope with oxidative stress [114]. Many isothiocyanates, in common with other inducers of phase II enzymes, raise tissue GSH levels [115], by stimulating the antioxidant response elements (ARE) in the 5'-upstream region of the gene for the heavy sub- unit of g-glutamylcysteine synthetase [116]. This enzyme catalyses the rate-limiting step in GSH synthesis. As GSH is already present in millimolar concentrations in virtually all cells, such increases in GSH presumably augment cellular antioxidant defenses. Moreover, recent evidence supports the view that enzymes induced by sulforaphane such as glutathione transferases, NAD(P)H: quinone reductase (DT-diaphorase), and heme oxygenase can all function as protectors against oxidative stress [117]. Thus, sulforaphane, a very potent inducer of Phase II enzymes and cellular glutathione levels present in broccoli, facilitates to combat the diseases related to oxidative damage.

D. Antimicrobial Activity

Isothiocyanates and the glucosinolate / myrosinase system that leads to their production in cruciferous vegetables like broccoli plays a major role in plant defense against fungal diseases and pest infestation [118]. The antimicrobial

effects and mode of action of allyl isothiocyanate have been examined against an array of bacteria and fungi including *E. coli* [119, 120]. Over 40 years ago, the antibacterial effects of 15 isothiocyanates were evaluated on 10 test organisms, including both gram positive and gram negative organisms and including *E. coli* [121]. Kim *et al*. utilized allyl isothiocyanate (from the glucosinolate sinigrin) as an antimicrobial on cooked rice [122] . Sulforaphane an isothiocyanate from broccoli appears to exhibit broad spectrum-like activity, similar to certain classes of antibiotics, such as ceftriaxone (a third generation cephalosporin used as a positive antimicrobial control for these experiments), by virtue of its ability to inhibit the growth of both Gram-positive and Gram-negative bacteria such as *E. coli* 0157:H7*, Salmonella* and *Shigella*, *S. aureus, Streptococcus pyogenes, P. aeruginosa*, and *Cryptococcus neoformans* [123]. Moreover, it has been identified that 3,3'-Diindolylmethane found in broccoli is a potent modulator of the innate immune response system with anti-viral, anti-bacterial and anti-cancer activity potentiating through synergetic effect of other phytoconstituents [124]. As a whole, broccoli consumption acts as a good antimicrobial healthy food.

Gastric infection with *Helicobacter pylori* is a cosmopolitan problem, and is especially common in developing regions where there is also a high prevalence of gastric cancer. These infections are known to cause gastritis and peptic ulcers, and dramatically enhance the risk of gastric cancer. Eradication of this organism is an important medical goal that is complicated by the development of resistance to conventional antimicrobial agents and by the persistence of a low level reservoir of *H. pylori* within gastric epithelial cells. Sulforaphane [(−)-1-isothiocyanato-(4*R*)-(methylsulfinyl) butane], an isothiocyanate abundant as its glucosinolate precursor in certain varieties of broccoli and broccoli sprouts, is a potent bacteriostatic agent against 3 reference strains and 45 clinical isolates of *H. pylori* [minimal inhibitory concentration (MIC) for 90% of the strains is ≤4 μg/ml], irrespective of their resistance to conventional antibiotics. Further, brief exposure to sulforaphane was bactericidal, and eliminated intracellular *H. pylori* from a human epithelial cell line (Hep-2) [85].

5. SUMMARY AND CONCLUSION

Broccoli constituents mediate a variety of physiological functions by acting as antioxidants, regulating enzymes, and controlling apoptosis and cell cycle. This emphasizes the consumption of this vegetable associated to the prevention of disease condition such as cancer, cardiovascular disorders, ulcers and diabetes. The organosulfur phytochemicals namely glucosinolates and the S-methyl cysteine sulphoxide found in broccoli in concert with other constituents such as vitamin E, C, and K, and minerals such as iron, zinc, and selenium, and the polyphenols namely kaempferol, quercetin, glycosides and isorhamnetin are presumably responsible for the various health benefits of broccoli. Hence, broccoli can be considered as a potential functional food. Further work is needed to better define the molecular mechanism by which the constituents of broccoli can act at the cellular level, modifying various disease conditions and translating the information to human beings.

REFERENCES

[1] Cornblatt, B. S.; Ye, L.; Dinkova-Kostova, A. T.; Erb, M.J.; Fahey, W.; Singh, N. K.; Chen, M.-S. A.; Stierer, T.; Garrett-Mayer, E.; Argani, P.; Davidson, N. E.; Talalay, P.; Kensler, T. W.; Visvanathan, K. Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast. *Carcinogenesis*, **2007**, *28*, 1485-90.

