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Chapter 15

BROCCOLI: AGRICULTURAL CHARACTERISTICS, HEALTH BENEFITS AND POST-HARVEST PROCESSING ON GLUCOSINOLATE CONTENT

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ABSTRACT

This chapter outlines some of the cultivation characteristics of broccoli as well as the nutrient composition of broccoli, including trace elements, vitamins, fatty acids and phytochemicals with a particular emphasis on glucosinolate profiles. A review of the recent history of world production of broccoli is given. Of the glucosinolates, the focus of this chapter is on glucoraphanin and its hydrolysis metabolite sulforaphane. Glucosinolate/sulforaphane content of some different cultivars and different portions of the plant (florets, stalks, leaves and seeds) is reviewed. The effect of growth conditions are also examined, including soil nutrients, temperature and photoperiod as well as post-harvest processing and cooking on glucosinolate profiles. Sulforaphane absorption and metabolism is briefly considered as well as the mechanisms whereby sulforaphane inhibits tumor development. Finally, a brief consideration is given to methods being researched to improve the stability of sulforaphane.

1. INTRODUCTION

Broccoli is a vegetable of great interest due to its nutritional contributions, which are a rich source of fiber, proteins, lipids, vitamins and minerals. Additionally positive effects for the human health are attributed to the plant because of its high content of phytochemicals,

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such as flavonoids and glucosinolates. The products of the glucosinolates hydrolysis are the isothiocyanates, which have antimicrobial, chemoprotective and anticarcinogenic properties. Glucoraphanin is the major glucosinolate in broccoli, whose hydrolysis product with biological activity is the sulforaphane isothiocyanate. Our investigation group has studied the content of this compound in the seed, the freshly harvested vegetable, leaves and stems. Also the effect of the processing in the content of sulforaphane, such as drying, fermented, germination and some storage conditions has been evaluated.

2. BROCCOLI *(BRASSICA OLERACEA L. VAR. ITALICA)*

2.1. Botanical Characteristics

The plants of the *Brassicales* order and the *Cruciferae* or *Brassicaceae* family cover around 350 genera with a total of around 2000 species, among which some plants of commercial interest are included such as the cabbage, cauliflower, Brussels sprouts and broccoli [1]. Broccoli originated through a selection process of the wild cabbage in the Southern Europe and Asia Minor [2]. The word broccoli is derived of the Italian *brocco* and the Latin *brachium*, which mean branch or arm. Broccoli is a plural word, and it refers to its numerous sprouts in inflorescence shape (Figure 1). The plant develops an erect stem, pulpy and thick with limp and spaced leaves. Those stems emerge with leaf axils creating inflorescences; generally a central one of higher size and others laterals. The central inflorescence measure between 7.5 and 20 cm of diameter, and the height average in the plant is 30-60 cm. Because of its edible quality the part of the plant with commercial interest is the thick inflorescence, which is formed by a group of flower buds with its pulpy stems but, unlike the cauliflower, can produce others of small size that grow on the principal stem leaf axils [1, 3, 4].

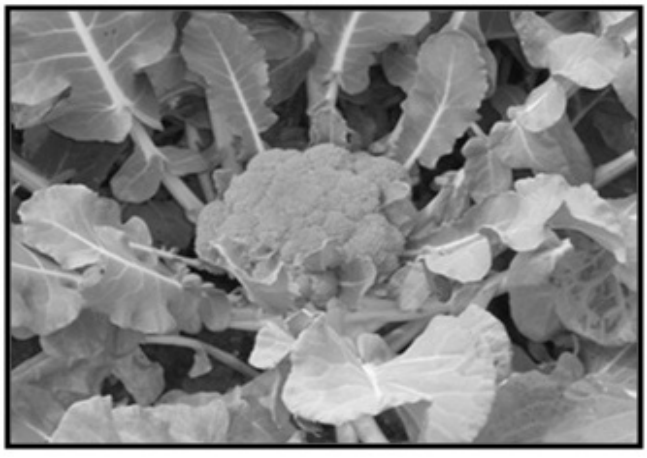


Figure 1. Inflorescence of the broccoli plant.

2.2. Agricultural Characteristics

Broccoli is a cold season crop that is sowed in a great diversity of soils. However, the best results are obtained on loamy, deep soils with a large content of organic matter and pH between 5.5 and 6.5. In order to establish a hectare, a seedbed of approximately 150 m^2 is made using 250 and 300 grams of seed [4]. Broccoli seed can germinate between 4 and 35°C, but the optimal growth is reached when temperatures are between 16 and 18°C. The seedlings are transplanted between 30 and 45 days. In commercial sowings of broccoli under optimal conditions large and leafy plants are obtained that produce compact inflorescences with a large and branched stem [5]. The consumption of fresh broccoli implies a simple chain of cold or a fast freezing process. Industrially broccoli is used in the production of pickles [4]. In Mexico, the inflorescences with commercial quality are harvested manually off the field and packed in boxes (Figure 2), and then transported to the plant to make disinfection and fast cooling.

