Food Chemistry 190 (2016) 771–776

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Antioxidant and antiproliferative activities in different maturation stages of broccoli (*Brassica oleracea* Italica) biofortified with selenium

Patricia Bachiega [a,*], Jocelem Mastrodi Salgado [a], João Ernesto de Carvalho [b], Ana Lúcia T.G Ruiz [b], Kélin Schwarz [c], Tiago Tezotto [d], Maressa Caldeira Morzelle [a]

[a] Departamento de Agroindústria, Alimentos e Nutrição, Laboratório de Bromatologia, Escola Superior de Agricultura "Luiz de Queiroz"/Universidade de São Paulo (Esalq/USP), Avenida Pádua Dias, 111, 13418-900 Piracicaba, SP, Brazil
[b] Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas, UNICAMP, CP 6171, 13083-970 Paulínia, SP, Brazil
[c] Centro de Energia Nuclear na Agricultura (CENA)/Universidade de São Paulo (USP), Avenida Centenário, 303, 13418900 Piracicaba, SP, Brazil
[d] Departamento de Produção Vegetal, Laboratório Multiusuário em Produção Vegetal, Escola Superior de Agricultura "Luiz de Queiroz"/Universidade de São Paulo (Esalq/USP), Avenida Pádua Dias, 111, 13418-900 Piracicaba, SP, Brazil

ARTICLE INFO

*Article history:*
Received 6 March 2015
Received in revised form 5 June 2015
Accepted 8 June 2015
Available online 12 June 2015

*Keywords:*
Antioxidant
Antiproliferative
Broccoli
Glucosinolates
Sodium selenate

ABSTRACT

In this work, three different broccoli maturity stages subjected to biofortification with selenium were evaluated for antioxidant and antiproliferative activities. Antioxidant trials have shown that the maturation stages biofortified with selenium had significantly higher amounts of phenolic compounds and antioxidant activity, especially seedlings. Although non-polar extracts of all samples show antiproliferative activity, the extract of broccoli seedlings biofortified with selenium stood out, presenting cytocidal activity for a glioma line (U251, $GI_{50}$ 28.5 mg $L^{-1}$).



## 1. Introduction

Several scientific studies have proven that consumption of plant foods rich in phytochemical is one of the crucial factors for the welfare and the promotion of health through the prevention of various diseases including cancer, inflammation, cardiovascular and neurodegenerative diseases (Alzheimer's disease, Parkinson's disease and cataracts) and depression (Khalaj et al., 2013).

Plants have a high structural diversity and for this reason constitute a major source of substances with biological potential, having anti-bacterial, anticoagulant, anti-interference, immunosuppressive and anti-cancer activities (Pascoal et al., 2014). Recent studies have shown that among the 128 anticancer drugs marketed between 1981 and 2010, approximately 12 are natural products and 21 are derived from these products (Newman, Cragg, & Snader, 2012), justifying the importance of the search for new strategies in cancer therapy in alliance with natural products, with an emphasis on functional foods.

Plants belonging to the family of Brassicas are known worldwide for their rich bioactive composition, highlighted by glucosinolates, which are enzymatically cleaved by the action of myrosinase to isothiocyanates, giving these plants pronounced chemopreventive activity (Medina et al., 2015). Broccoli (*Brassica oleracea* Italica), a nutritionally important crop grown worldwide, has a high content of organosulfur compounds that promote an increase in the activity of enzymes involved in the detoxification of carcinogens and other foreign compounds (Chaudhary, Sharma, Vig, Singh, & Arora, 2014). The inflorescences of broccoli are consumed worldwide and are recognised for their anticarcinogenic properties, a new section of the world market has been gaining attention: the consumption of sprouts. During the germination period, broccoli sprouts undergo significant changes in metabolism of both biochemical and physiological co-factors, including the interconversion and synthesis of new compounds. Furthermore, there are also increased in the myrosinase enzyme activity and reduced epithiospecifier proteins (ESP) (Pérez-Balibrea, Moreno, & García-Viguera, 2011). Thus, broccoli sprouts have become more promising for isothiocyanate production than adult broccoli (Guo, Guo, Wang, Zhuang, & Gu, 2013).

Human health and plant nutrition, most of the time, have been seen as totally distinct areas, however, when deep analysed, can be

* Corresponding author.
*E-mail addresses:* patriciabachiega@hotmail.com, patriciabachiega@usp.br (P. Bachiega).

http://dx.doi.org/10.1016/j.foodchem.2015.06.024
0308-8146/

extremely favourable to establish relationships between them. Many nutrients that are essential for plant development are also proven essential for humans and animals, playing in many cases, similar mechanisms of action (Grusak & DellaPenna, 1999). As an example of this relationship there are selenium and broccoli. Selenium is an essential trace element for mammals that functions as a structural component of enzymes when in the form of selenocysteine; it is found in approximately 25 selenoproteins, including glutathione peroxidase, which has antioxidant functions (Rayman, 2012).

