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Antioxidant Properties of *Brassica* Vegetables

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ABSTRACT

Brassica vegetables include some economically interesting crops such as cabbage, broccoli, cauliflower, Brussels sprouts, kale and turnip, which are consumed all over the world. A high intake of *Brassica* vegetables reduces the risk of age-related chronic illness such as cardiovascular health and other degenerative diseases and reduces the risk of several types of cancer, thanks in part to the antioxidant properties of different compounds. Compared to other vegetables, *Brassica* vegetables have higher antioxidant potential which makes them very interesting crops from the consumer's point of view. This review focuses on the composition and antioxidant capacity of both lipid- and water-soluble extracts of *Brassica* vegetables. Here, we will provide an overview of the role of phenolic compounds, vitamins and carotenoids present in *Brassica* vegetable crops in relation to antioxidant properties and human health. Both climatic conditions and agronomic practices influence the phytochemical content of the plant. The effects of genotype and plant organ on the stability of bioactive components and antioxidant activity are discussed, as well as post-harvest storage, processing and different cooking methods. Furthermore, we summarize in this review the current knowledge on the role of the antioxidant compounds present in *Brassica* vegetables in relation to human health. As conclusion, *Brassica* vegetables contain bioactive substances with a potential for reducing the physiological as well as oxidative stress and this could explain the suggested cancer preventive effect of these plants as well as their protective role on other major diseases.

Keywords: antioxidant activity, carotenoids, cruciferae; health, polyphenols, vitamins

Abbreviations: ABTS, 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid); **DNA**, deoxyribonucleic acid; **DPPH**, 2, 2-diphenyl-1-picrylhydrazyl; **ET**, single electron transfer reaction; **FC**, Folin-Ciocalteau assay; **FRAP**, ferric reducing power assay; **GPx**, glutathione peroxidase; **HAT**, hydrogen atom transfer reaction; **LDL**, low density lipoprotein; **ORAC**, oxygen radical absorbance capacity; **ROS**, reactive oxygen species; **RNA**, ribonucleic acid; **RNS**, reactive nitrogen species; **SOD**, superoxide dismutase; **TEAC**, Trolox equivalent antioxidant capacity

CONTENTS

INTRODUCTION

Clinical trials and epidemiological studies have established an inverse correlation between the intake of fruits and vegetables and the occurrence of diseases such as inflammation, cardiovascular disease, cancer and aging-related disorders. Dietary antioxidants, including polyphenolic compounds, vitamins E and C and carotenoids, are believed to be the effective nutrients in the prevention of these oxidative stress related diseases (Huang *et al.* 2005). For this reason, an important effort has been dedicated to identify potential antioxidant-rich cultivars and genotypes for breeding programs (Vinson *et al.* 1998; Chu *et al.* 2002; Zhang *et al.* 2004; Chun *et al.* 2005; Heimler *et al.* 2006; Charanjit *et al.* 2007; Cartea *et al.* 2008).

Among plant foods with health benefits, crops from the family *Brassicaceae* (also known as *Cruciferae*) have been the focus of numerous epidemiological and clinical studies (Podsedek 2007). Cruciferous vegetables, in particular those included into the *Brassica* genus, are good sources of a variety of nutrients and health-promoting phytochemicals (Liu 2004). It has been demonstrated that a high intake of *Brassica* vegetables reduces the risk of age-related chronic illnesses such as cardiovascular health and other degenerative diseases (Kris-Etherton *et al.* 2002) and reduces the risk of several types of cancer (Kristal *et al.* 2002; Wang *et al.* 2004; Bjorkman *et al.* 2011). The family *Brassicaceae* is a large group, having about 3,000 species grouped in 350 genera, including several types of edible plants. Petals of plants from this family have a distinctive cross form arrangement, which is the origin of the initial term '*Cruciferae*'. These plants may be annuals, biennials or perennials (Cartea *et al.* 2011a). The genus *Brassica* belongs to this family and, economically speaking, it is the most important

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Invited Review

genus within the tribe *Brassiceae*, containing 37 different species. The taxonomy of this genus is complex. Gómez-Campo (1999) presented a complete classification of the genus *Brassica* and its allied genera, indicating subgenera, sections, species and subspecies, which was later updated by the same author (Gómez-Campo 2003). The genus includes a group of six interrelated species of worldwide economic importance. U (1935) studied the cytology of the genus and established the relationships among the genomes of the six species. The three diploid *Brassica* species: *Brassica nigra* (L.) Koch (2n=16), *Brassica oleracea* L. (2n=18) and *Brassica rapa* L. (2n=20), form the classic Triangle of U. In nature, these species have hybridized in different combinations to give rise to the three amphidiploid species, namely *Brassica carinata* A. Braun (2n=4x=34), *Brassica juncea* (L.) *Czern*. (2n=4x=36) and *Brassica napus* L. (2n=4x=38). The genus is categorized into oilseed, forage, condiment and vegetable crops by using their buds, inflorescences, leaves, roots, seeds and stems. The same species may be utilized for several uses according to different forms or types (Cartea *et al.* 2011a).

The principal vegetable species into this genus is *B. oleracea*, which includes vegetable and forage forms, such as kale, cabbage, broccoli, Brussels sprouts, cauliflower and others; *B. rapa* includes vegetable forms, such as turnip, Chinese cabbage and pak choi, along with forage and oilseed types; *B. napus* crops are mainly used as oilseed (rapeseed), although forage and vegetable types like leaf rape and 'nabicol' are also included; finally, the mustard group which is formed by three species, *B. carinata*, *B. nigra* and *B. juncea*, is mainly used as a condiment because of their seeds, although leaves of *B. juncea* are also consumed as vegetables.

BRASSICA VEGETABLES AS A SOURCE OF DIETARY ANTIOXIDANTS

As it was pointed out by Huang *et al.* (2005), a general definition of antioxidant is rather straightforward as 'a substance that opposes oxidation or inhibits reactions promoted by oxygen or peroxides, many of these substances being used as preservatives in various products'. A more biologically relevant definition of antioxidants is 'synthetic or natural substances added to products to prevent or delay their deterioration by action of oxygen in air'. In terms of biochemistry and medicine, antioxidants are defined as 'organic substances that are capable of counteracting the damaging effects of oxidation in animal tissues' (Huang *et al.* 2005). Halliwell defined biological antioxidants as 'molecules which, when present in small concentrations compared to the biomolecules they are supposed to protect, can prevent or reduce the extent of oxidative destruction of biomolecules' (Halliwell 1990).

Biological antioxidants include enzymatic antioxidants, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), and nonenzymatic antioxidants, such as oxidative enzyme (e.g., cyclooxygenase) inhibitors, antioxidant enzyme cofactors, reactive oxygen/nitrogen species (ROS/RNS) scavengers and transition metal chelators. A dietary antioxidant can scavenge ROS/RNS to stop radical chain reactions or it can inhibit the reactive oxidants from being formed in the first place (Huang *et al.* 2005).

Human health benefits associated to *Brassica* consumption could be explained, in part, by their 'dietary antioxidants' and consequently, *Brassica* crops have been the focus of intense research based on the content of secondary metabolites (Traka and Mithen 2009; Verkerk *et al.* 2009). The antioxidant potential of *Brassica* vegetables is high compared to other vegetable crops. In fact, broccoli and kale are among the ones having the highest potential in the group of vegetable foods, including spinach, carrot, potato, purple onion, green pepper, beet, rhubarb or green bean (Cao *et al.* 1996; Ou *et al.* 2002; Zhou and You 2006). These vegetables possess high levels of antioxidant compounds, including vitamins, especially vitamin B6 (exceeded by garlic, pepper and spinach), vitamin A, β-carotene (only exceeded by carrot), lutein, zeaxanthin and vitamin K (Dekker *et al.* 2000; Vallejo *et al.* 2002, 2004a), folate, fiber, soluble sugars (Pedroche *et al.* 2004), lignin carotenoids, and phenolic compounds (Heimler *et al.* 2006). Therefore, *Brassica* vegetables can be considered as a good source of dietary antioxidants, including water soluble and water insoluble antioxidants.