[2] Gill, C.I.R.; Haldar, S.; Porter, S.; Matthews, S.; Sullivan, S.; Coulter, J.; McGlynn, H.; Rowland, I. The effect of cruciferous and leguminous sprouts on genotoxicity, *in vitro* and *in vivo*. *Cancer Epidemiol. Biomarkers Prev.*, **2004**, *13*, 1199-1205.

[3] Michaud, D.S.; Spiegelman, D.; Clinton, S.K.; Rimm, E.B.; Willett, W.C.; Giovannucci, E.L. Fruit and vegetabl intake and incidence of bladder cancer in a male Prospective cohort. *J. Natl. Cancer Inst.*, **1999**, *91*, 605-13.

[4] Parnaud, G.; Li, P.; Cassar, G.; Rouimi, P.; Tulliez, J.; Combaret, L.; Gamet-Payrastre, L. Mechanism of sulforaphane-induced cell cycle arrest and apoptosis in human colon cancer cells. *Nutr. Cancer*, **2004**, *48*, 198-206.

[5] Brandi, G.; Schiavano, G.F.; Zaffaroni, N.; Marco, C.D.; Paiardini, M.;Cervasi, B.; Magnani, M. Mechanisms of action and antiproliferative properties of Brassica oleracea juice in human breast cancer cell lines. *J. Nutr.*, **2005**, *135*, 1503-9.

[6] Jackson, S.J.; Singletary, K.W. Sulforaphane inhibits human MCF-7 mammary cancer cell mitotic progression and tubulin polymerization. *J. Nutr.*, **2004**, *134*, 2229-36.

[7] Jeffery, E.H.; Brown, A.F.; Kurililch, A.C.; Keck, A.S.; Matusheski, N.; Klein, B.P.; Juvik, J.A. Variation in content of bioactive components in broccoli. *J. Food Compos. Anal.*, **2003**, *6*, 323-30.

[8] Finley, J.W.; Ip, C.; Lisk, D.J.; Davis, C.D.; Hintze, K.J.; Whanger. P.D. Cancer-protective properties of high-selenium broccoli. *J. Agric. Food Chem.*, **2001**, *49*, 2679-83.

[9] Finley, J.W. Proposed criteria for assessing the efficacy of cancer reduction by plant foods enriched in carotenoids, glucosinolates, polyphenols and selenocompound. *Ann. Bot.*, **2005**, *95*, 1075-96.

[10] Fenwick, G.R.; Heaney, R.K.; Mullin, W.J. Glucosinolates and their breakdown products in food and food plants. *Crit. Rev. Food Sci. Nutr.*, **1983**, *18*, 123-201.

[11] Keck, A.S.; Finley, J.W. Cruciferous vegetables: cancer protective mechanisms of glucosinolate hydrolysis products and selenium. *Integr. Cancer Ther.*, **2004**, *3*, 5-12.

[12] Rouzaud, G.; Rabot, S.; Ratcliffe, B.; Duncan, A.J. Influence of plant and bacterial myrosinase activity on the metabolic fate of glucosinolates in gnotobiotic rats. *Br. J. Nutr*., **2003**, *90*, 395-404.

[13] Fahey, J.W.; Zalcmann, A.T.; Talalay, P. Corrigendum to "The chemical diversity and distribution of glucosinolates and isothiocyanates among plants". *Phytochemistry*, **2001**, *56*, 5-51.

[14] Fahey, J.W.; Zalcmann, A.T.; Talalay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, **2002**, *59*, 237.

[15] Galan, M.V.; Kishan, A.A.; Silverman, A.L. Oral broccoli sprouts eradicate *Helicobacter pylori* infection. *Am. J. Gastroenterol.*, **2003**, *9*, S-57.

[16] Haristoy, X.; Fahey, J.W.; Scholtus, I.; Lozniewski, A. Evaluation of the ntimicrobial effects of several isothiocyanates on *Helicobacter pylori*. *Planta Med.*, **2005**, *71*, 326-30.

[17] Sarkar, F.H.; Rahman, K.M.W.; Li, Y. Bax translocation to mitochondria is an portant event in inducing apoptotic cell death by indole-3-carbinol (I3C) treatment of breast cancer cells. *J. Nutr.*, **2003**, *133*, 2434-39.

[18] Visanji, J.M.; Thompson, D.G.; Padfield, P.J.; Duthie, S.J.; Pirie, L. Dietary isothiocyanates inhibit Caco-2 cell proliferation and induce G_2/M phase cell cycle arrest, DNA damage, and G_2/M checkpoint activation. *J. Nutr.*, **2004**, *134*, 3121-26.