In this sense, this survey aimed to evaluate the effect of selenium supplementation during broccoli cultivation and to evaluate the antioxidant and antiproliferative activities observed in three different broccoli maturity stages.

## 2. Materials and methods

### 2.1. Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethyl benzthiazoline-6-sulfonic acid) diammonium salt (ABTS), (±)-6-h ydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), perchloric acid ($ClHO_4$), 2,4,6-tripyridin-2-yl-1,3,5-triazine (TPTZ), sodium selenate, gallic acid, iron sulphate, potassium persulphate ($K_2O_8S_2$), sodium carbonate ($CNa_2O_3$), trichloroacetic acid ($C_2HCl_3O_2$), sulforhodamine B and Folin–Ciocalteu were purchased from Sigma–Aldrich Chemical Co. Reagents were of analytical grade. Doxorubicin hydrochloride was purchased from Europharma (Sao Paulo, Brazil).

### 2.2. Plant material

Broccoli seeds Avenger were supplied by Sakata Seed Sudamerica. For the experiment with the sprouts, seeds were sown on two sheets of blotter paper soaked with treatment solutions in germination boxes and were grown in a growth chamber under the following conditions: 8 h at 30 °C in the light and 16 h in the dark at 20 °C. The treatments consisted of a distiled water and 50 μM sodium selenate solution (Ávila et al., 2013) and were labelled as sprouts without selenium (BS) and sprouts with selenium (BC). The sprouts were collected 8 days after sowing (DAS), stored in plastic bags and sent to the laboratory for processing.

The broccoli seedlings were produced in black polyethylene trays of 200 cells filled with commercial substrate (coconut fibre) in an arched agricultural greenhouse. The treatments consisted of distiled water and 50 μM sodium selenate solution (Ávila et al., 2013) and were applied when the seedlings were at 15 days of germination. The seedlings were labelled as seedlings without selenium (MS) and seedlings with selenium (MC). The broccoli seedlings were collected with 30 DAS. The roots were removed and the leaves were stored in plastic bags and sent to the laboratory for processing.

The broccoli inflorescences were grown in arch-type greenhouses. Forty-five days after sowing, the seedlings were transplanted into eight-litre pots filled with commercial substrate of Pinus bark base. Irrigation was performed daily through autocompensation drippers with a flow of 2.0 L $h^{-1}$ with nutrient solutions properly tailored to cultivate the plants in question. The treatments consisted of distiled water and a 1.5 mM sodium selenate solution and were labelled as inflorescences without selenium (IS) and inflorescences with selenium (IC) (Ávila et al., 2013). When the plants entered the floral period, 100 mL of the solution was applied twice a week until the inflorescences were fully formed. The broccoli inflorescences were collected with 127 DAS. The stem and the leaves were removed and the inflorescences were stored in plastic bags and sent to the laboratory for processing.

In the laboratory, all samples were sanitized, freeze-dried, crushed and stored at −20 °C in amber vials until used in the experiment. The freeze-dried was employed because this technique preserves glucosinolates and prevents inactivation of myrosinase (Townsend, Chen, Jeffery, & Johnson, 2014).

### 2.3. Energy dispersive X-ray fluorescence spectrometry (EDXRF)

One gram of BS, BC, MS, MC, IS and IC was packed into a polyethylene cup with an internal diameter of 20 mm and covered with 6-μm-thick polypropylene film. The samples were irradiated in triplicate for 300 s under vacuum using an energy-dispersive X-ray fluorescence spectrometer (Shimadzu EDX-720). The samples were irradiated using a Rh X-ray tube operated at 15 kV (Na to Sc) and 50 kV (Ti to U). The current was automatically adjusted (maximum of 1 mA). A 10-mm collimator was chosen. The detection was performed using a Si (Li) detector cooled with liquid nitrogen. The intensity of the element Kα counts per second (cps/μA) was determined from the sample X-ray spectrum deconvolution using the EDX Shimadzu software package (Tezotto et al., 2013).

### 2.4. Antioxidant activity

Food matrices are composed of a complex of compounds that have functional groups with different polarities and chemical behaviour. The tests to determine the antioxidant capacity have peculiarities as their way of assessing such activity and in ABTS is considered lipophilic and hydrophilic compounds, DPPH is more sensitive to hydrophilic compounds and FRAP is a method more stable and easily reproducible. Thus, this study used three chemical methods most commonly used to assess this potential: sequestration capacity of radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6 sulfonic acid (ABTS) and iron reduction capacity (FRAP).

The extracts were obtained from the percolation method of plant material in 60% methanol 1:10 (plant material (g):Solvent (mL)). The extraction was conducted under stirring in a water bath at 40 °C for 2 h. After this period, the extracts were centrifuged, filtered and stored at −20 °C in amber bottles for protection from light until the time of analysis of the antioxidant activity and phenolic compounds (Jaiswal, Abu-Ghannam, & Gupta, 2012).