Water-soluble antioxidants

1. Phenolic compounds

The contribution of *Brassica* vegetables to health improvement has generally been partly associated with their antioxidant capacity, being phenolic compounds the major antioxidants of these plants (Podsedek 2007; Jahangir *et al.* 2009). These antioxidants have proved to be good for human health and also useful as food preservatives (Kroon and Williamson 1999). Phenolics range from simple, low molecular-weight, single aromatic-ringed compounds to large and complex tannins and derived polyphenols (Crozier *et al.* 2009; Pereira *et al.* 2009). Phenolics are able to scavenge reactive oxygen species due to their electron-donating properties. The chemical properties of polyphenols in terms of the availability of phenolic hydrogens as hydrogen-donating radical scavengers predict their antioxidant activity (Rice-Evans *et al.* 1996). Phenolics can scavenge superoxide and peroxyl radicals, although there is conflicting evidence, and they have inhibitory effects on lipid peroxidation (Rice-Evans *et al*. 1996).

The most widespread and diverse group of polyphenols in *Brassica* species are flavonoids (mainly flavonols, but also anthocyanins) and hydroxycinnamic acids. The major polyphenolic constituents of *Brassica* foods, flavonols such as quercetin and kaempferol, and anthocyanidins, show a greater efficacy as antioxidants on a mole for mole basis than the antioxidant nutrients vitamin C, vitamin E and carotenoids (Rice-Evans *et al.* 1995; Vinson *et al.* 1995; Rice-Evans *et al.* 1996). An efficient peroxynitrite scavenger activity has been described for sinapic acid, which has shown to contribute to the cellular defense avoiding peroxynitrite-mediated disorders (Zou *et al.* 2002). *Brassica* plants accumulate glucose esters (1,6-di-O-sinapoylglucose), gentiobiose esters (1-O-caffeoylgentiobiose and 1,2,60-tri-O-sinapoylgentiobiose) of phenolic acids and kaempferol conjugates (Baumert *et al.* 2005).

Several studies have reported the presence of phenolic compounds in different vegetable crops of the genus *Brassica* (Nielsen *et al.* 1993; Llorach *et al.* 2003b; Vallejo *et al.* 2004b; Ferreres *et al.* 2005; Francisco *et al.* 2009, 2011; Velasco *et al.* 2011). Among crops included into *Brassica* vegetables, broccoli has been the most exhaustively studied with regard to polyphenol composition. Numerous and recent studies have shown that this crop (leaves, florets and sprouts) contains a high antioxidant potential linked to a high level of phenolic compounds (Llorach *et al.* 2003a; Vallejo *et al.* 2003; Moreno *et al.* 2006). Heimler *et al.* (2006) compared the main phenolic compounds in several *B. oleracea* crops and stated that broccoli and kale varieties exhibit the highest content of both total phenolics and flavonoids.

Quercetin, kaempferol and phenolic acids derivatives from the external and internal leaves, seeds and sprouts leaves of tronchuda cabbage have also been reported by several authors (Ferreres *et al.* 2005; Sousa *et al.* 2005; Ferreres *et al.* 2006; Sousa *et al.* 2007) and the different composition seems to be conclusive for the antioxidant activity displayed by each plant part.

Anthocyanins have also been identified in *Brassica* vegetables (Wu *et al.* 2005; Jahangir *et al.* 2009; Moreno *et al.* 2010). For example, the red pigmentation of red cabbage, purple cauliflower and purple broccoli is caused by anthocyanins. The major anthocyanins identified in these crops are cyanidin derivatives. Cauliflower and red cabbage showed differences in their anthocyanin profiles: cyanidin-3, γ5-diglucoside was absent in cauliflower, while it was well represented in red cabbage, together with the characteristic anthocyanin of the genus *Brassica*, cyanidin-3-sophoroside-5-glucoside. The p-coumaryl and feruloyl esterified forms of cyanidin-3-sophoroside-5-glucoside were predominant in cauliflower, while the sinapyl ester was mostly present in red cabbage (Lo Scalzo *et al.* 2008; Jahangir *et al.* 2009). Red cabbage contains more than 15 different anthocyanins, which are acylglycosides of cyanidin (Dyrby *et al.* 2001). Seventeen different anthocyanins were present in broccoli (Moreno *et al.* 2010), the main peaks corresponding to cyanidin-3-O-digluco-side-5-O-glucoside acylated and double acylated with p-coumaric, sinapic, caffeic, ferulic or sinapic acids.

Lignans are diphenolic compounds. A large variety of plant lignans exist, but only a few of them are converted into the enterolignans, absorbed into the human body. Lignans possess several biological activities, such as antioxidant and (anti) oestrogenic properties. Several studies have shown that lignans are prevalent in the *Brassicaceae* family, and particularly in kale, broccoli and Brussels sprouts (Heinonen *et al.* 2001), with lariciresinol and pinoresinol the most abundant (Milder *et al.* 2005).

2. Vitamin C and folic acid

Vitamin C, which includes ascorbic acid and its oxidation product, dehydroascorbic acid, performs many biological activities in the human body. The biological function of L-ascorbic acid can be defined as an enzyme cofactor, as a radical scavenger and as a donor/acceptor in electron transport at the plasma membrane. Ascorbic acid is able to scavenge the superoxide and hydroxyl radicals, as well as regenerate α-tocopherol (Davey *et al.* 2000). The content of vitamin C in *Brassica* vegetables varies significantly among and within species. Vitamin C levels varied over 4-fold in broccoli and cauliflower, 2.5-fold in Brussels sprouts and white cabbage and 2-fold in kale. The cause of these variations in vitamin C content might be related to the differences in genotype (Kurilich *et al.* 1999; Vallejo *et al.* 2002). Generally, white cabbage, one of the most popular *Brassica* vegetables, is the poorest source of vitamin C among this group of crops (Podsedek 2007).

In addition to ascorbic and dehydroascorbic acid, *Brassica* vegetables include ascorbigen, which is formed as the result of the reaction between ascorbic acid and indolyl-3-carbinole, one of the degradation products of a glucosinolate, glucobrassicin. It is likely that some of the biological effects attributed to ascorbigen are mediated by its breakdown to ascorbic acid.

Raw broccoli, cauliflower and cabbage contain folic acid, a scarce and important vitamin that acts as a coenzyme in many single carbon transfer reactions, in the synthesis of DNA, RNA and protein components (Kurilich *et al.* 1999).

Lipid-soluble antioxidants

1. Carotenoids

Carotenoids (carotens and xanthophylls) are yellow, orange and red pigments present in many fruits and vegetables. Several of them are precursors of vitamin A (i.e. β-carotene, γ-carotene and β-cryptoxanthin) and due to conjugated double bonds, they are both radical scavengers and quenchers of singlet oxygen, particularly under low oxygen pressure (Kurilich *et al.* 1999). The most abundant carotenoids in *Brassica* species are lutein and β-carotene, but, at least 16 carotenoids have been identified in *Brassica* extracts (Wills and Rangga 1996). In *B. oleracea*, kale is one of the vegetables with the highest carotenoid content (over 10 mg/100 g edible portion) (Muller 1997), being Brussels sprouts intermediate (6.1 mg/100 g) and broccoli (1.6 mg/100 g), red cabbage (0.43 mg/100 g) and white cabbage (0.26 mg/100 g) low in total carotenoid content (Muller 1997). Lutein and β-carotene are the dominant carotenoids in cruciferous vegetables (Podsedek 2007). The highest lutein + zeaxanthin values were observed for kale (3.04–39.55 mg/100 g), being the amount of these compounds moderately high (0.78–3.50 mg/100 g) in broccoli and Brussels sprouts (Podsedek 2007). In *B. rapa* species, 16 carotenoids were identified by Wills and Rangga (1996) in the *chinensis*, *parachinensis* and *pekinensis* subspecies, being lutein and *β*-carotene also the most abundant.

2. Vitamin E

In addition to carotenoids, vitamin E also belongs to a group of lipid-soluble antioxidants whose effect is mainly due to α-tocopherol. The predominant tocopherol in all *Brassica* vegetables is α-tocopherol with the exception of cauliflower, which predominantly contains γ-tocopherol (Piironen *et al*. 1986, cited by Podsedek 2007). The predominant reaction responsible for tocopherol antioxidant activity is the donation of hydrogen atoms, where a tocopheroxyl radical is formed (Podsedek 2007). The descending order of total tocopherols in *Brassica* vegetables is from broccoli (0.82 mg/100 g) to Brussels sprouts (0.40 mg/100 g), cauliflower (0.35 mg/100 g), Chinese cabbage (0.24 mg/100 g), red cabbage (0.05 mg/100 g) and white cabbage (0.04 mg/100 g) (Piironen *et al*. 1986, cited by Podsedek 2007). Kurilich *et al*. (1999) reported a similar rank on the basis of concentration and, therefore, they pointed out that the best sources of lipid-soluble antioxidants are kale and broccoli. Brussels sprouts have moderate levels of the above-mentioned compounds, while cauliflower and cabbage are characterized by their low levels.