Figure 2. Harvesting of broccoli on the field.

Afterwards, the boxes of fresh broccoli are stored in low temperatures for a short period of time to immediately transport it to its final destination. A summary of the general characteristics of the broccoli is presented in Table 1.

2.3. Chemical Composition

Crucifer family vegetables provide nutrients such as vitamin C, folic acid, calcium, potassium, fiber and low fat content [6]. Broccoli has been rated as the vegetable that contains a greater quantity of nutrients and less calories per unit of weight of edible product; this vegetable contributes around 3 g of proteins, 2.6 of dietary fiber and only 34 kcal each per 100 g of fresh product. Some authors emphasize that its medicinal and nutritional value reside mainly in its high content of vitamins A, B2 and C, carbohydrates, proteins and chemoprotective substances as glucosinolates and flavonoids [7, 8]. It is recommended that

broccoli be consumed fresh and of recent harvest since this vegetable undergoes a fast senescence, which is characterized by the change of color in the vegetable head (yellow inflorescence), loss of texture, unpleasant odors and reduction of the nutritional value [9]. Complex factors are involved in the senescence process such as ethylene biosynthesis, temperature and the vegetal respiration process [10]. The chemical composition of the fresh broccoli per edible portion reported by the USDA [11] is shown in Table 2.

Table 1. Technical specifications of broccoli

Common name	Broccoli, Bróculi, Brécol, Brécoles
Scientific name	*Brassica oleracea L. var itálica*
Order	*Brassicales*
Family	*Cruciferae, Brassicaceae*
Origin	Southern Europe and Asia Minor
Cultivation	It requires loamy and deep soils. Optimal temperatures of growth are low (18°C). It is recommended that broccoli is cultivated in rotation with other vegetables.
Plants	The plants of broccoli are vigorous and leafy with deep roots.
Varieties	Precocious or early cultivars whose harvesting time is under 90 days after the sowing. Intermediate cultivars that are harvested between 90 and 110 days after the sowing. Late cultivars that take more than 110 days to achieve the adequate development.
Collection	The harvest begins when the inflorescences have achieved a decent development, diameter greater than 13 cm and before flower buds are open.
Uses	Generally it is consumed fresh in salads, cooked in soups, dressings.

Source: FAO [4]

Table 2. Chemical composition of broccoli raw (value per 100 g)

Nutrient	**Florets**	**Leaves**	**Stalks**
Proximates			
Water (g)	89.30	90.69	90.69
Energy (kcal)	34	28	28
Protein (g)	2.82	2.98	2.98
Total lipid (g)	0.37	0.35	0.35
Carbohydrated, by difference (g)	6.64	5.24	5.24
Fiber, total dietary (g)	2.6	----	----
Minerals			
Ca (mg)	47	48	48
Fe (mg)	0.73	0.88	0.88
Mg (mg)	21	25	25
P (mg)	66	66	66
K (mg)	316	325	325
Na (mg)	33	27	27
Zn (mg)	0.41	0.40	0.40
Vitamins			
Vitamin C (mg)	89.2	93.2	93.2
Thiamin (mg)	0.071	0.065	0.065
Riboflavin (mg)	0.117	0.119	0.119
Niacin (mg)	0.639	0.638	0.638

Nutrient	Florets	Leaves	Stalks
Vitamin B-6 (mg)	0.175	0.159	0.159
Folate (µg)	63	71	71
Vitamin A (IU)	623	16000	400
Vitamin E (mg)	0.78	----	----
Vitamin K (µg)	101.6	----	----
Lipids			
Fatty acids, total saturated (g)	0.039	0.054	0.062
Fatty acids, total monounsaturated (g)	0.011	0.024	0.027
Fatty acids, total polyunsaturated (g)	0.038	0.167	0.190

Source: USDA [11]

Table 3 shows the approximate composition, the total phenolic compounds content, total isothiocyanates and antioxidant capacity of the inflorescence of the commercial quality broccoli analyzed in our laboratory.