[19] Rangkadilok, N.; Nicolas, M.E.; Bennet, R.N.; Premier, R.P.; Eagling, D.R.; Taylor, P.W.J. Determination of sinigrin and glucoraphanin in *Brassica* species using a simple extraction method combined with ion-pair HPLC analysis. *Sci. Hortic.*, **2002**, *96*, 27-41.

[20] Ettlinger, M.G.; Lundeen, A.J. The mustard oil of Limnanthese douglasii seed, m-methyoxybenzyl isothiocyanate. *J. Am. Chem. Soc.*, **1956**, *78*, 1952-54.

[21] Betz, J.M.; Fox, W.D. Separation by solid phase extraction and quantification by reverse phase HPLC of sulforaphane in broccoli. *Food Chem.*, **1994**, *63*, 417-421.

[22] Bennett, R.N.; Mellon, F.A.; Kroon, P.A. Screening crucifer seeds as sources of specific intact glucosinolates using ion-pair high-performance liquid chromatography negative ion electrospray mass spectrometry. *J. Agric. Food Chem.*, **2004**, *52*, 428-438.

[23] Font, R.; Rio-Celestino, M.; Cartea, E.; Haro-Bail´on, A. Quantification of glucosinolates in leaves of leaf rape (Brassica napus spp. pabularia) by near-infrared spectroscop**.** *Phytochemistry*, **2005**, *66*, 175-185.

[24] Rungapamestry, V.; Duncan Zoe, A.J. Effect of cooking brassica vegetables on the subsequent hydrolysis and metabolic fate of glucosinolates. Fuller and Brian Ratcliffe. *Proc. Nutr. Soc.*, **2007**, *66*, 69-81.

[25] Verkerk, R.; Dekker, M. Glucosinolates and Myrosinase Activity in Red Cabbage (Brassica oleracea L. Var. Capitata f. rubra DC.) after Various Microwave Treatments. *J. Agric. Food Chem.,* **2004**, *52*, 7318-23.

[26] Price, K.R.; Casuscelli, F.; Colquhoun, I.J.; Rhodes, M.J.C. Composition and content of flavonol glycosides in broccoli florets (*Brassica oleracea*) and their fate during cooking. *J. Sci. Food Agric.*, **1998**,*77*, 468-472.

[27] Vallejo, F.; Tom´as-Barber´an, F.A.; Ferreres F. Characterisation of flavonols in broccoli (*Brassica oleracea* L. var. *italica*) by liquid chromatography-UV diode-array detection-electrospray ionisation mass spectrometry. *J. Chromatogr.*, **2004**, *1054*, 181-193.

[28] Vallejo, F.; Tom´as-Barber´an, F.A.; Ferreres, F. Potential bioactive compounds in health promotion from broccoli cultivars grown in Spain. *J. Sci. Food Agric.*, **2002**, *82*, 1293-1297.

[29] Rice-Evans, C.; Miller, N.J.; Botwell, P.G.; Bramley, P.; Mand Pridham, J.B.; The relative antioxidant activities of plant derived polyphenolic flavonoids. *Free. Radic. Res.,* **1994**, *22*, 375-383.

[30] Kaur, C.; Kapoor, H.C. Antioxidant level and total phenolic content of some Asian vegetables. *Int. J. Food Sci. Technol.*, **2002**, *37*, 153-161.

[31] Suido, H.; Takeuchi, A.; Makino, T.; Tanaka, T. Serum cholesterol lowering effects of a broccoli and cabbage mixture in rats. *Proceedings of the XIIIth International Sympocium on Athrosclerosis*, Kyoto, Japan, **2003**, *238*.

[32] Manach, C.; Scalbert, A.; Morand, C.; R´em´esy, C.; Jim´enez, L. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.*, **2004**, *79*, 727-747.

[33] Williamson, G.; Manach, C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am. J. Clin. Nutr.*, **2005**, *81*, 243S-255S.

[34] Zielinski, H.; Frias, J.; Piskula, M.K.; Kozlowska, H.; Vidal-Valverde; C. Vitamin B1 and B2, dietary fiber and minerals content of Cruciferae Sprouts. *Eur. Food Res. Technol.*, **2005**, *221*, 78-83.

[35] Damon, M.; Zhang, N.Z.; Haytowitz, D.B.; Booth, S.L. Phylloquinone Vitamin K1 Content of Vegetables. *J. Food Comp. Anal.*, **2005**, *18*, 751-758.

[36] Kauri, C.; Kumari, K.; Dahuja, A.; Kapoor, H.C. Variations in antioxidant activity in broccoli (*Brassica oleraceal*) cultivars. *J. Food Biochem.*, **2007**, *31*, 621-638.