#### 2.4.1. Assay by DPPH method

The test samples were measured in terms of hydrogen-donating or radical-scavenging ability using the stable radical DPPH (Brand-Williams, Cuvelier, & Berset, 1995). Briefly, the reaction mixture contained 500 μL of different extracts and 300 μL of DPPH. The absorbance of the reaction mixture was measured at 517 nm against the blank, which did not contain a test sample. The results were expressed in μM Trolox 100 $g^{-1}$ whole sample, and the full equivalence values were calculated using the standard curve of Trolox.

#### 2.4.2. Assay by ABTS method

The ABTS assay is based on the formation of the radical $ABTS^{\cdot+}$ by the reaction of ABTS (7 mm) with 2.45 mM potassium persulphate. For this assay, 20 μL of the different extracts were reacted with 2.0 mL of ABTS radical. The absorbance of the reaction mixture was measured at 734 nm against the blank, which did not contain a test sample. The results of the antioxidant activity were expressed as μM Trolox 100 $g^{-1}$ whole sample (Re et al., 1999).

*2.4.3. Assay by FRAP method*

The determination of the total antioxidant activity through the reduction of iron (FRAP) was based on the methodology described by Oyaizu (1986) and is based on direct measurement of the ability of the antioxidants (reducing) in the sample to reduce, under acidic conditions (pH 3.6), the complex $Fe^{3+}$/tripyridyltriazine (TPTZ) to form $Fe^{2+}$ with an intense blue colour and an absorption maxima of 595 nm. An aliquot of 90 μL of different extracts was added to 2.7 mL of the FRAP reagent. A calibration curve was plotted using data for iron sulphate (500–2000 mM), and the results were expressed in μM iron sulphate 100 $g^{-1}$ whole sample.

*2.5. Phenolic compounds*

The content of the phenolic compounds in the extracts was determined via the Folin–Ciocalteu method, using gallic acid as the standard. The reaction occurred with 0.5 mL of each extract, 2.5 mL of Folin–Ciocalteu reagent diluted 1:10 in distiled water and 2 mL of a 4% solution of sodium carbonate. The absorbance was measured at 740 nm. The results of the total phenolics were expressed as gallic acid 100 $g^{-1}$ whole sample (Woisky & Salatino, 1998).

*2.6. Determination of antiproliferative activity*

The antiproliferative effect of broccoli was investigated on five human tumour cell lines (glioma (U251), breast (MCF-7), kidney (786-0), lung (NCI-H460), colon adenocarcinoma (HT-29)) provided by the Frederick Cancer Research & Development Center – National Cancer Institute – Frederick, MA, USA. Samples were also evaluated against one spontaneously transformed keratinocytes from histologically normal skin (HaCaT cell line) kindly provided by Prof. Dr. Ricardo Della Coletta (University of Campinas, UNICAMP). Stock cultures were grown in 5 mL of RPMI-1640 supplemented with 5% foetal bovine serum (RPMI/FBS 5%). Penicillin:streptomycin mixture 1000 U $mL^{-1}$:1000 μg $mL^{-1}$ (1 mL $L^{-1}$ RPMI-1640) was added to the experimental cultures (Araújo et al., 2013).

Freeze-dried broccoli was extracted by successive maceration (1:10 sample: solvent, 3 × 1 h) with dichloromethane (non-polar extract) and ethanol (polar extract), at room temperature. Both fluid extracts (non-polar and polar extract) were concentrated under reduced pressure until dryness. The aliquots (10 mg) of dried polar and non-polar broccoli extracts were diluted in DMSO (100 μL) and then successively diluted in RPMI/FBS 5% affording the final concentration 0.25, 2.5, 25 and 250 μg $mL^{-1}$ (Leite-Legatti et al., 2012). As a positive control we used the chemotherapeutic doxorubicin at concentrations of 0.025, 0.25, 2.5 and 25 μg $mL^{-1}$ (100 μL compartment$^{-1}$) in triplicate.

Cells in 96-well plates (100 μL cells well$^{-1}$) were exposed to different concentrations of samples (0.25, 2.5, 25 and 250 μg $mL^{-1}$) in triplicate, for 48 h at 37 °C and 5% of $CO_2$. Final DMSO concentration did not affect cell viability (<0.25%). Before (T0 plate) and after (T1 plates) sample addition, cells were fixed with 50% trichloroacetic acid, submitted to sulforhodamine B assay for cell proliferation quantitation at 540 nm (Monks et al., 1991). The $GI_{50}$ value (concentration that produces 0% cell growth or totally cytostatic effect) was determined through non-linear regression, type sigmoidal, analysed using Origin 8.0® software (OriginLab Corporation).