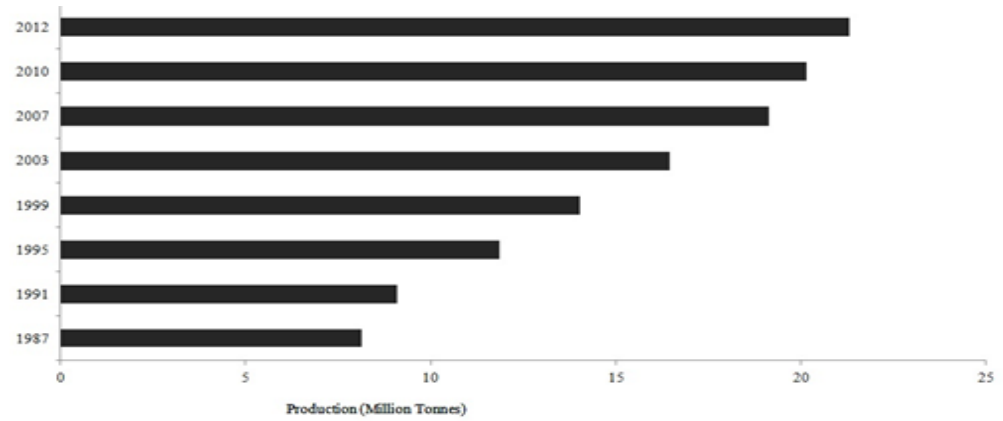
Table 3. Proximate composition, total polyphenols, total isothiocyanates and antioxidant capacity of broccoli raw

Analysis	Florets
Proximates (value per 100 g).	
Water (g)	89.47
Protein (g)	3.12
Total lipid (g)	0.88
Total ash (g)	0.5
Fiber, total dietary (g)	6.1
Polyphenols	
Total polyphenols (mg GAE/g dry matter)	17.21
Isothiocyanates (mg/g dry matter)	
Total isothiocyanates	31.93
Antioxidant capacity (mmol TE/g dry matter)	
DPPH Radical scavenging capacity	112
ABTS Radical scavenging capacity	123

Data expressed as the mean ± standard deviation of three assays (in triplicate).
GAE, Gallic acid equivalent
TE, trolox equivalent

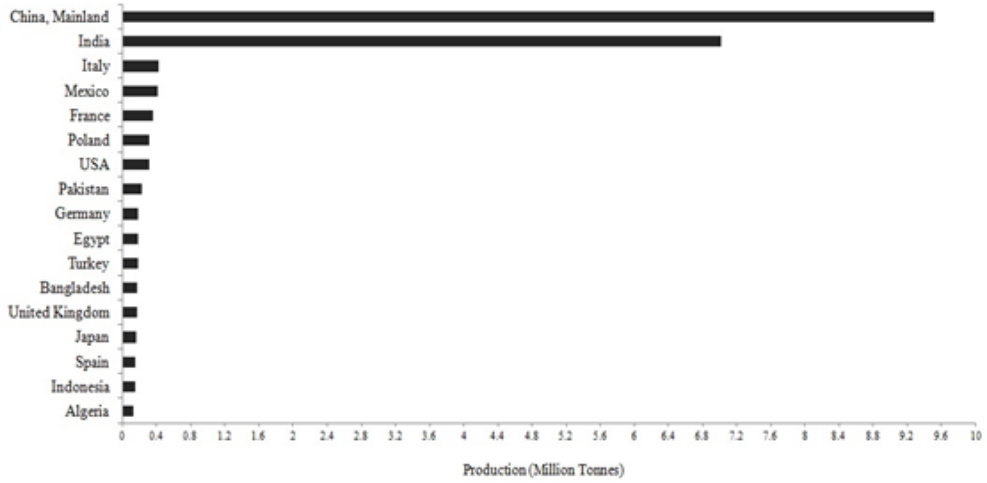
2.4. Production

During recent years the vegetables demand of the *Brassica* genus has been increased, particularly broccoli and cauliflower, because its frequent consumption promotes numerous health benefits. This has generated in the international context, a constant increase of its production in the last 25 years (Figure 3). Currently, China, India, Italy, Mexico, France, Poland, and the United States of America are the seven worldwide major producers of cauliflower and broccoli (Figure 4) [12].



Source: FAOSTAT [12]

Figure 3. World production of cauliflower and broccoli.



Source: FAOSTAT [12]

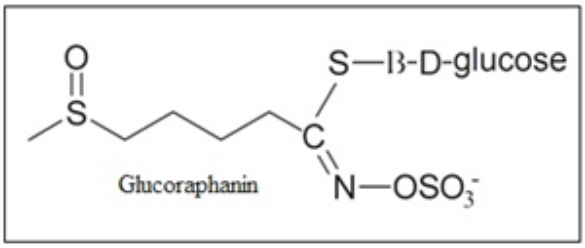
Figure 4. Major producer's countries of cauliflower and broccoli worldwide in 2012.