[37] Jagdish, S.; Rai, M.; Upadhyay, A.K.; Bahadur, A.; Chaurasia, S.N.S.; Sing, K.P. Antioxidant phytochemicals in broccoli (*Brassica oleracea* var. *italica* Plenck) cultivars. *J. Food Sci. Technol.*, **2006**, *43***,** 391-393.

[38] Guo, J.T.; Lee, H.L.; Chiang, S.H.; Lin, F.I.; Chang, C.Y. Antioxidant properties of the extracts from different parts of Taiwan. *J. Food Drug Anal.*, **2001**, *9*, 96-101.

[39] West, L.G.; Meyer, K.A.; Balch, B.A.; Rossi, F.J.; Schultz, M.R.; Haas, G.W. Glucoraphanin and 4-hydroxyglucobrassicin contents in seeds of 59 cultivars of broccoli, raab, kohlrabi, radish, cauliflower, brussels sprouts, kale, and cabbage. *J. Agric. Food Chem.,* **2004**, *52*, 916-926.

[40] Charron, C.S.; Saxton, A.M.; Sams, C.E. Relationship of climate and genotype to seasonal variation in the glucosinolate-myrosinase system. II. Myrosinase activity in ten cultivars of *Brassica oleracea* grown in fall and spring seasons. *J. Sci. Food Agric.*, **2005**, *85*, 682-690.

[41] L´opez-Berenguer, C.; Garc´ıa-Viguera, C.; Carvajal, M. Are root conductivity responses to salinity controlled by aquaporins in broccoli plants? *Plant Soil*, **2006**, *279*,13-23.

[42] Rangkadilok, N.; Nicolas, M.E.; Bennett, R.N.; Eagling, D.R.; Premier, R.R.; Taylor, P.W.J. The effect of sulfur fertilizer on glucoraphanin levels in broccoli (*B. oleracea* L. var. *italica*) at different growth stages. *J. Agric. Food Chem.*, **2004**, *52*, 2632-39.

[43] Vallejo, F.; García-Viguera, C.; Tom´as-Barber´an, F.A. Changes in broccoli (*Brassica oleracea* var. *italica*) health-promoting compounds with inflorescence development. *J. Agric. Food Chem.*, **2003**, *51*, 3776-82.

[44] Rosa, E.A.S.; Rodrigues, P.M.F. The effect of light and temperature on glucosinolates concentration in the leaves and roots of cabbage seedlings. *J. Sci. Food Agric.*, **1998**, *78***,** 208-212.

[45] Vallejo F, Tom´as-Barber´an FA and Garc´ıa-Viguera C, Glucosinolates and vitamin C content in edible parts of broccoli inflorescences after domestic cooking. *Eur. Food Res. Technol.,* **2002**, *215*, 310-316.

[46] Dekker, M.; Verkerk, R. Dealing with variability in food production chains: a tool to enhance the sensitivity of epidemiological studies on phytochemicals. *Eur. J. Nutr.* **2003**, *42*, 67-72.

[47] Jones, R.B.; Faragher, J.D.; Winkler, S. A review of the influence of postharvest treatments on quality and glucosinolate content in broccoli (*Brassica oleracea* var. *italica*) heads. *Postharv. Biol. Technol.,* **2006**, *41***,** 1-8.

[48] House, W.A. Trace element bioavailability as exemplified by iron and zinc. *Field Crops Res*., **1999**, *60*, 115-141.

[49] Farnham, M.W.; Grusak, M.A.; Wang, M. Calcium and magnesium concentration of inbred and hybrid broccoli heads. *J. Am. Soc. Hortic. Sci.*, **2000**, *125*, 344-349.

[50] Lyi, S.M.; Heller, L.I.; Rutzke, M.; Welch, R.M.; Kochian, L.V.; Li. L. Molecular and biochemical characterization of the selenocysteine *Se*-methyltransferase gene and *Se*-methylselenocysteine synthesis in broccoli. *Plant Physiol.*, **2005**, *138*, 409-420.

[51] Antonia Murcia, M.; López-Ayerra, B.; García-Carmona, F. Effect of processing methods and different blanching times on broccoli: proximate composition and fatty acids lebensm. *Wiss. u.-Technol.*, **1999**, *32*, 238-243.

[52] Dolores, M.; Moreno, J.C. ; Moral, R. ; Espinosa, A.P. ; Pards, C. ; Rufete, B. Use of Composted sewage sludge as horticultural growth media; Effects on germination and trace element extraction. *Commun. Soil Sci. Plant Anal.*, **2005**, *36*, 571-582

[53] Cefalu William, T.; Frank Hu, B. Role of Chromium in Human Health and in Diabetes. *Diabetes Care,* **2004**, *27*, 2741-51.