ANTIOXIDANT ACTIVITY IN *BRASSICA* VEGETABLES

Antioxidant capacity assays

In assessments of the effectiveness of particular antioxidants or antioxidant-rich foods, there are several complementary approaches that can be taken. In the first place, it is necessary to assess if the substance acts as an antioxidant *in vitro*. In the second place, if it protects cells in culture from oxidative damage and then, if when administered to human subjects, the substance acts as an antioxidant *in vivo* thus, decreasing the level of oxidative damage to biomolecules such as lipids, proteins and DNA (Collins 2005).

The antioxidant potential of *Brassica* extracts can be measured *in vitro* as a first step in determining if they have a potential effect in health. Sample preparation is the crucial first step in the study of the antioxidant property of plants. Several methods have been used for the extraction of antioxidants from vegetables *in vitro*, such as solvent extraction (Kaur *et al.* 2007; Kusznierewicz *et al.* 2008; Pérez-Balibrea *et al.* 2011) or ultrasonic extraction (Kim *et al.* 2004; Pant *et al.* 2009). Liophilized samples are mixed and macerated in the solvent for different periods of time (Lin and Chang 2005; Pérez-Balibrea *et al.* 2011) or are boiled in the solvent (Barillari *et al.* 2006; Ferreres *et al.* 2009).

The antioxidant capacity of *Brassica* vegetables depends on antioxidant levels and their composition. In order to determine their total antioxidant capacity, the activity of both water and lipid-soluble antioxidants must be considered (Podsedek 2007). In the studies published on the antioxidant activity of these vegetables, different extraction procedures and several methods measuring this activity have been employed (**Table 1**). For this reason, comparing the results is very difficult. A lot of studies have been made to evaluate the antioxidant potential of hydrophilic antioxidants, which are extracted from the food matrix with polar solvents. For example, Wachtel-Galor *et al.* (2008) extracted hydrophilic antioxidants from several *Brassica* crops, including broccoli, cauliflower, cabbage and choy-sum using water as solvent. Phosphate buffer 50 mM was employed by Serrano *et al.* (2006) to extract hydrophilic anti-

Table 1 Summary of works evaluating the antioxidant potential of *Brassica* crops *in vitro*. Crop studied, type of assay employed and extraction method are described.

Crop	Antioxidant activity assays[§]	Sample extraction	References
Brassica oleracea			
Broccoli	DDPH, FC, OH^- scavenging activity	70% methanol	Borowski *et al.* 2008
	DPPH, FC	methanol	Cogo *et al.* 2011
	DPPH, lipid peroxidation	ethanol	Costa *et al.* 2005
	FC, ORAC	hexane, water	Eberhardt *et al.* 2005
	DPPH, FC, lipid peroxidation	50% metanol (v:v)	Gawlik-Dziki 2008
	ORAC	water	Girard-Lalancette *et al.* 2009
	DPPH, FC, lipid peroxidation, O_2^-, H_2O_2 scavenging activity	ethanol, water	Gulcin *et al.* 2004
	DPPH	acetone, methanol, water	Guo *et al.* 2001
	DPPH, FC, FRAP, lipid peroxidation	80% methanol	Kaur *et al.* 2007
	ORAC	hexane, water	Kurilich *et al.* 2002
	DPPH, FC	ethanol	Lemoine *et al.* 2010
	DPPH, lipid peroxidation	methanol	Lin and Chang 2005
	ABTS, DPPH, FRAP	water	Miglio *et al.* 2008
	DPPH, FC	methanol	Mrkic *et al.* 2006
	ABTS, DPPH, FRAP	water	Ng *et al.* 2011
	ABTS, DPPH	methanol, water	Perez-Balibrea *et al.* 2011
	FC, ORAC	hexane, ethanol, methanol, water	Roy *et al.* 2009
	ABTS, FC	50 mM phosphate buffer	Serrano *et al.* 2006
Brussels sprouts	DPPH	ethanol	Vina *et al.* 2007
Cabbage	ABTS, FC	80% methanol	Kim *et al.* 2004
	ABTS, DPPH, FC	methanol	Kusznierewicz *et al.* 2008
	ABTS, DPPH	water	Kusznierewicz *et al.* 2010
	DPPH, FC, lipid peroxidation	water	Tanongkankit *et al.* 2010
	FC, FRAP, ORAC	(10 mM HCl)-methanol	Volden *et al.* 2008
Cauliflower	ABTS, DPPH, FRAP, lipid peroxidation	ethanol, water	Llorach *et al.* 2003
	FC, lipid peroxidation	water	Lo Scalzo *et al.* 2007
	FC, FRAP, ORAC	(10 mM HCl)-methanol	Volden *et al.* 2009a
	FC, FRAP, ORAC	(10 mM HCl)-methanol	Volden *et al.* 2009b
Kale	DPPH, OH^-, NO scavenging activity	water	Ferreres *et al.* 2009
	FC, ORAC	methanol	Hagen *et al.* 2009
Tronchuda cabbage	DPPH, O_2^-, OH^-, HClO scavenging activity	water	Ferreres *et al.* 2006
	DPPH, O_2^-, OH^-, HClO scavenging activity	water	Ferreres *et al.* 2007
	DPPH, O_2^-, OH^-, HClO scavenging activity	water	Vrchovska *et al.* 2006
Brassica rapa			
Cima di rapa	DPPH, FC	methanol-water (80:20)	Cefola *et al.* 2010
Friariello	ABTS, FC	60% methanol	De Pascale *et al.* 2007
Turnip	DPPH	water	Fernandes *et al.* 2007
Pak choi	FC, ORAC	ethanol/acetone (1:1 v/v)	Pant *et al.* 2009
Undefined	FC, DPPH	80% methanol	Gutierrez *et al.* 2008
Undefined	ABTS, DPPH, lipid peroxidation	ethanol	Samarth *et al.* 2008
Several *Brassica* crops			
Broccoli, cabbage, cauliflower, kale	ORAC	acetone, water	Cao *et al.* 1996
Broccoli, Brussels sprouts, cabbage, cauliflower, Chinese cabbage	FC, DPPH	methanol	Jagdish *et al.* 2009
Broccoli, kale	ABTS, DPPH, FC, ORAC, O_2^-scavenging activity	acetone/water (1:1 v/v)	Zhou and You 2006
Broccoli, radish	ABTS	phosphate buffered saline	Martínez-Villaluenga *et al.* 2010
Cabbage, cauliflower, kale	FRAP	acetone, 0.1 M sodium-acetate buffer	Nilsson *et al.* 2006
Broccoli, cabbage, cauliflower	FRAP, ORAC	acetone/water (1:1 v/v)	Ou *et al.* 2002
Broccoli, Brussels sprouts, cauliflower	ABTS, DPPH, FRAP	water	Pellegrini *et al.* 2010
Brussel sprouts, cabbage	ABTS, DPPH, FC, lipid peroxidation, O_2^- scavenging activity	hexane, 70% methanol, water	Podsedek *et al.* 2006
Cabbage, Chinese cabbage	ABTS, DPPH, FC, FRAP	water	Samec *et al.* 2011
Broccoli, Brussels sprouts, cauliflower, kale	ABTS, FC	70% methanol	Sikora *et al.* 2008
Kale, tronchuda cabbage, turnip	DPPH, O_2^-, OH^-, HClO scavenging activity	water	Sousa *et al.* 2008
Broccoli, cabbage, cauliflower, choysum	FC, FRAP	water	Wachtel-Galor *et al.* 2008
Other *Cruciferae*			
Radish	ABTS, FC	70% ethanol	Barillari *et al.* 2006
	DPPH, lipid peroxidation	70% ethanol	Barillari *et al.* 2008
Watercress, mizuna, wild rocket, salad rocket	ABTS, DPPH, FRAP	methanol-water (1:1 v/v)	Martínez-Sánchez *et al.* 2008

[§] ABTS: 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), DPPH: 2,2-diphenyl-1-picrylhydrazyl, FC: Folin-Ciocalteau, FRAP: ferric reducing antioxidant power, ORAC: oxygen radical absorbance capacity.

Table 2 *In vitro* antioxidant capacity assays, based on Apak *et al.* (2009), Huang *et al.* (2005), Mcdonald-Weeks *et al.* (2006) and Moon and Shibamoto (2007).