3. GLUCOSINOLATES

3.1. Chemical Nature

A characteristic of cruciferous plants is the synthesis of sulfur-rich compounds, such as glucosinolates [13]. The *Brassicas*, and a few other edible plants drawn from the order Capparales are the source of all glucosinolates in the human diet. Around 100 different compounds have been identified, which are distributed throughout the plant, although its concentration varies between tissues [14]. Glucosinolates are synthesized and stored in plants as relatively stable precursors of isothiocyanates.

Glucosinolates are water soluble, anionic, non-volatile, and heat-stable, they do not possess direct biological activity. These are located within the vacuole of plant cell [15]. Glucosinolates are classified as S-glycosides (Figure 5), because they are the result of binding a reducing sugar and sulfur in a molecule that has a carbohydrate character (known as aglycone) [16]. The glucose molecule, which imparts hydrophilic characteristics to glucosinolates, is unlike isothiocyanates that has hydrophobic properties [17].



Source: Bones & Rossiter [18].

Figure 5. Chemical structure of glucoraphanin.

Glucosinolates are synthesized by the Shikimic Acid Pathway, whose precursors are the three amino acids: phenylalanine, tryptophan and tyrosine. These three amino acids provide the carbon atoms for the production of glucosinolates in the family *Brassicaceae* [16]. Furthermore, hybrids can be obtained with high glucoraphanin (sulforaphane precursor) by implanting genome segments from the wild ancestor of *Brassica villosa* [19]. Some glucosinolates present in broccoli are shown in Table 4.

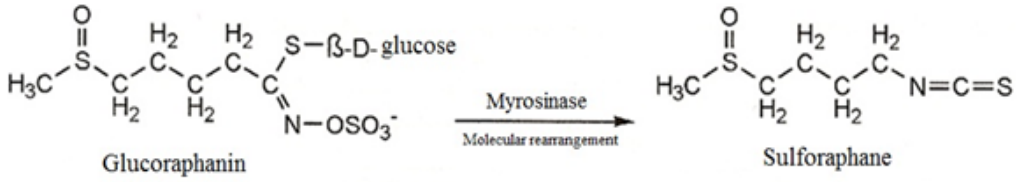
Table 4. Major glucosinolates found in broccoli

Glucosinolates (GLS)	Chemical name	Trivial names
Aliphatic-GLS	4-Metyl-sulphinyl-3-butenyl-glucosinolate	Glucoraphanin
	2(R)-Hidroxy-3-butenyl-glucosinolate	Progoitrin
	3-Metyl-sulphinyl-propyl-glucosinolate	Glucoiberin
	5-Metyl-sulphinyl-pentenyl-glucosinolate	Glucoalyssin
	3-Butenyl-glucosinolate	Gluconapin
	2-Propenyl-glucosinolate	Sinigrin
	2-Hidroxy-4-pentenyl-glucosinolate	Napoleiferin
Indole-GLS	4-Hydroxy-3-indolyl-methyl-glucosinolate	4-Hydroxy-Glucobrassicin
	3-Indolyl-methyl-glucosinolate	Glucobrassicin
	4-Methoxy-3-indolyl-methyl-glucosinolate	4-Methoxy-Glucobrassicin
	1-Methoxy-3-indolyl-methyl-glucosinolate	Neoglucobrassicin
Phenyl-GLS	2-Phenyll-ethyl-glucosinolate	Gluconasturtiin

Source: Baik et al. [20]; Delaquis & Mazza[7].

3.2. Hydrolysis

Glucosinolates are chemically stable in tissues and normal cells. However when the tissue and plant cells are damaged (chopped or chewed) glucosinolates are hydrolyzed (Figure 6) by a group of enzymes thioglucosidases (E.C. 3.2.3.1.) or myrosinases. These enzymes are contacted with the substrate and release glucose molecules, bisulfate and the corresponding aglycone (Thiohydroximate-O-sulphonate), which then undergoes an intramolecular (Lossen) arrangement that generates isothiocyanates, nitriles, methylisothiocyanates, methylnitriles and thiocyanates (all of low molecular weight), which are responsible for the typical odor and flavor of these products [15, 18, 21, 22]. The reaction conditions at 40°C and neutral pH are necessary for efficient conversion of sulforaphane from glucoraphanin. The isothiocyanates formed are insoluble in water, but easily soluble in organic solvents such as methanol, dichloromethane, acetonitrile and ethyl acetate [15].