*2.7. Statistical analysis*

Statistical analyses were performed using the SAS version 9.0 for Windows. Results were expressed as mean values ± standard deviations. The difference between the means of treatments with and without selenium was tested by the Student's *t* test for paired samples for each maturity stage of broccoli.

## 3. Results

*3.1. Quantification of selenium*

To determine the selenium content present in broccoli samples, energy dispersive X-ray fluorescence spectrometry (EDXRF) was used. This technique has been studied as an efficient alternative for the quantification of elements in plant samples, with advantages such as comparative simplicity, ability to analyse multiple samples simultaneously occur without its destruction, thus dispensing the use of pre-chemical treatments (Tezotto et al., 2013). No selenium was detected in the samples that received only distiled water (BS, MS and IS). For the BC, MC and IC samples, the amount of selenium increased with the development of the broccoli, and the concentrations were 132, 148 and 166 μg selenium $g^{-1}$ dry sample, respectively.

*3.2. Antioxidant activity and phenolic compounds*

To evaluate the influence of biofortification on the antioxidant activity and the amount of total phenolic content of different broccoli maturation, in vitro methods were used, as described in Table 1.

Sample BC showed higher antioxidant activity when measured by the ABTS and FRAP methods than did sample BS. This increase was also evident in the amount of phenolic compounds. The MC sample had significantly higher antioxidant activity than the MS sample when evaluated using the three methods (DPPH, ABTS, FRAP) and higher amounts of phenolic compounds in relation to the MS sample. For the inflorescences, the IC sample showed larger results compared to the IS sample for antioxidant activity and the amount of phenolic compounds. The antioxidant activity measured using the FRAP method was the highest compared to those measured using the DPPH and ABTS methods.

The samples with a higher content of phenolic compounds also had higher antioxidant activity, which can be explained by the fact that the phenolic compounds are the main natural antioxidants of brassicas, especially broccoli. Moreover, the biofortification of broccoli with selenium significantly increased the amount of phenolic compounds.

*3.3. In vitro antiproliferative activity*

To achieve greater amplitude for the antiproliferative activity of different broccoli maturity stages (sprouts, seedlings and inflorescence) biofortified or not with selenium and knowing that the same sample can present this differentiated activity against the cell lines, we used human tumour cell lines of different histological and genetic origins. The concentrations of broccoli extracts at different maturation stages required for 50% ($GI_{50}$) cell growth inhibition are summarised in Table 2.

The non-polar BS extract showed antiproliferative activity that was slightly higher than that of sample BC with cytostatic activity for lines 786-0 (kidney, $GI_{50}$ = 123.6 μg $mL^{-1}$) and MCF-7 (breast, $GI_{50}$ = 96.7 μg $mL^{-1}$). The non-polar extracts of MS and MC showed selective cytostatic activity for line 786-0 with $GI_{50}$ 12.0 and 20.7 μg $mL^{-1}$, respectively. Furthermore, the non-polar extract of MC was the only extract to show cytocidal activity for line U251 (glioma, $GI_{50}$ = 28.5 μg $mL^{-1}$) (Fig. 1). The non-polar extracts of IS and IC showed weak antiproliferative activity.

The polar extracts were obtained from the non-polar extracts, and the latter initially remove compounds that exhibit low

**Table 1**
Antioxidant activity and phenolic compounds of broccoli samples biofortified and not biofortified with selenium.

| Sample* | DPPH (μM Trolox 100 $g^{-1}$ whole sample) | ABTS (μM Trolox 100 $g^{-1}$ whole sample) | FRAP (μM ferrous sulphate 100 $g^{-1}$ whole sample) | Phenolic compounds (mg of gallic acid 100 $g^{-1}$ whole sample) |
|---|---|---|---|---|
| BS | 531.68 ± 13.69 | 448.03 ± 5.28 | 2257.00 ± 17.32 | 95.64 ± 1.36 |
| BC | 548.02 ± 3.55 | 559.77 ± 30.52 | 2804.50 ± 55.60 | 120.09 ± 1.39 |
| Student *t*-test | $p = 0.089^{ns}$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ |
| MS | 629.07 ± 7.50 | 812.71 ± 39.18 | 4152.00 ± 62.72 | 179.08 ± 3.10 |
| MC | 730.86 ± 10.93 | 897.02 ± 31.28 | 4587.00 ± 80.21 | 188.84 ± 2.19 |
| Student *t*-test | $p < 0.001$ | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ |
| IS | 531.68 ± 10.56 | 703.00 ± 31.28 | 2217.00 ± 84.26 | 94.65 ± 1.39 |
| IC | 512.83 ± 36.13 | 754.81 ± 33.43 | 3194.50 ± 75 | 105.94 ± 0.55 |
| Student *t*-test | $p = 0.425^{ns}$ | $p = 0.06^{ns}$ | $p < 0.001$ | $p < 0.001$ |

The comparison of means was performed for each broccoli maturation stage separately.
[ns] Not significant.
[*] BS: sprout without selenium; BC: sprout with selenium; MS: seedling g without selenium; MC: seedlings with selenium; IS: inflorescence without selenium; IC: inflorescence with selenium.