Assays involving hydrogen atom transfer reactions (HAT-based assays)	Assays by electron-transfer reaction (ET-based assays)	Other assays
Crocin bleaching assay	CUPRAC (cupric ion reducing antioxidant capacity)	TOSC (total oxidant scavenging capacity)
Inhibition of lipid peroxidation	DPPH (diphenyl-1-picryhydrazyl)	Inhibition of Briggs-Rauscher oscillation reaction
IOU (inhibited oxygen uptake)	FOX (ferrous oxidation-xylenol orange)	Chemiluminiscence
ORAC (oxygen radical absorbance capacity)	FRAP (ferric ion reducing antioxidant parameter)	Electrochemiluminiscence
TRAP (total radical trapping antioxidant parameter)	FTC (ferric thyocianate assay)	Scavenging activity against O_2^-, OH^-, HClO, H_2O_2
	TEAC (Trolox equivalent antioxidant capacity)	
	Total phenols assay by Folin-Ciocalteau reagent	

oxidant components from broccoli florets. Nilsson *et al.* (2006) employed acetate buffer 0.1 M to extract antioxidant components from cauliflower, cabbage and kale. Short-chain alcohols such as ethanol and methanol are commonly employed to obtain hydrophilic extracts. As examples, Viña *et al.* (2007) employed ethanol 95% and Cefola *et al.* (2010) methanol: water (80:20) as solvents to extract antioxidant components from *B. rapa*. Lipid-soluble antioxidants from *Brassica* vegetables are easily extracted with the use of organic solvents such as acetone (Guo *et al.* 2001), or hexane (Kurilich *et al.* 2002; Podsedek *et al.* 2006; Roy *et al.* 2009).

The antioxidant capacity of the plant extract also depends on the test system and it is influenced by many factors, which cannot be fully described with one single method. Therefore, it is necessary to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant actions. Many methods have been developed to evaluate antioxidant activity. But, due to the lack of a standard assay, it is difficult to compare the results reported from different research groups (Huang *et al.* 2005).

On the basis of the chemical reactions involved, free radical scavenging assays can be roughly divided into two categories (Huang *et al.* 2005; Mcdonald-Wicks *et al.* 2006): 1.-hydrogen atom transfer reaction based assays (HAT-based assays) and 2.-single electron transfer reaction based assays (ET-based assays). ET-based assays measure antioxidant reducing capacity and the HAT-based assays quantify hydrogen atom donating capacity. The hydrogen atom transfer reaction is a key step in the radical chain reaction. Therefore, the HAT-based method is more relevant to the radical chain-breaking antioxidant capacity. ET-based assays include the total phenols assay by Folin-Ciocalteau reagent (FC) and the measure of antioxidant capacity by using the reagents ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid, usually called TEAC: Trolox equivalent antioxidant capacity) and DPPH (2, 2-diphenyl-1-picrylhydrazyl), the ferric ion reducing antioxidant power (FRAP), the ferrous oxidation-xylenol orange (FOX), the ferric thyocianate assay (FTC) and the cupric ion reducing antioxidant capacity (CUPRAC).

Assays are carried out in acidic (FRAP), neutral (ABTS) or basic (FC) conditions. HAT-based assays include the Inhibited Oxygen Uptake (IOU) method, which measures the rate of oxygen uptake in the presence of antioxidant extracts. Another HAT-based assay measures the inhibition of induced lipid autoxidation. This method artificially induces autoxidation of linoleic acid or LDL by either Cu(II) or an azo initiator. Several colorimetric assays use molecular probes to monitorize kinetics of the inhibited autoxidation of lipids. Assays with this feature include a total radical trapping antioxidant parameter (TRAP assay), oxygen radical absorbance capacity (ORAC assay) and crocin bleaching assay (**Table 2**).

Besides general methods, some assays intended to measure a sample's scavenging capacity of biologically relevant oxidants such as singlet oxygen, superoxide anion, peroxyl radicals, hydroxyl radical, singlet oxygen and peroxynitrite. A summary of different works about the antioxidant potential of *Brassica* vegetables is presented in **Table 1**. As it can be observed, the most popular methods employed in *Brassica* crops are the ET-based assays. Among the HAT-based assays, ORAC is commonly used.

Generally speaking there is a good correlation among results given by ABTS, FRAP and DPPH antioxidant assays and, among these and total phenolic content (FC) (Zhou and You 2006; Podsedek *et al.* 2006; Kusznierewicz *et al.* 2008; Martínez-Sánchez *et al.* 2008; Samec *et al.* 2011). There was also a high correlation between ORAC values obtained in the hydrophilic extract and total phenolic content (Eberhardt *et al.* 2005). However, this is not always the case when correlations are computed among ET-based assays and the inhibition of lipid autoxidation (Kaur *et al.* 2007) or ET-based assays and superoxide and hydroxyl radical scavenging activity (Sousa *et al.* 2008). Differences could be due to the differential reaction mechanism involved in different assay systems.

The antioxidant potential of *Brassica* vegetables may be influenced by different parameters, such as the genotype under evaluation, environmental conditions in which plants are grown and the way vegetables are processed or cooked. All these factors should be taken into account to keep or to increase the antioxidant capacity of vegetables as much as possible.

Variation of the antioxidant potential of *Brassica* vegetables

1. Influence of the genotype

The antioxidant potential of *Brassica* vegetables has been measured in the most economically important crops, mainly in *B. oleracea* crops (**Table 2**), although the antioxidant potential of several *B. rapa* crops has also been tested. Among *B. oleracea* crops, most works have been focused in studying the antioxidant potential of broccoli (**Table 2**) but the antioxidant potential of kale, Brussels sprouts, tronchuda cabbage and cabbage (including red, white and savoy cabbage) have also been studied extensively (**Table 2**). Data shows that the antioxidant potential of Brussels sprouts is quite high, as it is that of kale, tronchuda cabbage and cauliflower. The *B. rapa* crops studied include cima di rapa, turnip, pak choi, Chinese cabbage, choy-sum, friariello and mizuna. The antioxidant potential of other species of the *Brassicaceae* family has also been tested (**Table 2**).

As a result of these works, we can affirm that there is variability among species, crops of the same species and cultivars of the same crop regarding the antioxidant potential. In this section, the results of different works comparing the antioxidant potential of different *Brassica* crops are going to be exposed. Because of the difficulties in comparing works carried out by different authors who follow different methodologies in extracting and measuring the antioxidant potential, only those comparisons carried out by the same research group using the same methodology are going to be discussed.

1.1. Brassica oleracea

Podsedek *et al.* (2006) measured the antioxidant potential of different crops of *B. oleracea* including red, white and savoy cabbage and Brussels sprouts. Red cabbage and Brussels sprouts had higher antioxidant potential than savoy and white cabbages. Nilsson *et al*. (2006) measured the antioxidant potential of several cultivars of cauliflower, white cabbage and curly kale, finding that the antioxidant potential in curly kale was at least 10-fold higher than that in cauliflower and white cabbage. Sikora *et al*. (2008) compared the antioxidant potential of kale, broccoli, Brussels sprouts and green and white cauliflower and they found that kale showed the highest antioxidant potential and cauliflower showed the lowest. Jagdish *et al*. (2009) studied the radical scavenging activity of methanolic extracts from red cabbage, Chinese cabbage, cauliflower, Brussels sprouts and broccoli and they found the highest antioxidant activity in red cabbage and the lowest in white cabbage extracts.

Podsedek *et al*. (2007) did a review of several works about antioxidant potential in *B. oleracea* crops. Brussels sprouts, broccoli and red cabbage belong to the group of the ones having the highest antioxidant capacity whereas cabbage demonstrated a rather low antioxidant activity. Controversial results had been demonstrated for cauliflower, which showed high activity in liposomal phospholipid suspension system, but low activity in oxygen radical absorption capacity (ORAC) method (reviewed by Podsedek *et al*. 2007). The same author reached the conclusion that the best sources of lipid-soluble antioxidants are kale and broccoli. Brussels sprouts have moderate levels of the above-mentioned compounds, while cauliflower and cabbage are characterized by their relatively low amounts.