Source: Vermeulen et al. [23].

Figure 6. Reaction of hydrolysis of glucoraphanin to sulforaphane.

The type of products generated during the enzymatic hydrolysis of glucosinolates will depend on the pH conditions in which to carry out the reaction, the presence of metal ions (such as Fe^{2+} ions) and other protein elements (ESP, epithiospecifier protein) [18]. Glucosinolates with consistent aliphatic chain on a β-hydroxyl group spontaneously form isothiocyanates, which cyclize to form oxazolidine-2-thiones; these compounds and their hydrolysis products are known for their toxic effects (mainly as goitrogenic, see also Chapter 2) in man and animals in high doses, in contrast to low doses that act as chemoprotective agents [24]. Intact glucosinolates have limited biological activity, but increase their activity when hydrolyzed to isothiocyanates [13].

Farming practices, storage conditions, and food preparation conditions have a potential impact on the hydrolysis of glucosinolates. The system glucosinolate-myrosinase present in cruciferous vegetables has a significant variability due to the myrosinase activity and the concentration of cofactors [25]. Table 5 shows the bioactive compounds (isothiocyanates) of great importance in broccoli, which result from the hydrolysis of glucosinolates.

3.3. Effect of Growth Conditions

During evolutionary and cultivation processes brassica plants may have developed typical profiles of glucosinolates, which are involved in defense mechanisms against insects and phytopathogens [28]. Genetic factors and environmental factors contribute significantly to the variation in levels of glucosinolates in brassica plants. Other factors that can influence this variation include growing sites, soil type, sulphate and nitrate fertilization, climate and date of

harvest [29]. Growing broccoli under optimal temperatures will produce broccoli with higher nutritive and bioactivity values through activated glucosinolates biosynthesis [30]. Sulfur fertilization will accelerate the biosynthesis of aliphathic glucosinolates and nitrogen fertilization enhanced indolic glucosinolates [31]. Specifically, the application of $ZnSO_4$ as a sulphur (S)-source enhanced glucoraphanin content in broccoli sprouts [32]. Mølmann et al. [33] found that both temperature and light can influence sensory and phytochemical contents of broccoli florets. Low temperature and long photoperiod seem to produce the highest aliphatic glucosinolate content, while the indolic glucosinolate content seems to favor a short photoperiod and high temperature.

Table 5. Major isothiocyanates found in broccoli

Glucosinolate (precursor)	Isothiocyanates (hydrolysis products of glusosinolates)	
	Chemical name	Trivial name
Glucoraphanin	1-isothiocyanate-4-(methylsulphinyl)butane	Sulforaphane
Progoitrin	1-cyano-2-hydroxy-3-butene	Crambene
Glucoiberin	1-isothiocyanate-3-methylsulphinylpropane	Iberin
Sinigrin	3-isothiocyanate-1-propene	Allyl-isothiocyanate
Glucobrassicin	Indole-3-carbinol	I3C

Source: Moreno et al. [26]; Van Eylen et al. [27]

3.4. Post-Harvest Stability

Hydrolysis of glucosinolates can take place during harvest and storage caused by senescence. Domestic and commercial treatments including chopping, blanching, cooking, steaming, microwaving and freezing have wide impact on glucosinolates content [29]. The postharvest process that has the most effect on glucosinolate content is the cooking. In general microwaving and boiling resulted in the largest losses of glucosinolates, and steaming minimizes the loss of glucosinolates. Moreover, the leaching of glucosinolates into of cooking water is a major cause of loss [34]. According to Cieślik et al. [35] the blanching of cruciferous vegetables results in significant decreases in total glucosinolates ranging from 2.7% to 30%. Nevertheless, boiling leads to higher losses of total glucosinolates compared to blanching, ranging from 35.3% to 72.4%. Glucobrassicin content in broccoli and cauliflower was not affected (even increased) by steam cooking. However, boiling treatment during 5, 10 and 20 minutes showed a slight decrease, but pickling and fermentation treatments showed a significant decrease in the concentration of this glucosinolate [36].