**Table 2**
$GI_{50}$ values of doxorubicin and crude extracts (non-polar and polar) of each sample in six human tumour cell lines (μg $mL^{-1}$).

| | U251 | MCF-7 | 786-0 | NCI-H460 | HT29 | HaCaT |
|---|---|---|---|---|---|---|
| Doxorubicin | <0.025 | <0.025 | 0.025 | <0.025 | 0.089 | 0.035 |
| *Extract non-polar* | | | | | | |
| BS | >250 | 96.7 | 123.6 | >250 | >250 | >250 |
| BC | >250 | >250 | >250 | >250 | >250 | >250 |
| MS | 61.7 | 94.6 | 12.0 | 94.9 | >250 | 58.4 |
| MC | 28.5 | 68.1 | 20.7 | 149.2 | >250 | 64.9 |
| IS | 209.7 | 98.9 | >250 | >250 | 178.1 | >250 |
| IC | >250 | 132.4 | >250 | >250 | >250 | >250 |
| *Extract polar* | | | | | | |
| BS | >250 | >250 | >250 | >250 | >250 | >250 |
| BC | >250 | >250 | >250 | >250 | >250 | >250 |
| MS | >250 | >250 | >250 | >250 | >250 | >250 |
| MC | >250 | >250 | >250 | >250 | >250 | >250 |
| IS | >250 | >250 | >250 | >250 | >250 | >250 |
| IC | >250 | >250 | >250 | >250 | >250 | >250 |

U251 – glioma; MCF-7 – breast; 786-0 – kidney; NCI-H460 – lung; HT29 – colon; HaCaT – keratinocyte.

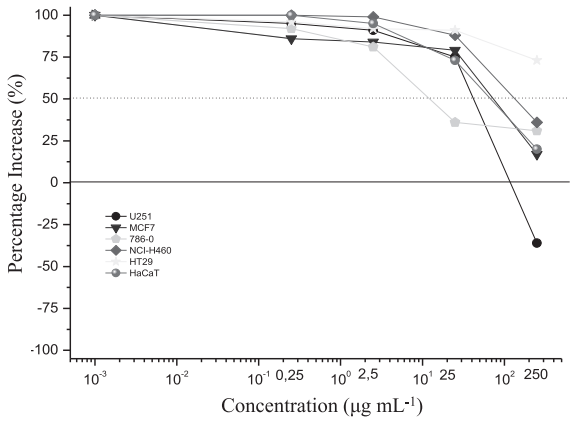


**Fig. 1.** Antiproliferative activity of the non-polar extracts of MS (A) and MC (B) samples on the cell lines, correlating the percentage increase versus the sample concentration (U251 – glioma, MCF-7 – breast, 786-0 – kidney, NCI-H460 – lung, HT29 – colon, HaCaT – keratinocyte).

polarity, whereas the first remove the remaining compounds that have higher polarity. The non-polar extracts of each maturation stages were more active than the polar extracts and although the maturity stages were not compared with each other, MC sample it seems to have presented most pronounced antiproliferative activity.

## 4. Discussion

Broccoli is a rich source of glucosinolates, which constitute the rich secondary metabolites in sulphur derivatives of sugars or amino acids, and their hydrolysis products (isothiocyanates) are responsible for the beneficial health effects through antioxidant and antiproliferative activity in addition to the characteristic flavours and odours of crucifers (Razis & Noor, 2013). However, to date, there are no reports on the influence of biofortification with selenium on the antioxidant and antiproliferative activities in different stages of maturation of this vegetable.

Among the crops that have the ability to accumulate selenium, broccoli can be highlighted, and it is classified as a secondary accumulator of selenium (Ramos et al., 2011). Another important fact is that biofortifying selenium as sodium selenate is more effective in promoting the accumulation of selenium than using sodium selenite, and it leads to more rapid absorption and distribution (Sharma, Bansal, Dhillon, & Dhillon, 2010). Research by Ávila et al. (2013) showed that the broccoli sprouts total selenium concentration in the shoots previously treated with selenate was 35% higher than for sprouts treated with selenite. It was also proven that shoots with 7 days of age exposed to concentration of 100 mM of selenate and 75 and 100 μM selenite showed clear symptoms of toxicity (decreased root growth and purple cotyledons), which justifies the use of selenate sodium in this study.