Genotypic variation for antioxidant potential among cultivars of the same crop has also been noted. Volden *et al*. (2009a) studied five different varieties of cauliflower (two white, one green, one purple and one green/romanesco). The antioxidant potential was higher in the purple variety, most probably due to the presence of polyphenolic pigments and the white cultivars had the lowest antioxidant capacity values. Kaur *et al*. (2007) evaluated eight cultivars of broccoli grown in two different years in India, finding variability in antioxidant capacity among cultivars, having cv. 'Aishwarya' the best antioxidant potential. Pérez-Balibrea *et al*. (2011) found differences in the antioxidant potential of seeds and sprouts among three cultivars of broccoli, being the cultivar 'Viola' the one with the highest antioxidant potential, probably due to its red pigmentation.

Kurilich *et al*. (2002) studied the variability in antioxidant capacity of several cultivars. Cultivar 'Majestic' showed the highest antioxidant potential. Kim et al. (2004) studied the antioxidant potential of 10 varieties of green cabbage and significantly higher levels of total phenols and antioxidants were observed in the varieties 'Fresco' and 'Bobcat'. Kusznierewick *et al*. (2008) found variability in the antioxidant potential of different genotypes of white cabbage grown in four different sites. The cabbage from Belgium displayed the highest antioxidant potential and the lowest one was from Poland.

1.2. Brassica rapa and other cruciferous crops

Samec *et al*. (2011) compared the antioxidant potential of white cabbage (*B. oleracea*) and Chinese cabbage (*B. rapa*). The phenolic content and the antioxidant potential were higher in Chinese cabbage than in white cabbage. Sousa *et al*. (2008) studied the antioxidant potential in the inflorescences of three different *Brassica* crops, two belonging to *B. oleracea* (tronchuda cabbage and kale) and one to *B. rapa* (turnip). Based on their results, it was not possible to suggest one variety as the best in terms of antioxidant potential because different assays gave different results.

Wachtel-Galor *et al.* (2008) compared the antioxidant activity of three *B. oleracea* crops (broccoli, cauliflower and cabbage) and one *B. rapa* crop (choy sum). Choy sum showed the highest antioxidant potential, followed by broccoli, cabbage and cauliflower. After analyzing the extracts of six species with radioprotective effects, including *Adhatoda vasica*, *Amaranthus paniculatus*, *B. rapa*, *Mentha piperita* and *Spirulina fusiformis*, Samarth *et al*. (2008) found that the most radical-scavenging were the extracts of *M. piperita*, *A. vasica* and *B. rapa*.

Martínez Sánchez *et al*. (2008) studied the antioxidant potential of *Brassica* vegetables used for salads, including watercress (*Nasturtium officinale*), mizuna (*B. rapa*), wild rocket (*Diplotaxis tenuifolia*) and salad rocket (*Eruca vesicaria*). The different methods tested showed similar results, being watercress the crop with the highest antioxidant potential and salad rocket the one with the lowest antioxidant potential. Authors concluded that watercress, wild rocket, mizuna and salad rocket are good sources of antioxidants. Martínez-Villaluenga *et al*. (2010) studied three cultivars of broccoli and one of horseradish (*Armoriacea rusticana*) finding that radish seeds had higher values than broccoli seeds.

2. Influence of plant part and development

The antioxidant potential of a particular crop also depends on the plant part assessed. Sometimes, parts of the plant which are not usually consumed can be rich in phytochemicals and antioxidant potential. Fernandes *et al*. (2007) studied the antioxidant potential of different parts of turnip plants, including leaves and stems, flower buds and roots. Flower buds were found to be the most active part followed by leaves and stems and turnip roots showed a significantly lower antioxidant capacity. Internal leaves of tronchuda cabbage are eaten raw in salads, or most usually, cooked. However, despite its high antioxidant capacity exhibited according to Ferreres *et al*. (2006) in several assays (**Table 1**), they showed lower antioxidant potential than external leaves, which are not normally consumed. In another study, Ferreres *et al*. (2009) concluded that kale seeds had higher antioxidant potential than kale leaves, despite the fact that leaves are richer in phenolic compounds than seeds.

During the processing of *Brassica* vegetables, an important amount of by-products is produced. In the case of cauliflower, leaves constitute about 50% of the total, the rest is mainly stem. A number of studies have proposed some vegetable by-products as sources of natural antioxidants in order to give value to these wastes. Guo *et al*. (2001) measured antioxidant activity and reducing power of flower, stem and leaf of broccoli showing that leaf and stem parts of broccoli exhibit certain levels of antioxidant properties. A proper stem or leaf processing or treatment to develop a new type of product could enhance the utilization of broccoli. Antiradical activity of cauliflower byproducts was measured by Llorach *et al*. (2003a) in water, ethanolic and purified fractions and all fractions showed antioxidant activity. Authors concluded that, although the aqueous fraction showed less antioxidant activity, it would be cheaper to extract antioxidants from it.

Antioxidant potential also varies depending on the maturity stage of the plant. This factor should be taken into account when choosing the harvest date of the plant products. The antioxidant potential is high in broccoli and horseradish seeds (Martínez-Villaluenga *et al.* 2010). However, the germination process caused a gradual decrease of antioxidant potential after the fifth day. Kim *et al*. (2004) found that juvenile cabbages possessed relatively higher amounts of flavonols compared with mature cabbage, suggesting that maturity may affect the phenolic composition in cabbage and therefore their antioxidant potential. Samec *et al.* (2011) studied the variation of antioxidant potential and total phenolic content depending on the harvesting date on white cabbage and Chinese cabbage finding that there was an increase in the antioxidant capacity of cabbage in the first 8 or 12 weeks, after which it assumed a gradual decrease. This is probably a consequence of a more active plant metabolism which accompanies active/rapid growth in the first few months.

3. Influence of environment

Even though environment plays an important role in vegetables development and their content on phytochemicals, few works have investigated its effect on the antioxidant potential of final products. This knowledge is very useful when choosing the appropiate environmental factors which can enhance the healthy potential of vegetables. Therefore, this review includes the effects of environmental factors like watering, fertilization or harvest time on the antioxidant activity of different *Brassica* crops.

Kim *et al*. (2004) studied the variation in the antioxidant potential of green cabbage grown under nutritional soil supplements derived from agricultural and food-processing sources and found that the application of nutritional soil supplements resulted in an increase of the antioxidant capacity. De Pascale *et al*. (2007) searched for the effects of sulphur availability on the antioxidant potential of two ecotypes of *B. rapa* subsp. *sylvestris.* Authors found that sulphur fertilization improved the antioxidant activity of both ecotypes. Pant *et al*. (2009) studied the effect of vermicompost teas applied as a foliar spray on plants grown under both organic and chemical fertilization. The treatment effect on the antioxidant potential was not significant under chemical fertilisation. The effect of all vermicompost teas on antioxidant activity was lower than that of the control under vermicompost fertilisation. More recently, Cogo *et al*. (2011) studied the influence of soil water content during plant growth and postharvest storage conditions on the antioxidant activity of broccoli. Low soil water content during plant growth and postharvest cold storage were the conditions that, when combined, gave the best preservation of antioxidant activity.

Harvest time is a very important factor to determine the vegetable quality and this fact assumes great importance for cauliflower, which has a narrow harvest period. Lo Scalzo *et al*. (2007) studied the antioxidant potential of cauliflower harvested in three years and in two different dates. There were significant differences among years, but samples showed no clear trend between early and late harvest. Vrchovska *et al*. (2006) evaluated the antioxidant potential of the aqueous extracts of external leaves of tronchuda cabbage under different cultivations (organic and conventional) and in different sample dates. They found that samples grown under organic cultivation and early harvested had the highest antioxidant potential.

4. Influence of postharvest treatments

The health-promoting capacity of plant foods strictly depends on their processing history. The conditions of storage, processing and preparation have been proved to have significant effects on the antioxidant content, but the impact of processing on the antioxidant activity of vegetables is still a neglected area and little information is available (Mrkic *et al.* 2006).

Most of the works about the influence of postharvest treatments in the antioxidant potential of *Brassica* vegetables have been carried out in broccoli. Broccoli florets are highly perishable vegetables with an accelerated senescence during postharvest life. Lipid degradation, lipid peroxidetion and protein degradation are usually correlated with tissue deterioration after harvesting (Costa *et al.* 2005; Serrano *et al.* 2006). The degradation of chlorophyll and the membranes causes an increase in the production of free radicals. In addition, the amount of reduced oxygen, e.g. hydrogen peroxide, increases greatly during senescence (Lemoine *et al.* 2010).