[54] Kovács, R.; Béni, Á.; Karosi, R.; Sógor, C.; Posta, J. Investigation of chromium content in foodstuffs and nutrition supplements by GFAAS and determination of changing Cr(III) to Cr(VI) during baking and toasting bread. *Food Chem.*, **2007**, *105*, 1209-12.

[55] lbarracin, C.A.; Fuqua, B.C.; Evans, J.L.; Goldfine, I.D. Chromium picolinate and biotin combination improves glucose metabolism in treated uncontrolled overweight to obese patients with type 2 diabetes. *Diabetes Metab. Res. Rev.* **2008**, *24*, 41-51.

[56] Antonia Murcia, M.; López-Ayerra, B.; Martínez-Tomé, M. Francisco García-Carmona. Effect of industrial processing on amino acid content of broccoli. *J. Sci. Food Agric.,* **2001**, *81*, 1299-1305.

[57] Robbins, R.; Keck, A. S.; Banuelos, G.; Finley, J. Cultivation conditions and selenium fertilization alter the phenolic profile glucosinolate and sulforaphane content of broccoli. *J. Med. Foods,* **2005**, *8*, 204-14.

[58] Gorelik, S.; Lapidot, T.; Shaham, I.; Granit, R.; Ligumsky, M.; Kohen, R.; Kanner, J. Lipid peroxidation and coupled vitamin oxidation in simulated and human gastric fluid inhibited by dietary polyphenols: health implications. *J. Agric. Food Chem.*, **2005**, *53*, 3397-3402.

[59] Hintze, K.J.; Keck, A.S.; Finley, J.W.; Jeffery, E.H. Induction of hepatic thioredoxin reductase activity by sulforaphane, both in Hepa1c1c7 cells and in male Fisher 344 rats. *J. Nutr. Biochem.*, **2003**, *14*, 173-179.

[60] Finley, J.W.; Sigrid-Keck, A.; Robbins, R.J.; Korry, J. Hintze Selenium Enrichment of Broccoli: Interactions between Selenium and Secondary Plant Compounds. *J. Nutr. Suppl.,* **2005**, 1237-39.

[61] Pappa, G.; Strathmann, J.; Löwinger, M.; Bartsch, H.; Gerhäuser, C. Quantitative combination effects between sulforaphane and 3'3'-diindolylmethane on proliferation of human colon cancer cells *in vitro*. *Carcinogenesis*, **2007**, *28*, 1477-1477.

[62] Wallig, M.A.; Heinz-Taheny, K.M.; Donna, L.; Epps, D.L.; Gos, T. Synergy among phytochemicals within crucifers: Does It translate into chemoprotection? *J. Nutr.,* **2005**, *135*, 2972S-77S.

[63] Verhoeven, D.T.; Goldbohm, R.A.; van Poppel G.; Verhagen, H.; van den Brandt, P.A. Epidemiological studies on brassica vegeta-

bles and cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **1996**, *5*, 733-748.

[64] Kristal, A.R.; Lampe, J.W. Brassica vegetables and prostate cancer risk: a review of the epidemiological evidence. *Nutr. Cancer*, **2002**, *42*, 1-9.

[65] Cohen, J.H.; Kristal, A.R.; Stanford, J.L. Fruit and vegetable intakes and prostate cancer risk. *J. Natl. Cancer Inst.*, **2000**, *92*, 61-68.

[66] Fahey, J.W.; Zhang, Y.; Talalay, P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 10367 -72.

[67] Zhang, Y.; Kensler, T.W.; Cho, C.G.; Posneret A.L. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 3147-50.

[68] Fahey, J. W.; Haristoy, X.; Dolan, P. M.; Kensler, T. W.; Scholtus, I.; Stephenson, K. K.; Talalay, P.; Lozniewski, A. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzo[a]pyrene-induced stomach tumors. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 7610 - 7615.

[69] Chung, F. L.; Conaway, C.C.; Rao, C. V.; Reddy, B. S. Chemoprevention of colonic aberrant crypt foci in Fischer rats by sulforaphane and phenethyl isothiocyanate. *Carcinogenesis*, **2000**, *21*, 2287-91.

[70] Khor, T.O.; Hu, R.; Shen,G.; Jeong, W.S.; Hebbar, V.; Chen, C.; Xu, C.; Nair, S.; Reddy, B.; Chada, K.; Kong, A.N. Pharmacogenomics of cancer chemopreventive isothiocyanate compound sulforaphane in the intestinal polyps of ApcMin/+ mice. *Biopharm. Drug Dispos.*, **2006**, *27*, 407 - 420.