Several countries, including the USA, the UK, China, Finland and Brazil, have reported selenium intakes below the daily recommendation of 55 μg per day (Bendich, 2001; Mccann & Ames, 2011), which can result in diseases and disorders, such as thyroid gland dysfunction, irreversible brain injury, heart disease, decreased immune response against viral infections, increased risk of various cancers, type 2 diabetes and reduced fertility, due to a significant reduction in the activity of selenoproteins (Ansong, Yang, & Diamond, 2014). Our results indicate that the consumption of 5.07, 3.31 and 2.30 g of sprouts, seedlings and inflorescence biofortified with selenium, respectively, supplies the daily needs of this mineral. Biofortification is thus a viable alternative as observed in this study biofortifying increases the concentration of selenium in broccoli. The biofortification of foods with selenium was also highlighted and verified by Bañuelos, Arroyo, Pickering, Yang, and Freeman (2015).

In addition to the glucosinolates, broccoli is also rich in phenolic compounds, which are the main compounds responsible for the antioxidant activity of this plant (Fernández-León, Fernández-León, Lozano, Ayuso, & González-Gómez, 2013). Thus, a greater amount of phenolic antioxidant activity was verified in

this work. There is still a contradictory effect of the biofortification of phenolic compounds (Kovacik & Klejdus, 2008; Yuan, Wang, Guo, & Wang, 2010). Research indicates that abiotic stress, such as the application of selenium, can result in increased production of phenolic compounds (Dutta & Maharia, 2012). Robbins, Keck, Banuelos, and Finley (2005) evaluated different varieties of broccoli and showed that phenolic compounds increased with the application of selenium, results similar to those in the present study.

Our results showed that the seedlings of broccoli, compared to sprouts and inflorescences, showed the highest amount of phenolic compounds and antioxidant activity. This result is in agreement with studies by Soengas, Cartea, Francisco, Sotelo, and Velasco (2012) and Samec, Piljac-Zagarac, Bogovic, Habjanic, and Gruz (2011), who found that the antioxidant activity in this species begins when the buds are formed, reaching a maximum three months after sowing. Subsequent to this period, the antioxidant activity suffers significantly.

Chemotherapeutic drugs have the ability to induce cytotoxicity in tumour cells by several mechanisms, acting primarily on cell signalling (Hoshino et al., 1991). There is currently a great need to search for new anticancer drugs that can kill cancer cells with low toxicity (Chaudhary et al., 2014), for example, compounds found in plants and food.

Broccoli has been highlighted in several studies due to its important antiproliferative activity. Chaudhary et al. (2014) evaluated the antiproliferative activities sprouts broccoli and inflorescence in human prostate lines and reported that all samples tested had $IC_{50}$ values ranging from 25 to 143 $\mu g\,mL^{-1}$ extracts. The results found in this study were similar to those reported by 41. Wang, Zhu, Meckling, and Marcone (2013) when evaluating broccoli inflorescences in MCF-7 lines.

Sulforaphane, a principal component of broccoli, demonstrated potent antiproliferative activity in pancreatic cancer, inhibiting cell growth with an $IC_{50}$ of approximately 10–15 μM (Li, Fu, Watkins, Srivastava, & Shankar, 2013). Additionally, in breast cancer cell lines, this compound was able to inhibit the invasion of cancer cells by inhibiting the expression of enzymes related to metastasis (Lee et al., 2013). Li et al. (2012) also demonstrated the synergism between the efficiency of selenium and isothiocyanates, wherein the combination of selenium with sulforaphane resulted in increased expression of thioredoxin reductase 1, contributing to efficient protection against oxidative damage mediated by free radicals in hepatocytes in humans.

In vitro analyses are widely used in scientific research and have the ability to evaluate the performance of one or more samples against different cell lines (Houghton et al., 2007). Therefore, this technique becomes the first step in the discovery of new potential anticancer compounds. Our studies demonstrated for the first time the antiproliferative activity of different broccoli maturity stages biofortified with selenium, and each of the maturation stages presented particular activity, highlighting the cytocidal activity for the glioma line of the seedlings of broccoli biofortified with selenium.

## 5. Conclusion

This study indicates the potential use of broccoli as a source of natural antioxidants and potent antiproliferative activity. Furthermore, this vegetable biofortified with selenium is an important alternative that combines the reduction of the mineral deficiency with increased antioxidant activity, an increased amount of phenolic compounds and increased antiproliferative activity. Among the broccoli maturation stages, the seedlings stood out because of their wealth of phenolic compounds and antioxidant activity and their antiproliferative activity, as highlighted by the cytocidal activity for the glioma line, and they are therefore a significant source of anti-cancer compounds. The mechanisms of action of this plant are still unclear and need to be further explored to assist in providing useful information for its application as an alternative for the prevention of cancer.

## References


Ansong, E., Yang, W., & Diamond, A. M. (2014). Molecular cross-talk between members of distinct families of selenium containing proteins. *Molecular Nutrition & Food Research, 58*, 117–123.