Methods for improving the shelf life of fresh-cut vegetables and delaying senescence include refrigeration, modification of the surrounding atmosphere or heat treatments, among others. All these methods can have an effect on the antioxidant potential of *Brassica* vegetables compared to untreated samples. The preservation of antioxidant potential is crucial, not only in order to preserve dietary antioxidant components but to maintain the defenses of vegetables against ROS, in order to delay senescence processes.

Cold storage can have a positive effect by preserving high levels of natural antioxidants (phenols, vitamin C) and *in vitro* antioxidant capacity in curly kale (Hagen *et al.* 2009). The use of modified atmosphere package reduces the respiration rate, maintains sensory attributes and increases the vegetables' shelf life. In broccoli heads packaged with micro-perforated and non-perforated films, total antioxidant activity, total phenolic content and ascorbic acid content remained almost unchanged compared to unpacked controls (Serrano *et al.* 2006). After studying different modified atmospheres in cold storage conditions, Cefola *et al*. (2010) concluded that low oxygen atmosphere did not affect the nutritional quality, maintaining the initial phenolic, antioxidant potential and vitamin C contents during the entire cold storage.

Blanching is necessary as a pre-treatment to freezing procedure, mainly in order to inactivate degradative enzymes. After blanching, the vegetable is frozen and maintained at constant temperature. Volden *et al*. (2009b) studied the effect of a combined treatment of blanching followed by long-term frozen storage on cauliflower. Three treatments were applied: untreated, blanched and freezer-stored. Blanching caused significant reductions in the levels of phenolics and other phytochemicals and antioxidant potential. In general, freezer storage gave diminishing levels of antioxidant potential over time. Recently, Tanongkankit *et al*. (2010) studied the effect of sample slicing and blanching by using either hot water or steam, as well as hot air drying, on the evolution of various phytochemicals and antioxidant activity in cabbage outer leaves. Slicing led to higher losses of antioxidants during either hot water or steam blanching. Water blanching led to a lower retention of water-soluble antioxidants and all antioxidants degraded significantly during drying. Viña *et al*. (2007) evaluated the effect of several blanching methods on Brussels sprouts: direct blanching, immersion in hot water (50°C) prior to blanching and microwave heating prior to blanching. Pre-blanching methods induced the greatest increases in radical scavenging activity.

Drying of different products has been studied and their beneficial effects have been well documented. Costa *et al*. (2005) evaluated the effect of different heat treatments on broccoli extracts. Antioxidant potential decreased in the control but heat treatment contributed to the conservation of tissue integrity by maintaining their antioxidant status. In other cases, heat combined to other treatments can increase the antioxidant potential of *Brassica* extracts. Mrkic *et al*. (2006) analyzed the effect of heat drying after blanching broccoli florets. Air drying treatments increased the antioxidant activity of broccoli, in particular, high-temperature and short-time drying processes maximised the antioxidant activity. This could be explained by an increased release of compounds from the matrix, or hydrolysis of polyphenols. In a recent study, Lemoine *et al*. (2010) analyzed the effect of the influence of a combined method of heat treatment combined with a UV-C treatment on the antioxidant activity of broccoli stored at 20°C. Broccoli showed a significant increase in the antioxidant activity after the application of combined treatment. Then, the antioxidant activity decreased in both control and treated samples, but the treated ones kept the antioxidant potential significantly higher.

As a conclusion of the works reviewed here, it seems that cold storage, modified atmospheres and drying treatments decrease the decay of antioxidant potential that normally happen after harvest; in the case of air drying systems, the antioxidant potential may even be increased. However, blanching had a negative effect compared to untreated controls by increasing the decay of antioxidant potential.

5. Influence of cooking methods

Variation in cooking treatment can strongly affect the texture, nutritional value and antioxidant capacity of vegetables. Cooking methods may affect quality parameters of vegetables negatively, but in some experiments, the antioxidant potential can even be increased after heat treatments. In fact, it has been reported that cooking treatments did not affect, or affected the antioxidant capacity of *Brassica* vegetables in a positive way.

Many studies have revealed that most vegetables, precooked at a moderate temperature (50-80°C) for a suitable period of time and subsequently cooked in boiling water, showed greater firmness than those cooked directly without precooking. Lin and Chang (2005) evaluated the effect of three different treatments on antioxidant potential and reducing power on broccoli. Treatments were: precooking at 50°C for 10 min, cooking in boiling water for 8 min and precooking followed by cooking. This study indicated that a precooking and/or cooking treatment had no profound effect on the antioxidant properties of broccoli. Kusznierewicz *et al*. (2010) determined the antioxidant potential of white cabbage cooked by different methods. Cabbage heads were cut and divided into two parts: one was submitted to fermentation and the other one was non-treated. Portions of fresh cabbage and sauerkraut were stewed for 2 h at 100°C under cover. Fresh cabbage juice displayed relatively low antioxidant activity, and spontaneous fermentation consistently increased this activity three to 4-fold. In the case of fresh cabbage, heating at 100°C increased the ability to scavenge radicals linearly. Pellegrini *et al*. (2010) evaluated the effect of boiling, microwaving and steaming in broccoli, Brussels sprouts and cauliflower analyzed fresh and frozen. For fresh broccoli, boiling and oven steaming generally led to an increase of antioxidant potential. Microwaving always has a detrimental effect and the effect of basket steaming strongly depends on the antioxidant assay. Fresh *Brassica* vegetables retained phytochemicals and antioxidant activity better than frozen samples. This behaviour was more evident for broccoli, probably due to the different structural matrix of cell walls. Miglio *et al*. (2008) evaluated the effect of boiling, steaming and frying on phytochemical contents and total antioxidant capacities in broccoli. Water-cooking treatments preserved the antioxidant compounds better, particularly carotenoids. An overall increase of antioxidant capacity was observed in all cooked vegetables, probably due to a matrix softening and increased extractability of compounds. Ng *et al*. (2011) compared the antioxidant potential of broccoli subjected to boiling, microwaving and pressure cooking. The total antioxidant activity was increased in boiling broccoli. Pressure cooking did not cause any significant decline in the antioxidant property. Boiling generally improved the overall antioxidant activity. Roy *et al*. (2009) evaluated the effect of steaming on the nutritional quality of broccoli by assessing the total antioxidant activity of raw and steamed broccoli. Steaming significantly increased the extractability of these compounds in broccoli and its antioxidant potential. These findings contradict the notion that processed fruits and vegetables have lower nutritional values than their unprocessed counterparts do.

Other authors have found that cooking treatments have a detrimental effect on the antioxidant potential of *Brassica* vegetables. Sikora *et al*. (2008) studied the effect of four different combinations of postharvest and cooking treatments (boiling, blanching, freezing after blanching and boiling after freezing) in the antioxidant activity of kale, broccoli, Brussels sprouts and green and white cauliflower. There was a decrease in the polyphenols content in vegetables subjected to aquathermal processes compared to the raw ones. Generally, the largest changes were caused by boiling fresh or previously frozen vegetables. Blanching caused a smaller loss of polyphenols than boiling did in all crops except for kale. After blanching and boiling, there was a reduction in the antioxidant activity of these vegetables. The freezing process does not cause a decrease in antioxidant activity in all vegetables, except for Brussels sprouts. In a similar work, Volden *et al*. (2008) studied the effect of blanching, boiling and steaming on the antioxidant activity of red cabbage and found that processing results in significant losses in the antioxidant potential, undergoing blanching and boiling. The reduction in blanched samples was more than twice the reduction in boiled samples. Steamed red cabbage was unaffected in both assays. Volden *et al*. (2009b) studied the effect of the same treatments on five cultivars of cauliflower. The reductions in antioxidant parameters with treatmens ocurred in descending order: boiling > blanching > steaming. In general, for all processes reductions in the antioxidant-related parameters in the florets were accounted for in the processing water. The effect of boiling was studied in the extracts of fresh raw and frozen broccoli by Gawlik-Dziki (2008). Boiling reduced the phenol compounds content in fresh broccoli, but increased it in frozen broccoli.

Antioxidant changes during cooking largely depend on the crop analyzed. Watchel-Galor *et al*. (2008) studied the antioxidant potential of broccoli, cauliflower, cabbage and Chinese cabbage processed by boiling, steaming and microwaving. Antioxidants were higher in steamed > boiled > microwaved. The antioxidant capacity of Chinese cabbage was unchanged or slightly decreased, while boiling decreased that of the cabbage markedly. Boiling of broccoli and cauliflower led to apparent increases in antioxidant capacity. This effect is perhaps due to the production of redox-active secondary plant metabolites or breakdown products, highly likely to be related to the release of antioxidants from intracellular proteins, changes in plat cell wall structure, matrix modifications and more efficient release of antioxidants during homogenization.