[71] Walters, D.G.; Young, P.J.; Agus, C.; Knize, M.G.; Boobis, A.R.; Gooderham, N.J.; Lake, B.G. Cruciferous vegetable consumption alters the metabolism of the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in humans. *Carcinogenesis*, **2004**, *25*, 1659-69.

[72] Guerrera, P.M. Traditional phytotherapy in Central Italy (Marche, Abruzzo, and Latium). *Fitoterapia*, **2005**, *76*, 1-25.

[73] Eberhardt, M.V.; Kobira, K.; Keck, A.S.; Juvik, J.A.; Jeffery, E.H. Correlation analyses of phytochemical composition, chemical, and cellular measures of antioxidant activity of broccoli (*Brassica oleracea* L. Var. italica.). *J. Agric. Food Chem.,* **2005**, *19*, 7421-31.

[74] Perocco, P.; Bronzetti, G.; Canistro, D.; Valgimigli, L.; Sapone; A.; Affatato, A.; Pedulli, G.F.; Pozzetti, L.; Broccoli, M.; Iori, R.; Barillari, J.; Sblendorio, V.; Legator, M.S.; Paolini, M.; Abdel-Rahman, S.Z. Glucoraphanin, the bioprecursor of the widely extolled chemopreventive agent sulforaphane found in broccoli, induces phase-1 xenobiotic metabolizing enzymes and increases free radical generation in rat liver. *Mutat. Res.*, **2006**, *595*, 125-136.

[75] Bjcldanes, L.F.; Kim, J.Y.; Grose, K.R.; Bartholomew, J.C.; Bradfield, C.A. Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol *in vitro* and *in vivo*: Comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 9543-47.

[76] Talalay, P.; De Long, M.J.; Prochaska, H.J. Identification of a Common Chemical Signal Regulating the Induction of Enzymes that Protect against Chemical Carcinogenesis. *Proc. Natl. Acad. Sci. USA*, **1988**, *85*, 8261-8265.

[77] Preslera, T.; Hollzclaw, W.D.; Zhang, Y.; Talalay, P. Chemical and molecular regulation of enzymes that detoxify carcinogens. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 2965- 69.

[78] PresterÃ, T.; Zhang, Y.; Spencer, S. R.; Wilczak, C.; Talalay, P. The lectrophile counterattack response: proteclion against neoplasia and toxicity. *Adv. Enzyme Regul.,* **1993**, *33*, 281-296.

[79] Yang, C.S.; Smith, T.J.; Hong, J.Y. Cytochrome P-450 enzymes as targets for chemoprevention against chemical carcinogenesis and toxicity: opportunities and limitations. *Cancer Res*., **1994**, *54*, 1982s-1986s.

[80] Hang, Y.; Talalay, P. Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. *Cancer Res.*, **1994**, *54*, 1976s-1981s.

[81] Maheo, K.; Morel, F.; Langouet, S.; Kramer, H.; Le Ferrec, E.; Ketterer, B.; Guillouzo, A. Inhibition of cytochromes P-450 and induction of Glutathione S-transferases by sulforaphane in primary human and rat hepatocytes. *Cancer Res.,* **1997**, *57*, 3649-52.

[82] Barcelo, S.; Gardiner, J. M.; Gescher, A.; Chipman, J.K. CYP2E1-mediated mechanism of anti-genotoxicity of the broccoli constituent sulforaphane. *Carcinogenesis,* **1996**, *17*, 277 - 282.

[83] Talalay, P. Chemoprotection against cancer by induction of phase 2 enzymes. *Biofactors*, **2000**, 12, 5-11.

[84] Prochaska, H.J.; Santamaria, A.B.; Talalay, P. Rapid detection of inducers of enzymes that protect against carcinogens. *Proc. Natl. Acad. Sci. USA*, **1992**, *89*, 2394-98.

[85] Basten, G.P.; Bao, Y.; Williamson, G. Sulforaphane and its glutathione conjugate but not sulforaphane nitrile induce UDP-glucuronosyl transferase (UGT1A1) and glutathione transferase (GSTA1) in cultured cells. *Carcinogenesis,* **2002**, *23*, 1399 - 1404.

[86] Matusheski, N.V.; Jeffery, E.H. Comparision of the bioactivity of two glucoraphanin hydrolysis products found in broccoli,sulforaphane and sulforaphane nitrile. *J. Agric. Food Chem.,* **2001**, *49*, 5743-9.