Araújo, M. E. M. B., Franco, Y. E. M., Alberto, T. G., Sobreiro, M. A., Conrado, M. A., Priolli, D. G., et al. (2013). Enzymatic de-glycosylation of rutin improves its antioxidant and antiproliferative activities. *Food Chemistry, 141*, 266–273.

Ávila, F. W., Faquin, V., Yang, Y., Ramos, S. J., Guilherme, L. R. G., Thannhauser, T. W., et al. (2013). Assessment of the anticancer compounds Se-methylselenocysteine and glucosinolates in Se-biofortified broccoli (*Brassica oleracea* L. var. *italica*) sprouts and florets. *Journal of Agricultural and Food Chemistry, 61*, 6216–6223.

Bañuelos, G. S., Arroyo, I., Pickering, I. J., Yang, S. I., & Freeman, J. L. (2015). Selenium biofortification of broccoli and carrots grown in soil amended with Se-enriched hyperaccumulator *Stanleya pinnata*. *Food Chemistry, 166*, 603–608.

Bendich, A. (2001). Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. *Nutrition, National Academy Press, 17*, 364.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology, 28*, 25–30.

Chaudhary, A., Sharma, U., Vig, A. P., Singh, B., & Arora, S. (2014). Free radical scavenging, antiproliferative activities and profiling of variations in the level of phytochemicals in different parts of broccoli (*Brassica oleracea italica*). *Food Chemistry, 148*, 373–380.

Dutta, R. K., & Maharia, R. S. (2012). Antioxidant responses of some common medicinal plants grown in copper mining areas. *Food Chemistry, 131*, 259–265.

Fernández-León, M. F., Fernández-León, A. M., Lozano, M., Ayuso, M. C., & González-Gómez, D. (2013). Altered commercial controlled atmosphere storage conditions for 'Parhenon' broccoli plants (*Brassica oleracea* L. var. *italica*). Influence on the outer quality parameters and on the health-promoting compounds. *LWT – Food Science and Technology, 50*, 665–672.

Grusak, M. A., & DellaPenna, D. (1999). Improving the nutrient composition of plants to enhance human nutrition and health. *Annual Review of Plant Physiology and Plant Molecular Biology, 50*, 133–161.

Guo, Q., Guo, L., Wang, Z., Zhuang, Y., & Gu, Z. (2013). Response surface optimization and identification of isothiocyanates produced from broccoli sprouts. *Food Chemistry, 141*, 1580–1586.

Hoshino, T., Hara, A., Inoue, M., Honda, J., Imai, Y., Oizumi, K., et al. (1991). Flow cytometric measurement of NK cell cytotoxicity. *Journal of Clinical & Laboratory Immunology, 36*, 39–43.

Houghton, P., Fang, R., Techatanawat, I., Steventon, G., Hylands, P. J., & Lee, C. C. (2007). The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. *Methods, 42*, 377–387.

Jaiswal, A. K., Abu-Ghannam, N., & Gupta, S. (2012). A comparative study on the polyphenolic content, antibacterial activity and antioxidant capacity of different solvent extracts of *Brassica oleracea* vegetables. *International Journal of Food Science and Technology, 47*, 223–231.

Khalaj, L., Nejad, S. C., Mohammadi, M., Zadeh, S. S., Pour, M. H., Ashabi, G., et al. (2013). Assessing competence of broccoli consumption on inflammatory and antioxidant pathways in restraint-induced models: Estimation in rat hippocampus and prefrontal cortex. *BioMed Research International, 2013*, 1–13.

Kovacik, J., & Klejdus, B. (2008). Dynamics of phenolic acids and lignin accumulation in metal-treated *Matricaria chamomilla* roots. *Plant Cell Reports, 27*, 605–615.

Lee, Y. R., Noh, E. M., Han, J. H., Kim, J. M., Hwang, B. M., Kim, B. S., et al. (2013). Sulforaphane controls TPA-induced MMP-9 expression through the NF-κB signaling pathway, but not AP-1, in MCF-7 breast cancer cells. *BMB Reports, 46*, 201–206.

Leite-Legatti, A. V., Batista, A. G., Dragano, N. R. V., Marques, A. C., Malta, L. G., Riccio, M. F., et al. (2012). Jaboticaba peel: Antioxidant compounds, antiproliferative and antimutagenic activities. *Food Research International, 49*, 596–603.

Li, S. H., Fu, J., Watkins, D. N., Srivastava, R. K., & Shankar, S. (2013). Sulforaphane regulates self-renewal of pancreatic cancer stem cells through the modulation of Sonic hedgehog–GLI pathway. *Molecular and Cellular Biochemistry, 373*, 217–227.

Li, D., Wang, W., Shan, Y., Barrera, L. N., Howie, A. F., Beckett, G. J., et al. (2012). Synergy between sulforaphane and selenium in the up-regulation of thioredoxin reductase and protection against hydrogen peroxide-induced cell death in human hepatocytes. *Food Chemistry, 133*, 300–307.

