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Time dependence of bioactive compounds and antioxidant capacity during germination of different cultivars of broccoli and radish seeds

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ABSTRACT

Optimisation of the germination process of different cultivars of broccoli (*Brassica oleracea* var. *italica* cv. Lucky, cv. Tiburon and cv. Belstar) and radish (*Raphanus sativus* cv. Rebel and cv. Bolide) seeds in relation to the content of glucosinolates (GLS), vitamin C and total antioxidant capacity was carried out in order to maximise the health-promoting properties of *Brassica* sprouts. The content of total and individual GLS varied between species, among cultivars, and germination time. Glucoraphanin in broccoli and glucoraphenin in radish were the predominant GLS in raw seeds (61–77 and 63–129 μmol/g DM, respectively) and, although their content decreased during germination, they were maintained in rather large proportions in sprouts. Vitamin C was not detected in raw seeds and its content increased sharply in broccoli and radish sprouts (162–350 and 84–113 mg/100 g DM, respectively). Raw brassica seeds are an excellent source of antioxidant capacity (64–90 and 103–162 μmol Trolox/g DM in broccoli and radish, respectively) and germination led to a sharp increase. Germination of broccoli cv. Belstar and radish cv. Rebel for 4 days provided the largest glucoraphanin and glucoraphenin content, respectively, and also brought about large amounts of vitamin C and antioxidant capacity.

1. Introduction

There is epidemiological evidence that diets rich in *Brassica* vegetables (broccoli, cabbage, Brussels sprouts, cauliflower, radish, garden cress, etc.) are associated with a lower risk of lung and colorectal cancer (Higdon, Delage, Williams, & Dashwood, 2007). *Brassica* vegetables are good sources of antioxidant vitamins and phytochemicals termed glucosinolates (GLS) that may work synergistically to aid in the prevention of cancer (Liu, 2004). Recently, the International Agency for Research on Cancer (IARC, Washington, DC, USA) concluded that high *Brassica* vegetable consumption could affect cancer risk by inducing the activity of various xenobiotic metabolising enzymes and by affecting important cellular mechanisms of cancer development, such as apoptosis and cell growth arrest (International Agency for Research on Cancer, 2004). Several studies have recently demonstrated that *Brassica* vegetables such as broccoli (*Brassica oleracea* var. *italica*) and radish (*Raphanus sativus*) may provide protection against oxidative damage and, consequently, prevent chronic diseases due to their composition in antioxidant vitamins and GLS (Barillari et al., 2006, 2007; Podsedek, 2007).

The most distinctive characteristic of *Brassicaceae* is its high GLS (β-thioglucoside N-hydrosulfates) content. These compounds have a wide variety of chemical structures and can be divided into three basic categories: aliphatic, aromatic, and indole, according to the type of side chain they possess. When GLS comes into contact with myrosinase – a plant thioglucosidase enzyme – during tissue damage because of handling, processing, or chewing, a complex mixture of phytochemicals are yielded, including isothiocyanates (ITC), thiocyanates, nitriles, cyanides and epithio-containing compounds (Fenwick, Heaney, & Mulin, 1983). In broccoli, one of the predominant GLS is glucoraphanin which is transformed by myrosinase into sulphoraphane which has been acknowledged as one of the compounds with the most important anticarcinogenic properties (Fahey & Talalay, 1999; Zhang, Talalay, Cho, & Posner, 1992). Similarly, in radish, high levels of glucoraphenin may be found. This is transformed into sulphoraphene by the action of myrosinase and, like sulphoraphane from broccoli, has anti-cancer potential as an inducer of phase II detoxification enzymes (Lee & Lee, 2006; Posner, Cho, Green, Zhang, & Talalay, 1994).

Vitamin C, an antioxidant vitamin, is involved in the protection against harmful free radicals and it has been strongly associated with a reduced risk of chronic diseases (Davey et al., 2000). Antioxidants scavenge radicals inhibit chain initiation or break down the chain propagation in different chemical and cellular systems

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(Cadenas & Parker, 2002). The Trolox Equivalent Antioxidant Capacity (TEAC) assay is one of the most established methods to measure antioxidant capacity in plant foods (Re et al., 1999).

It has been widely reported that seed sprouts provide higher nutritive value than raw seeds and their production is simple and inexpensive. Brassica species sprouts are becoming a popular health food which has been recommended for human diet since they have the advantages of germinated seeds (enhanced nutritional value, low fat food, rich in health-promoting phytochemicals, safe and fresh) and consumers are demanding fresh and safe vegetables that may promote health and well-being.

The aim of this work was to carry out a kinetic study on bioactive compounds (GLS and vitamin C) and antioxidant capacity during 3, 4 and 5 days of germination of three Spanish cultivars of broccoli (cv. Tiburon, cv. Belstar and cv. Lucky) and two Spanish cultivars of radish (cv. Rebel and Bolide), in order to select suitable brassica cultivars and germination times to provide the maximum concentration of health-promoting compounds.

2. Materials and methods

2.1. Plant material

Broccoli seeds (*B. oleracea* L. var. italica cv. Tiburon, cv. Belstar and cv. Lucky) and radish seeds (*R. sativus* cv. Rebel) were purchased at Bejo Iberica S.L. (Madrid, Spain). Radish seeds (*R. sativus* cv. Bolide) were kindly provided by Man Fong Pacific Trading (Madrid, Spain).