[87] Gerhauser, C.; You, M.; Liu, J.; Moriarty, R.M.; Hawthorne, M.; Mehta, R.G.; Moon, R.C.; Pezzuto, J.M. Cancer chemo preventive potential of sulforamate, a novel analogue of sulforaphane that induces phase 2 drug-Metabolizing enzymes. *Cancer Res.,* **1997**, *57*, 272-278.

[88] Posner, G.H.; Cho, C.G.; Green, J.V. Design and synthesis of bifunctional isothiocyanate analogs of sulforaphane: correlation between structure and potency as inducers of anticarcinogenic detoxication enzymes. *J. Med. Chem.,* **1994**, *37*, 170-176.

[89] Zhang, Y.; Talalay, P.; Cho, C.G.; Posner, G.H. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc. Natl. Acad. Sci. USA*, **1992**, *89*, 2399-403.

[90] Combs, G.F.; Clark, L.C.; Turnbull, B.W. An analysis of cancer prevention by selenium. *Biofactors*, **2001**, *14*, 153-159.

[91] Ip, C.; Ganther, H.E. Activity of Methylated Forms of Selenium in Cancer Prevention. *Cancer Res.*, **1990**, *50*, 1206-1211.

[92] Ip, C.; Birringer, M.; Block, E.; Kotrebai, M.; Tyson, J.F.; Uden, P.C.; Lisk, D.J. Chemical speciation influences comparative activity of selenium-enriched garlic and yeast in mammary cancer prevention. *J. Agric. Food Chem.*, **2000**, *48*, 2062-70.

[93] Lingyun, W.; Hossein, M.; Ashraf, N.; Facci, M.; Wang, R.; Paterson, P. G.; Ferrie, A.; Juurlink, B.H. J. Dietary approach to attenuate oxidative stress, hypertension, and inflammation in the cardiovascular system. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 7094-7099.

[94] Murashima, M.; Watanabe, S.; Zhuo, X.G.; Uehara, M.; Kurashige, A. Phase I study of multiple biomarkers for metabolism and oxidative stress after one-week intake of broccoli sprouts. *Biofactors,* **2004**, *22*, 271-275.

[95] Yochum, L.; Kushi, L.H.; Meyer, K.; Folsom, A.R. Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. *Am. J. Epidemiol*., **1999**, *149*, 943-949.

[96] Mukherjee, S.; Gangopadhyay, H.; Das, D.K. Broccoli: a unique vegetable that protects mammalian hearts through the redox cycling of the thioredoxin superfamily. *J. Agric. Food Chem.,* **2008**, *56*, 609-17.

[97] Tanito, M.; Masutani, H.; Kim, Y.C.; Nishikawa, M.; Obira, A; Yodoi, J. Sulforaphane induces thioredoxin through the antioxidant-responsive element and attenuates retinal light damage in mice. *Invest. Opthalmol. Visual Sci.*, **2005**, *46*, 979-987.

[98] Das, D.K.; Maulik, N. Conversion of death signal into survival signal by redox signaling. *Biochemistry,* **2004**, *69*, 10-17.

[99] Xue, M.; Qian, Q.; Antonysunil, A.; Rabbani, N.; Jadidi, R.B.; Paul, J. Thornalley. Activation of NF-E2-related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease. *Diabetes*, **2008**, *57*, 2809-17.

[100] Zhang, D.; Hamauzu, Y. Phenolics, ascorbic acid, carotenes and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chem.,* **2004**, *88*, 503-509.

[101] Lin, C.H.; Chang, C.Y. Textural changes and antioxidant properties of broccoli under different *cooking* treatments. *Food Chem.*, **2005**, *90*, 9-15.

[102] Piao, X.; Kim, H.Y.; Yokozawat, L.Y.A.; Piao, X.S.; Cho, E.J. Protective effects of broccoli (*Brassica oleracea*) and its active components against radical-induced oxidative damage. *J. Nutr. Sci. Vitaminol.*, **2005**, *51*, 142-147.

[103] Nilsson, J.; Olsson, K.; Enggvist, G.; Ekvall, J.; Olsson, M.; Nyman, M.; Akessin, B. Variation in the content of glucosinolates,hydroxycinnamic acids, carotenoids, total antioxidant capacity and low molecular weight carbohydrates in *Brassica* vegetables. *J. Sci. Food Agric.*, **2006**, *86*, 528-538.

[104] Jeffery, E.H.; Brown, A.F.; Kurilich, A.C.; Wallig, M.A.; Klein, B.P.; Kushad, M.M.; Juvik, J.A. Content variation in bioactive food components. *Nutr. Today*, **2002**, *37*, 208-210.

[105] Vallejo, F.; Tomas-Barberan, F.A.; Garcia-Viguera, C. Effect of climatic and sulphur fertilization conditions, on phenolic compounds and vitamin C in the inflorescence of eight broccoli cultivars. *Eur. Food Res. Technol.*, **2003**, *216*, 395-401.