Mccann, J. C., & Ames, B. N. (2011). Adaptive dysfunction of selenoproteins from the perspective of the triage theory: Why modest selenium deficiency may increase risk of diseases of aging. *FASEB Journal, 25*, 1793–1814.

Medina, S., Domínguez-Perles, R., Moreno, D. A., García-Viguera, C., Ferreres, F., Gil, J. I., et al. (2015). The intake of broccoli sprouts modulates the inflammatory and vascular prostanoids but not the oxidative stress-related isoprostanes in healthy humans. *Food Chemistry, 173*, 1187–1194.

Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paull, K., Vistica, D., et al. (1991). Feasibility of a high-flux anticancer drug screen using a diverse panel of

cultured human tumor cell lines. *Journal of the National Cancer Institute, 83*, 757–766.
Newman, D. J., Cragg, G. M., & Snader, K. M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of Natural Products, 75*, 311–335.
Oyaizu, M. (1986). Studies on products of browning reaction – Antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition, 4*, 307–315.
Pascoal, A. C. R. F., Ehrenfried, C. A., Lopez, B. G. C., Araujo, T. M., Pascoal, V. D. B., Gilioli, R., et al. (2014). Antiproliferative activity and induction of apoptosis in PC-3 cells by the chalcone cardamonin from *Campomanesia adamantium* (Myrtaceae) in a bioactivity-guided study. *Molecules, 19*, 1843–1855.
Pérez-Balibrea, S., Moreno, D. A., & García-Viguera, C. (2011). Genotypic effects on the phytochemical quality of seeds and sprouts from commercial broccoli cultivars. *Food Chemistry, 125*, 348–354.
Ramos, S. J., Faquin, V., Guilherme, L. R. G., Castro, E. M., Avila, F. W., Carvalho, G. S., et al. (2011). Selenium biofortification and antioxidante activity in lettuce plants feed with selenate and selenite. *Plant Soil Environmental, 56*, 583–587.
Rayman, M. P. (2012). Selenium and human health. *Lancet, 379*, 1256–1268.
Razis, A. F. A., & Noor, N. M. (2013). Cruciferous vegetables: Dietary phytochemicals for cancer prevention. *Asian Pacific Journal of Cancer Prevention, Bangkok, 14*, 1565–1570.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine, 6*, 1231–1237.
Robbins, R. J., Keck, A. S., Banuelos, G., & Finley, J. W. (2005). Cultivation conditions and selenium fertilization alter the phenolic profile, glucosinolate, and sulforaphane content of broccoli. *Journal of Medicinal Food, 8*, 204–214.
Samec, D., Piljac-Zagarac, J., Bogovic, M., Habjanic, K., & Gruz, J. (2011). Antioxidant potency of white (*Brassica oleracea* L. var. *capitata*) and Chinese (*Brassica rapa* L. var. *pekinensis* (Lour.)) cabbage: The influence of development stage, cultivar choice and seed selection. *Scientia Horticulturae, 128*, 78–83.
Sharma, S., Bansal, A., Dhillon, S. K., & Dhillon, K. S. (2010). Comparative effects of selenate and selenite on growth and biochemical composition of rapeseed (*Brassica napus* L.). *Plant and Soil, 329*, 339–348.
Soengas, P., Cartea, M. E., Francisco, M., Sotelo, T., & Velasco, P. (2012). New insights into antioxidant activity of *Brassica* crops. *Food Chemistry, 134*, 725–733.
Tezotto, T., Favarin, J. L., Neto, A. P., Gratão, P. L., Azevedo, R. A., & Mazzafera, P. (2013). Simple procedure for nutrient analysis of coffee plant with energy dispersive X-ray fluorescence spectrometry (EDXRF). *Scientia Agricola, 70*, 263–267.
Townsend, B. E., Chen, H.-J., Jeffery, E. H., & Johnson, R. W. (2014). Dietary broccolimildly improves neuroinflammation in aged mice but does not reduce lipopolysaccharide-induced sickness behavior. *Nutrition Research, 34*, 990–999.
Wang, S., Zhu, F., Meckling, K. A., & Marcone, M. F. (2013). Antioxidant capacity of food mixtures is not correlated with their antiproliferative activity against MCF-7 breast cancer cells. *Journal of Medicinal Food, 16*, 1138–1145.
Woisky, R. G., & Salatino, A. (1998). Analysis of propolis: some parameters and procedures for chemical quality control. *Journal of Apicultural Research, 37*, 99–105.
Yuan, G., Wang, X., Guo, R., & Wang, Q. (2010). Effect of salt stress on phenolic compounds, glucosinolates, myrosinase and antioxidant activity in radish sprouts. *Food Chemistry, 121*, 1014–1019.