As conclusions, in most part of the works analyzed, aquathermal cooking processes seem to have a positive effect on health properties of *Brassica* vegetables by keeping or increasing their antioxidant potential. However, in several experiments, boiling has a negative effect on antioxidant potential. The effects of cooking treatments depend on the crop analyzed and on the previous postharvest treatment. Spontaneous fermentation, frying and steaming also have a positive effect, but this was not the case of microwaving process.

ANTIOXIDANT POTENTIAL OF *BRASSICA* VEGETABLES AND HEALTH

Reactive oxygen species (ROS) play a critical role in cardiovascular diseases, inflammatory diseases, neurodegenerative disorders, cancer and aging because they are highly reactive to biological molecules and can damage DNA, proteins, carbohydrates as well as lipids. Diets rich in foods containing antioxidant compounds, such as *Brassica* vegetables, could help prevent these pathologies since they contribute both to the first and second defense lines against oxidative stress. In numerous studies it has been demonstrated that ROS are able to modulate gene expression, cell growth and signal transduction pathways (Murashima *et al.* 2004; Li *et al.* 2008a; Kusznierewicz *et al.* 2010). Therefore, there is strong evidence that an adequate amount of ROS scavenger can minimize the deleterious effects of oxidative stress in the human body. As a result, antioxidant compounds are important for limiting damaging oxidative reactions in cells, which may affect to the development of heart diseases and cancer. When considering the ability of a plant food to prevent oxidative damage, even more important than amounts of antioxidants consumed may be the biological activities attributed to these compounds. These biological activities include: the modulation of the expression of endogenous antioxidant enzymes detoxifying ROS (e.g., SOD, GPx and catalase), or enzymes repairing DNA damages induced or products of oxidant-induced damage (e.g., GST/GSH conjugation of the products of lipid peroxidation). The ability of an excess of ROS to damage molecules like DNA, protein and lipids is often referred to the initiation and progression of carcinogenesis and abnormal vascular cell proliferation (Berlett *et al.* 1997).

According to this, the antioxidant potential of *Brassica* vegetables has been assessed at several levels: as a ROS scavenger, as a modulator of endogenous antioxidant defense and as an inducer of repair of oxidant-induced DNA damage. Even though *Brassica* vegetables include a large group of plants, most research has been focused on *B. ole-*

racea species, and mainly on broccoli, cabbage and Brussels sprouts since they have been found to have antioxidant, antihyperglycemic, anticancer and hypocholesterolemic properties (Ayaz *et al.* 2008; Kataya and Hamza 2008; Akhlaghi and Bandy 2010; Kusznierewicz *et al.* 2010). Some reports have demonstrated the antioxidant properties of *Brassica* vegetables in isolated compounds. In contrast, other studies have been carried out for natural juices obtained from fresh or culinary processed *Brassica* vegetables and for concentrations to which cells of alimentary tract may be exposed after consumption of a typical meal containing these crops. Both types of studies will be discussed in the following sections.

Health properties of isolated antioxidant compounds described on *Brassica* crops

As it was previously explained, the main antioxidative components present in *Brassica* vegetables are water-soluble and include phenolic compounds (mainly flavonoids) and vitamins (mainly ascorbic acid). Other antioxidant constituents are lipid-soluble such as carotenoids and tocopherols. All of these phytochemicals contribute to the reported antioxidant, anticarcinogenic and cardiovascular protective activities widely attributed to *Brassica* vegetables.

1. Water-soluble antioxidants

Phenolic compounds

Phenolic compounds, especially flavonoids, perform different biological activities, but the most important are the antioxidant activity, the capillary protective effect and the inhibitory effect elicited in various stages of tumor (Cartea *et al.* 2011b). Within phenolic compounds, flavonoids are involved in a vast array of biological functions and they may protect against cancer development through several biological mechanisms. Numerous preclinical studies have shown that kaempferol and some glycosides of kaempferol have a wide range of pharmacological activities, including antioxidant activities (Kim *et al.* 2004; Calderon-Montano *et al.* 2011). In fact, it is well known that higher intakes of kaempferol result in a lower risk of coronary heart disease. Glycosylated flavonoids such as 3-sophoroside-7-glucosides of kaempferol, quercetin and iso-rhamnetin and hydroxyl-cinnamates reported in the family *Brassicaceae* (Podsedek 2007; Sousa *et al.* 2008; Francisco *et al.* 2009; Jahangir *et al.* 2009; Velasco *et al.* 2011) are increasingly attributed beneficial health effects such as a reduced risk of age-related chronic diseases, like cancers and cardiovascular diseases (Podsedek 2007). In addition, quercetin, a major representative of the flavonol subclass and which is found at high concentration in broccoli, has received considerable attention. This flavonoid has displayed the ability to prevent the oxidation of LDL by scavenging free radicals and chelating transition metal ions. As a result, quercetin may aid in the prevention of certain diseases, such as cancer, atherosclerosis and chronic inflammation by retarding oxidative degradation and inducing enzymes that detoxify carcinogens (Ackland *et al.* 2005; Kim *et al.* 2006). Furthermore, isorhamnetin isolated from mustard leaf showed a strong activity in reducing serum levels of glucose in *Diabetes mellitus* through an antioxidant activity test (Yokozawa *et al.* 2002). However, epidemiological studies on dietary flavonoids and cancer risk have yielded inconsistent results. Lu *et al.* (2009) investigated the association between the intake of selected flavonoids and flavonoid-rich foods and risk of cancers in middle-aged and older women and their results did not find a significant association between the intake of flavonoid-rich foods and the incidence of specific cancers.

Anthocyanins have been found to have a high antioxidant power and antigenotoxic properties (Posmyk *et al.* 2009a, 2009b). These authors suggest that a mixture of anthocyanins not only prevents and limits but also repairs the cytological injury caused by Cu^{2+} stress on lymphocytes. Although cabbage does not possess high antioxidative potential compared with other plant foods, it may provide a very effective antioxidative barrier especially if culinary processing caused the release of antioxidants.

Lignans may reduce the risk of certain cancers as well as cardiovascular diseases. This is important since these compounds are efficiently converted into the 'enterolignans' enterodiol and enterolactone by the intestinal microflora. These are then readily absorbed and exert activities much like oestrogens. In essence the lignans present in *Brassica* crops are phytoestrogens. Inverse associations have been found between plasma or urinary lignans and breast cancer risk. For cardiovascular diseases, inverse associations with serum lignans were reported (Heinonen *et al.* 2001; Milder *et al.* 2005).

Vitamin C and folic acid

It is a well-known fact that vitamin C together with vitamin E and folic acid have the potential to prevent and treat malignant and degenerative diseases (Kurilich *et al.* 1999). On the other hand, it has now been recognized the potential therapeutic benefit and the pharmacological actions that the compound ascorbigen has, showing their antioxidant and anticancer activities *in vitro* and *in vivo* (Joshi *et al.* 2008). Authors concluded that ascorbigen has antioxidant properties and protects human umbilical cord endothelial cells against hyperglycemic toxicity *in vitro*. Additionally, ascorbigen also relaxed the vascular tone induced by L-phenylephrine, which is not mediated by an endothelial cell nitric oxide dependent mechanism. However, the therapeutic value of this substance in disease settings is scarce and it needs to be further investigated.

Folic acid reduces the risk of neural tube defects and may be associated with the reduced risk of vascular disease and cancer (Bailey *et al.* 2003), while low-folate intake has been identified as a main cause of anemia.

2. Lipid-soluble antioxidants

Carotenoids

Carotenoids such as α- and β-carotene present in dark green leafy vegetables like broccoli might be involved in the prevention of several diseases related to oxidative stress (Kurilich *et al.* 1999; Riso *et al.* 2003; Girard-Lalancette *et al.* 2009). An intake of these bioactive compounds has been implicated in a reduced risk of certain cancers and degenerative diseases, immune dysfunction and aged-related macular degeneration (Kurilich *et al.* 1999). Each carotenoid has characteristic functions such as cell cycle inhibition, induction of cell differentiation and apoptosis (Nagao 2009). Lower serum β-carotene levels have been linked to higher rates of cancer and cardiovascular diseases, as well as to an increased risk of myocardial infarction among smokers (Rice-Evans *et al.* 1997). However, the detailed mechanisms of these biological actions have not been fully revealed yet and deserve future studies.