On the other hand, some authors have studied the effect of the storage conditions in the content of glucosinolates in broccoli. According to Vallejo et al. [37], during storage at 1°C over 7 days, over 3 days at 15°C, the degraded quantity of glucosinolates was in the range of 71 to 80%. In another study, during broccoli storage the effect of postharvest treatments and packaging in the content of glucoraphanin was evaluated. It was determined that at 20°C**,** the concentration of glucoraphanin decreased by 55% the third day after being stored in open boxes. Moreover, a loss of 56% in broccoli stored in plastic bags at was found at 7 days. The glucoraphanin concentration fluctuated slightly during 25 days while stored in controlled atmospheres (1.5% O_2 + 6% de CO_2) with air at 4°C. Also, it was found that broccoli heads,

stored without packing at 4°C, last a week without important losses in glucoraphanin concentration [38]. Cooling and controlled atmosphere treatments (21% O_2 + 10% CO_2 at 5°C) maintain the glucosinolates content of the broccoli florets for 20 days [39]. Moreover, packaging of broccoli florets in a polyethylene film (4 μm thick, 20 cm x 30 cm) without holes is a simple, economical and effective method for maintaining the visual quality and chemopreventive glucosinolates content [40].

4. SULFORAPHANE

4.1. Chemical Nature

Sulforaphane has a molecular weight of 177.29 and a molecular formula $C_6H_{11}NOS_2$. Its chemical structure (Figure 7) has a sulfoxide group wherein the sulfur is a chiral center with four different groups around the sulfur: oxygen, a four-carbon chain terminating in an isothiocyanate, a methyl group and the electron pair solitary [41]. Because of this, the isothiocyanates are strongly electrophilic compounds that can react with nucleophilic groups such as thiol, hydroxyl, and amino groups to form dithiocarbamates, thiourea derivatives or thiocarbamates [42]. In addition, broccoli is the primary natural source of sulforaphane precursor glucoraphanin and its isothiocyanate that constitutes between 50% and 80% of total glucosinolates present in this vegetable [27, 43].

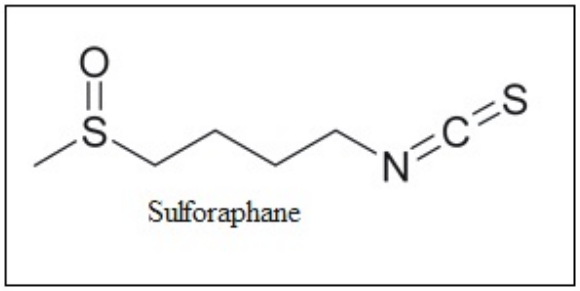


Figure 7. Chemical structure of sulforaphane.

4.2. Metabolism and Bioavailability

Sulforaphane is absorbed in the intestinal epithelium, enters the circulatory system and is subsequently conjugated with glutathione and eventually excreted in the urine as the corresponding conjugate of N-acetylcysteine or as mercapturic acids [25]. Glutathione-sulforaphane conjugates are the means of transport of this bioactive compound through the human body.

Sulforaphane that passes into the small intestine is absorbed by passive diffusion. Within epithelial cells sulforaphane can bind to glutathione (GSH + SFN) or in its simplest form, can be excreted into the intestinal lumen by active transport through P-glycoprotein (Pgp-1) or resistant protein multiple drugs (MRP-1) and discarded in feces together with unabsorbed

fraction. Sulforaphane and SFN+GSH that is not excreted from epithelial cells pass via passive diffusion into the bloodstream. Within the enterocytes, sulforaphane can spontaneously bind to glutathione or N-acetylcysteine, and is transported to the tissues subsequently processed in the metabolic pathway of the mercapturic acids and subsequently excreted in the urine [44].

The FDA [45] has defined the term bioavailability as the rate and extent to which active substances or therapeutic moieties contained in a drug are absorbed and becomes available at the site of action. The absorption and bioavailability of sulforaphane is affected by multiple factors. The first factor involves the hydrolysis of glucoraphanin to sulforaphane through myrosinase activity. This first step is critical because only the correct form of ITC is biologically active and has the desired properties against cancer. It is noteworthy that mammalian cells have no endogenous myrosinase activity. Myrosinases are found in the plant. However, myrosinase is heat labile and therefore cooking procedures inactivate the enzyme and can significantly reduce the bioavailability of sulforaphane up to three times. Another source of myrosinase activity is the intestinal microbial flora. Studies indicate that glucoraphanin can be converted to sulforaphane by the colonic flora [46, 47, 48].

In human studies [49] it has been found that the concentration of total isothiocyanates (this includes sulforaphane) in blood plasma begins to increase from 30 minutes after consumption, and reaching a peak between 1.5 to 3 hours, depending on the source of sulforaphane and food with which it is accompanied. After being absorbed and metabolized most of the ingested sulforaphane (around 60-65%) is excreted in urine as N-acetylcysteine+Sulforaphane (SFN-NAC) within 24 hours. In experiments performed in rodents it has been found that sulforaphane after being transported through the bloodstream is distributed in virtually all tissues of the body. In most cases a peak concentration is reached between 4 and 6 hours after consumption and becomes almost undetectable after 24 hours, except in prostate tissue [50].