[106] Afanas'ev; Dorozhko, A.I.; Brodskii, A.V.; Kostyuk, V.A.; Potapovitch, A.I. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem. Pharmacol.*, **1989**, *38*, 1763-1769.

[107] Amarowicz, R.; Pegg, R.B.; Rahimi-Moghaddam, P.; Barl, B.; Weil, J.A. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem.*, **2004**, *84*, 551-562.

[108] Rice-Evans, A.; Miller, N.J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.,* **1996**, *20*, 933-956.

[109] Robards, K.; Prenzler, P.D.; Tucker, G.; Swatsitang, P.; Glover, W. Phenolic compounds and their role in oxidative processes in fruits. *Food Chem.*, **1999**, *66*, 401-436.

[110] van Acker, S.A.; van den Berg, D.J.; Tromp, M.N.; Griffioen, D.H.; van Bennekom, W.P.; van der Vijgh, W.J.; Bast, A. Structural aspects of antioxidant activity of flavonoids. *Free Radic. Biol.Med.*, **1996**, *20*, 331-342 .

[111] Pietta, P.G. Flavonoids as antioxidants. *J. Nat. Prod.,* **2000**, *63*, 1035-42.

[112] Seeram, N.P.; Nair, M.G. Inhibition of lipid peroxidation and structure-activity related studies of the dietary constituents anthocyanins, anthocyanidins, and catechins. *J. Agric. Food Chem.,* **2002**, *50*, 5308-5312.

[113] Barton, D.; Ollis, W.D. Comprehensive Organic Chemistry: *The Synthesis and Reactions of Organic Compounds,* Pergamon, New York, **1979**, *3*, pp. 461-477.

[114] Fahey, J.W.; Talalay, P. Antioxidant Functions of Sulforaphane: a Potent Inducer of Phase II Detoxication Enzymes. *Food Chem. Toxicol.,* **1999**, *37*, 973-979.

Received: 06 January, 2009 Revised: 18 February, 2009 Accepted: 05 March, 2009

[115] Zhang Y. and Talalay P. Anticarcinogenic activities of organic isothiocyanates: Chemistry and Mechanisms. *Cancer Res.*, **1994**, *54*, 1976s-1981s.

[116] Mulcahy, R.T.; Wartman, M.A.; Bailey, H.H.; Gipp J.J. Constitutive and b-naphtho avone-induced expression of the human g-glutamylcysteine synthetase heavy subunit gene is regulated by a distal antioxidant response element/TRE sequence. *J. Biol. Chem.,* **1997**, *272*, 7445-7454.

[117] Prestera, T.; Talalay, P.; Alam, J.; Ahn Y.I.; Lee, P.J.; Choi, A.M.K. Parallel induction of heme oxyge- nase-1 and chemoprotective phase II enzymes by elec-trophiles and antioxidants: regulation by upstream antioxidant-responsive elements (ARE). *Mol. Med.,* **1995**, *1*, 827-837.

[118] Eas, R.; Heaney, R.K.; Fenwick, G.R.; Portas, C.A.M. Glucosinolates in crop plants. *Horticult. Rev.,* **1997**, *19*, 99-215.

[119] Brabban, A.D.; Edwards, C. The effects of glucosinolates and their hydrolysis products on microbial growth. *J. Appl. Bacteriol.,* **1995**, *79*, 171-177.

[120] Lin, C.M.; Preston, J.F.; Wei, C.I. Antibacterial mechanism of allyl isothiocyanate. *J. Food Prot.*, **2000**, *63*, 727-734.

[121] McKay, A.F.; Garmaise, D.L.; Gaudry, R.; Baker, H.A.; Paris, G.Y.; Kay, R.W.; Just, G.R.; Schwartz, R. Bacteriostats. II. The chemical and bacteriostatic properties of isothiocyanates and their derivatives. *J. Am. Chem. Soc.*, **1959**, *81*, 4328-4335.

[122] Kim, Y.S.; Ahn, E.S.; Shin, D.H. Extension of shelf life by treatment with allyl isothiocyanate in combination with acetic acid on cooked rice. *J. Food. Sci.,* **2002**, *67*, 274-279.

[123] Johansson1, N.L.; Pavia1, C.S.; Chiao, J.W. Growth inhibition of a spectrum of bacterial and fungal pathogens by sulforaphane, an isothiocyanate product found in broccoli and other cruciferous vegetables. *Planta Med*., **2008**, *74*, 747-750.

[124] Liu, R.H. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J. Nutr.*, **2004**, *134*, 3479S-85S.