2.2. Seed germination

Germination of broccoli and radish seeds was carried out according to Martínez-Villaluenga, Frias, Gulewicz, Gulewicz & Vidal-Valverde (2008). Ten grams of seeds were used for each germination stage. Brielfly, the seeds were soaked in 50 ml of 0.07% sodium hypochlorite for 30 min. These seeds were drained and washed with distilled water until they reached a neutral pH. Afterwards, seeds were soaked in 50 ml distilled water for $5\frac{1}{2}$ h and shaken every 30 min. The imbibed seeds were germinated at pilot scale by layering seeds over moist filter paper in a germination tray. The tray was placed in a seed germinator G-120 model (ASL Snijders International S.L., Holland) and seeds were continuously watered by capillarity. Germination of broccoli seeds was carried out at 25 °C in photoperiod conditions (16 h light and 8 h darkness). Germination of radish seeds was carried out at 25 °C in darkness. Sprouted seeds were harvested after 3, 4 and 5 days. The germination process was carried out in triplicate for each germination stage and the germination rate was over 90%. The sprouted seeds were freeze-dried and stored at −20 °C until further analyses.

2.3. Determination of glucosinolates

Individual GLS were extracted and analysed by HPLC following enzymatic desulfatation according to the Official Journal of the European Communities (1990), as described previously (Ciska, Martyniak-Przybyszewska, & Kozlowska, 2000; Martinez-Villalulenga et al., 2009). Briefly, 200 mg of freeze-dried samples were extracted twice with 70% boiling methanol. Since all the cultivars examined lacked glucotropaeolin (Merck, Darmstadt, Germany), a known amount was added to each sample just before the first extraction as an internal standard for HPLC analysis. The isolation, desulfatation and HPLC analyses of GLS were carried out according to the modified method of Heaney, Spinks, Hanley, and Fenwick (1986). Desulfo-GLS were separated in the HPLC system in a LiChrospher 100 RP-18 (250 × 4 mm, 5 μm) column (Merck, Darmstadt, Germany) using an auto injector (20 μl loop) and a 1.2 ml/min flow rate by eluting with a gradient of double distilled water (A) and 20% acetonitrile (B) as follows: 1% B for 1 min, linear gradient from 1% to 99% B for 30 min, 99% B for 6 min, linear gradient from 99% to 1% B for 5 min, and 1% B for 8 min. GLS were detected at $\lambda = 229$ nm. Individual GLS were identified by comparing the retention times with those for standards or on the basis of data from literature. The sample content of desulfo-GLS was quantified based on relevant relative response factors (Heaney et al., 1986).

2.4. Determination of vitamin C

Vitamin C quantification was performed by micellar electrokinetic capillary electrophoresis (MECC) according to Frias, Miranda, Doblado, and Vidal-Valverde (2005) using a P/ACE system 2050 (Beckman Instruments, Fullerton, CA, USA) and UV detection at 254 nm. One gram of sample was extracted with 20 ml metaphosphoric acid (Sigma–Aldrich, St. Louis, MO) and isoascorbic acid was added as internal standard (Sigma–Aldrich, St. Louis, MO). An aliquot of extract was treated with D,L-dithiothreitol (Sigma–Aldrich, St. Louis, MO) to convert any dehydroascorbic acid present in the sample to ascorbic acid (Sapers, Douglas, Ziolkowski, Miller, & Hicks, 1990). Extractions were carried out in triplicate. Ascorbic acid was quantified from a calibration curve prepared with pure ascorbic acid standard (Merck, Damstadt, Germany) and with the response factor relative to internal standard.

2.5. Determination of Trolox equivalent antioxidant capacity (TEAC)

The TEAC assay was based on the reduction of the ABTS radical ($ABTS^+$) by antioxidants present in phosphate buffer saline (PBS) extracts from broccoli and radish seeds, and sprouts, according to the procedure described by Re et al. (1999) and modified by Doblado et al. (2005). Briefly, ABTS radical was prepared by mixing ABTS (Sigma–Aldrich, St. Louis, MO) stock solution (7 mM in water) with 2.45 mM potassium persulfate (Sigma–Aldrich, St. Louis, MO). This mixture had to be kept for 12–24 h until the reaction was completed and the absorbance was stable (0.700 ± 0.020 at 734 nm at 30 °C). For the spectrophotometric assay, 1.48 ml of the $ABTS^+$ solution and 20 μl of the PBS sample extract or Trolox (Sigma–Aldrich, St. Louis, MO) solutions were mixed and measured immediately after 10 min at 734 nm at 30 °C. Trolox (2.5 mM) was prepared in PBS buffer to use as the standard solution.

2.6. Determination of moisture content

For determination of water content, aliquots of raw broccoli and radish seeds and fresh sprouts were dried to constant weight in a vacuum oven (20 mm/Hg, 35 °C).

2.7. Statistical analysis

Data were expressed as the mean ± standard deviation (SD) of three independent replicates. Data were subjected to multifactor analysis of variance (ANOVA) using the least-squared difference test with the Statgraphic 5.0 Programme (Statistical Graphic, Rockville, MD, USA).