4.3. Distribution and Postharvest Stability

The content of sulforaphane in broccoli has been widely studied for its biological activity. The concentration of the precursor of sulforaphane (glucoraphanin) varies greatly depending on the genotype, maturity of tissues and postharvest treatment. It is also important to mention that the chemical conditions (such as pH, presence of metal ions, the activity of the enzyme myrosinase and its cofactors) where the conversion of glucoraphanin to sulforaphane takes place are crucial to its final concentration [25]. In the mature plant, the inflorescence has the largest concentration. However sprouts and seeds have higher contents of sulforaphane compared to mature broccoli [51]. Furthermore, it is noteworthy that the processing of the vegetable has a significant effect on the content of sulforaphane, some data obtained in our laboratory show that the sulforaphane content decreases by 50% on the third day in fresh broccoli kept at room temperature. Furthermore broccoli frozen and refrigerated for 7 days had a sulforaphane loss of 14 and 29%, respectively [52]. Sulforaphane was not detected in broccoli flour obtained by convection drying at 60°C [53]. Moreover, during the lactic acid fermentation process of pickling broccoli it was not possible to detect quantifiable sulforaphane. In addition we found that blanching broccoli at 60°C for 3 minutes increases

the conversion efficiency of glucoraphanin to sulforaphane. Table 6 shows the results of sulforaphane content broccoli parts reported by different authors.

Table 6. Sulforaphane content in the broccoli plant

Sample	Sulforaphane content (µg/g dry matter)	Reference
Broccoli florets (Autolyzed samples)	17.99	Bertelli et al. [54]
Broccoli florets (Autolyzed samples)	12.69	
Broccoli florets (pH 3 hydrolysis)	31.82	
Broccoli florets (pH 3 hydrolysis)	49.28	
Broccoli stalks (pH 3 hydrolysis)	17.45	
Broccoli leaves (pH 3 hydrolysis)	110.03	
Broccoli commercial:		Matusheski et al. [55]
Cultivar Brigadier	335	
Cultivar Brigadier +	603	
Broccoli seeds:		Liang et al. [21]
Zhongqing II	4748	
Greenshirt	4548	
Greenyu	2036	
Broccoli 70	1619	
Greenball	1349	
Greenwave	2746	
Mature broccoli (supermarket) A:		Nakagawa et al. [56]
Florent	1713	
Stem	893	
Mature broccoli (supermarket) B:		
Florent	691	
Stem	443	
Mature broccoli (supermarket):		Campas-Baypoli et al. [52]
Florent	585	
Leaves	420	
Stalks	229	

The method to quantify sulforaphane used in our laboratory, consist in four phases, the first is the conversion of glucoraphanin to sulforaphane, the second is the extraction with dichloromethane, the third is purification in column of the solid phase extraction (SPE) and last the chromatography quantification HPLC-DAD [52]. Figure 8 shows the variation in the content of sulforaphane for different types of samples.

Although sulforaphane has been demonstrated to have great promise as a chemopreventive agent, its potential for clinical use is limited by the number of factors that can affect its bioavailability including the food matrix and the instability under normal storage conditions [57], due to its a sensitive compound to the oxidants, pH, temperature and heating time, favoring its degradation after exposure to these factors [58, 59].

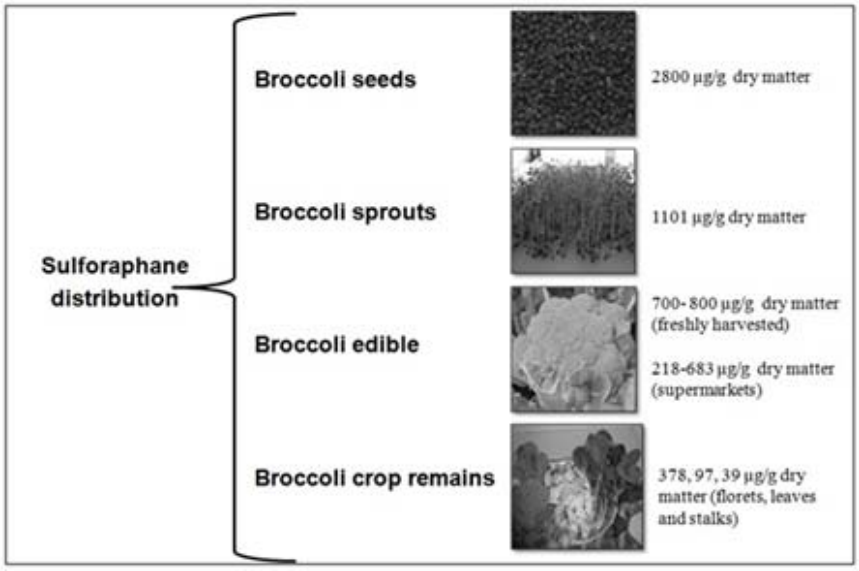


Figure 8. Sulforaphane concentration in seeds, sprouts, florets and crop remains of broccoli.