Vitamin E

Vitamin E activity is important for maintaining stable cell membranes and preventing oxidative damage to tissues (Kurilich *et al.* 1999). Elevated intake of tocopherols can protect against several degenerative diseases including cardiovascular disease, cancer, neurological disorders, and inflammatory diseases, in addition to inhibition of oxidative modification of low-density lipoprotein (LDL) (Ibrahim and Juvik 2009).

Health properties *in vivo* and *in vitro* for *Brassica* extracts

One of the most important 'indirect' antioxidative actions of *Brassica* phytochemicals would be the stimulation of the expression of genes involved in the improvement of undesirable effects of ROS. Assays for antioxidant status and oxidative damage are many and varied. The simplest ones are purely chemical *in vitro* reactions or tests in cell cultures. Supplementation with antioxidants *in vivo* seems to be the best approach and experiments should be performed with human subjects if possible. However, extrapolation to effects of dietary antioxidants *in vivo* is dangerous, because the uptake from the gastrointestinal tract and metabolism are not considered.

1. In vitro cell-based studies

As it was described in the previous sections, several *in vitro* methods have been used for the evaluation of the antioxidant activities of fruit and vegetable extracts. However, it must be taken into account that many factors may affect the antioxidant potential of these molecules, such as the affinity of the molecules for the aqueous or lipid phase, the oxidation conditions in the cell as well as the nature of the oxidizable substrate used in the assay. Therefore, cell culture models could be a useful complementary method to evaluate the antioxidant activities of fruit and vegetable extracts such as kale, cabbages and Brussels sprouts.

Chung *et al*. (2003) showed the beneficial effects of kale juice on nitrite scavenging *in vitro*, growth of cancer cells in culture and plasma lipid and antioxidant status in smokers. In another study, Gulcin *et al*. (2004) investigated the antioxidant activity *in vitro* of broccoli extracts, finding that they displayed effective reducing power, free-radical scavenging, superoxide anion radical scavenging and hydrogen peroxide scavenging and metal chelating activity. Brussels sprouts extracts inhibited DNA oxidation *in vitro* in human lymphocytes, possibly through scavenging oxygen radicals (Zhu and Loft 2001) suggesting that Brussels sprouts can enhance lymphocyte resistance towards H_2O_2-induced DNA strand breaks *in vitro*. So far, the mechanism involved in the protection of DNA against oxidative damage by constituents of Brussels sprouts is not well studied and it is not known whether there is any relationship with the ability to induce phase II enzymes. Recently, Girard-Lalancette *et al*. (2009) assessed the antioxidant activities of lipophilic and hydrophilic extracts from broccoli by using the HepG2 cell line and the ORAC assay and found that the antioxidant activities measured with the cell-based assay were, in general, in good agreement with values obtained using the ORAC assay, although the cell culture conditions favored the pro-oxidant properties of the broccoli juices. In another work, Kwon *et al*. (2006) found that chlorophyll-rich methanol extracts of *B. oleracea* showed antioxidant and antimutagenic activities *in vitro* through mechanisms that include inhibition of carcinogen activation and scavenging of reactive oxygen species. These authors confirmed the high antioxidant activity of these plant extracts in ORAC and FRAP assays and demonstrated the inhibition of H_2O_2-induced DNA damage, evaluated indirectly by using HCT116 colon cancer cells and directly using the plasmid pUC19 *in vitro*.

Keck and Finley (2006) showed that broccoli extracts reduced oxidative stress and inhibited DNA single-strand breaks in cultured cells (Hepa 1c1c7). The effect of broccoli extracts on the prevention of oxidation in a cellular system was also investigated by Roy *et al*. (2009). These authors demonstrated that broccoli extract gives significant cytoprotection in PC-12 cell line (neuroblastoma) and therefore, it showed a neuroprotective effect. The PC-12 cell is a ROS sensitive cell line often used as a model for evaluating neuronal cell damage, typically free radical mediated injury. Indeed, authors show that steam processed broccoli samples showed higher activity than fresh products.

The evaluation of induction of DNA repair enzymes in HT29 cells by cabbage juices was carried out recently by Kusznierewicz *et al*. (2010), who described the antioxidative potential of white cabbage as a modulator of endogenous antioxidative defense and a ROS scavenger as well as inducer of repair of oxidant-induced DNA damage. Interestingly, in this tumor cell line, cabbage juices displayed a moderate inhibitory effect on cell growth and induced DNA fragmentation. However, no protective effect was found after prolonged incubation with this juice prior to ROS exposure. Indeed, authors found that cabbage phytochemicals for natural juices obtained from fresh or after the typical culinary process and at concentrations expected during normal daily consumption, may prevent damage to molecules by removing ROS and probably toxic compounds generated by them. In a recent study, performed *in vivo* with rats, Akhlaghi and Bandy (2010) showed that a relatively short dietary treatment with broccoli sprouts can strongly protect the heart against oxidative stress and cell death caused by ischemia-reperfusion. Cell death, oxidative damage and Nrf2-regulated phase 2 enzyme activities were evaluated. Broccoli sprouts feeding inhibited markers of necrosis (lactate dehydrogenase release) and apoptosis (caspase-3 activity) and decreased indices of oxidative stress.

2. In vivo-based studies

Other studies have investigated the antioxidant activity of *Brassica* vegetables *in vivo* and support the view that these plant foods can enhance the antioxidant status in both culture cells as well as in intact organisms. As it was previously explained, one of the antioxidant defense systems consists in a series of endogenous antioxidant enzymes. With regard to studies conducted *in vivo*, Young *et al*. (2002) concluded that broccoli reduced the oxidation of muscle proteins and lipids in the liver and stabilized erythrocytes when it was supplemented in a diet of chicken. In another study, Murashima *et al*. (2004) investigated the effect of broccoli sprouts on the induction of various biochemical oxidative stress markers in 12 healthy subjects, finding that fresh broccoli sprouts reduced the oxidative stress markers including phosphatidylcholine hydroperoxide, urinary 8-isoprostane and 8-hydroxydeoxyguanosine and thus, improved the cholesterol metabolism. The protective action against oxidative stress of red cabbage extract was also investigated in rats by Kataya and Hamza (2008). After induction of diabetes in rats, these exhibited many symptoms due to diabetes induction including renal dysfunction, loss of body weight, and hyperglycemia. These symptoms were accompanied by a significant increase in malondialdehyde, a lipid peroxidation marker, in reduced glutathione, SOD and catalase activities, as well as in the total antioxidant capacity of the diabetic rats. Authors showed that after a daily oral ingestion of *B. oleracea* extracts for 60 days, the adverse effect of diabetes was reduced. *Brassica* extracts lowered blood glucose levels and restored renal function and body weight loss. In addition, *Brassica* extracts attenuated the adverse effect of diabetes on malondialdehyde, glutathione and superoxide dismutase activity as well as catalase activity and total antioxidant capacity of diabetic kidneys. They concluded that the antioxidant and antihyperglycemic properties of *B. oleracea* extract may offer a potentially therapeutic source for the treatment of diabetes.

In other studies, Li *et al*. (2008a, 2008b) reported the interaction of broccoli extracts on the induction of endogenous antioxidant enzymes in fruit flies (*Drosophila melanogaster*) reared on a high fat diet and concluded that the antioxidant activity of broccoli extracts in *D. melanogaster* was mediated by up-regulation of SOD and catalase at both the transcriptional and translational level. Li *et al*. (2008b) stated that broccoli extracts were the most effective in scavenging superoxide anion and hydrogen peroxide in *D. melanogaster* compared with other *Brassica* vegetables like cabbage and Chinese cabbage.

On the other hand, cancer preventive effects of cruciferous vegetables have been related to protection from mutagenic oxidative DNA damage. At this point, results from human studies with a diet rich in Brussels sprouts showed a reduction on DNA damage in terms of a decreased excretion 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) into human urine (Verhagen *et al.* 1997). Indeed, aqueous Brussels sprouts extracts had similar effects in rats (Deng *et al.* 1998). These authors found a relationship between the oral administration of cooked Brussels sprouts homogenate for four days and a reduction of the spontaneous urinary 8-oxodG excretion, which reflects the prevention of oxidative DNA damage in rats by this crop.

As a conclusion, all findings reported here demonstrate that *Brassica* vegetables contain bioactive substances with a potential for reducing the physiological as well as oxidative stress-induced DNA damage and this could explain the suggested cancer preventive effect of these plants as well as their protective role on other major diseases.

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