4.4. Beneficial Effects in Health

A balanced alimentation is essential for an adequate nutrition and health. The discovery of bioactive (phytochemicals) in food suggests the possibility to improve public health through the diet. Even so, the content of those phytochemicals in edible plants is very variable, which make the quality control and food intake recommendations really problematic. The variations depend on environmental and genetic factors, growing conditions, harvest, storage, processing, and preparation of food [60]. As some authors mention [61, 62, 63], fruit and vegetable consumption is not a guarantee of reduction in breast cancer. However, there exists sufficient evidence to state that the *Brassica* genus has a potential effect in the prevention of this type of cancer. Also, the isothiocyanate levels in urine in Asia populations have been correlated with a reduction in breast cancer risk in pre- and post-menopausal women [64].

The biotransformation process consists in the chemical conjugation that increases the solubility of the potentially toxic substances with the goal to facilitate excretion (see Chapter 4), this effect is called generally detoxication [65]. Sulforaphane is considered a powerful inducer of the phase II enzymes (e.g., glutathione-S-transferase, quinone reductase) [66, 67] that inactivate potential carcinogens. Sulforaphane acts as an indirect antioxidant. This has been shown in *in vivo* (animals) and in *in vitro* (cell culture) experimental models that reduce the incidence of some tumors [54]. Another benefit to health is its *in vitro* antimicrobial activity against *Helicobacter pylori* [68] (see Chapter 13). Furthermore, recent studies have shown that the topical application of broccoli sprout extract rich in sulforaphane protects against ultraviolet rays on animals and humans [69] (see Chapter 10).

A variety of *in vitro* and *in vivo* studies indicate that sulforaphane has high anticarcinogenic activity against breast, prostate, lung, stomach, skin and others types of cancer [69, 70, 71, 72]. Some of the proposed mechanisms about the chemopreventive and

anticarcinogenic mechanisms include: **a)** the detoxification of potentially carcinogenic substances by the induced phase II enzymes (e.g., glutathione-S-transferase, quinone reductase) [66, 67]. Sulforaphane forms a bond with Keap1-Nrf2 complex by direct reaction with the sulfhydryl groups of the cysteine residues of Keap1 [70], thereby activating the nuclear factor Nrf2 signalling pathway, which induces enzymes that repair some of the damages caused by the free radicals is induced [73]; **b)** through modulation of the phase I cytochrome P-450 (CYP) enzymes that are important in the normal metabolic processing of numerous endogenous and exogenous compounds, but can also activate certain chemical carcinogens [74]; **c)** by the induction of the apoptosis and cell cycle arrest, in tumor cells. It has been reported that sulforaphane can activate a variety of programmed cell death mechanisms including the mitochondria-mediated apoptosis, the death receptor mediated apoptosis, death cell by autophagy and the arrest of cell in phase G2/M of the cell cycle; **d)** by the inhibition of the angiogenesis and metastasis through inhibition of vascular endothelial cell growth factor thus inhibiting new blood vessels formation in tumors [70].

5. PERSPECTIVES ON SULFORAPHANE RESEARCH

The chemopreventive and anticarcinogenic capacity of the sulforaphane has been widely documented in a great quantity of *in vitro* and *in vivo* studies. A growing interest exist in the activity of sulforaphane against cancer has developed. This is why there has been, in the last years, a great amount of research to establish the main mechanisms of action of sulforaphane, both *in vivo* and *in vitro,* and the development of markers to monitor sulforaphane intake. We have discovered that the main disadvantage of sulforaphane is its rapid degradation in the food matrix as well as in the purified extracts. This is the reason why a variety of strategies are being studied to improve its chemical stability and effectiveness, for example the creation of encapsulates using micro-spheres with bovine serum albumin [75], with polymers such as poly-lactid acid/poli-glycolic acid [76], liposomes [75] and inclusion complex formation with β-cyclodextrin [59]. Currently, our investigation group is working to determine the bioavailability of natural sulforaphane extracts in different biological matrices, as well as in the research of alternatives to improve its stability through the microencapsulation process.

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