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Evaluating the impact of sprouting conditions on the glucosinolate content of *Brassica oleracea* sprouts

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ABSTRACT

The glucosinolates content of brassica plants is a distinctive characteristic, representing a healthy advantage as many of these compounds are associated to antioxidant and anti-carcinogenic properties. Brassica sprouts are still an underutilized source of these bioactive compounds. In this work, four varieties of brassica sprouts (red cabbage, broccoli, Galega kale and Penca cabbage), including two local varieties from the North of Portugal, were grown to evaluate the glucosinolate profile and myrosinase activity during the sprouting. Also the influence of light/darkness exposure during sprouting on the glucosinolate content was assessed. Glucosinolate content and myrosinase activity of the sprouts was evaluated by HPLC methods. All sprouts revealed a higher content of aliphatic glucosinolates than of indole glucosinolates, contrary to the profile described for most of brassica mature plants. Galega kale sprouts had the highest glucosinolate content, mainly sinigrin and glucoiberin, which are recognized for their beneficial health effects. Penca cabbage sprouts were particularly richer in glucoraphanin, who was also one of the major compounds in broccoli sprouts. Red cabbage showed a higher content of progoitrin. Regarding myrosinase activity, Galega kale sprouts showed the highest values, revealing that the use of light/dark cycles and a sprouting phase of 7–9 days could be beneficial to preserve the glucosinolate content of this variety.

1. Introduction

Brassica oleracea L. belongs to the Brassicaceae (Cruciferae) family which comprises many important vegetables, grown and consumed worldwide (Podsedek, 2007). Brassicaceae vegetables are a good source of antioxidants compounds, especially phenolics and glucosinolates (GLs) (Bruce and Pickett, 2007; Jahangir et al., 2008). In fact, only these plants and a few other edible plants from the Capparales order, are recognized as a source of all known GLs (Fahey et al., 2001).

GLs are sulfur-containing secondary plant metabolites from the β-thioglycosides group, derived from the amino acid biosynthesis (Chen and Andreasson, 2001; Podsedek, 2007), being related to the pungent flavor and odor of Brassica vegetables (Martinez-Sanchez et al., 2006; Jones et al., 2006; Padilla et al., 2007). The GLs have a great diversity of compounds and chemical structures (Agerbirk and Olsen, 2012), differing accordingly to species, cultivar, and even within varieties of the same species (Aires et al., 2006; Cartea et al., 2008). Besides genetic, many other factors can cause the variation of GL content in Brassica plants, namely agronomical (Aires et al., 2006), climatic (Padilla et al., 2007; Cartea et al., 2008) and environmental factors (Pereira et al., 2002; Schreiner, 2005). More than 132 individual GLs were detected and grouped into aliphatic, aromatic and indolic GLs, depending on the structure of their side-chain (Agerbirk and Olsen, 2012). From the GLs detected, 30–40 are present in the most economically important Brassica species (Halkier and Gershenzon, 2006).

GLs are considered health-promoting phytochemicals. The products of the enzymatic or non-enzymatic hydrolysis of GLs are biologically active compounds with diverse effects on human health (Ciska et al., 2000), including anti-carcinogenic, cholesterol-reducing, and other pharmacological effects (Cieslik et al., 2007; Verkerk et al., 2009). These substances may also act as indirect antioxidants by modulating the activity of xenobiotic metabolizing enzymes (phase I and phase II enzymes) that trigger the long lasting antioxidant activity (Vig et al., 2009), reducing the oxidative stress status responsible for triggering chronic degenerative diseases (Verkerk et al., 2009). On the other hand,

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intact GLs have limited biological activity (Smith et al., 2003). Their effect arises when GLs come in contact with plant myrosinase, a thioglucoside glucohydrolase, EC 3.2.3.1 belonging to glycoside hydrolase family 1 (GH1), which catalyzes the hydrolysis of GLs in Brassicas after tissue damage (Travers-Martin et al., 2008). Myrosinase is normally physically separated from the GLs in the cell, being localized in idioblasts (myrosin cells) (Andreasson et al., 2001). When plant cells are damaged (e.g., during food processing, ingestion and digestion, or injury by predators) the enzyme is released, promoting the hydrolysis of GLs, resulting in a range of breakdown biologically active compounds, like indoles and isothiocyanates (Kissen et al., 2009), and also glucose and sulphate (Singh et al., 2007). Different biological activities were attributed to these compounds, some beneficial, like the reduction of the risk of certain human's cancers (Fahey et al., 2001; Mithen et al., 2003), while others have a detrimental effect for humans and animals (Rosa et al., 1997). The isothiocyanates are one of the GLs breakdown products that present bio-protective effects, with proved anti-carcinogenic effects (Rouzaud et al., 2004), enhancing the activity of phase II enzymes and possibly inhibiting phase I enzymes (Fahey et al., 1997; Cartea et al., 2008).

Usually myrosinase stability and activity decreases during processing and domestic treatments (Oerlemans et al., 2006; Aires et al., 2012), specifically with the use of heat and occurrence of cell disruption, affecting the intake and bioavailability of GLs and their breakdown products (Getahun and Chung, 1999). However, food products containing active myrosinase, like Brassica sprouts and shortly cooked mature Brassica vegetables present an increased bioavailability of isothiocyanates as a result of high myrosinase activity (Verkerk et al., 2009). Sprouts, as fresh-cut products with a short shelf life, can be susceptible to GLs losses due to a high myrosinase activity that can also be affected by sprouting conditions (eg. sprouting time, light exposure, harvesting and storage) (Aires et al., 2012). Sprouts are a valuable but still under-appreciated healthy dietary option which may be considered a functional food, enhancing the concentration of health-promoting bioactive compounds in the diet (Fahey et al., 1997). Besides broccoli sprouts, that showed potential anti-carcinogenic activity (Munday et al., 2008; Keum et al., 2009; Yanaka et al., 2009; Li et al., 2010), other sprouts from *B. oleracea* varieties are still understudied. Portuguese tronchuda cabbage and Portuguese Galega kale are traditional varieties of *B. oleracea* consumed in Portugal. The sprouts of these varieties have already demonstrated a high "in vitro" antioxidant capacity (Vale et al., 2014), which raised even more the interest in these products. The current study focuses on characterization of GLs content and myrosinase activity of Brassica sprouts produced under different photoperiod conditions and collected with different sprouting ages, in order to better define healthier sprouts and best practices of production.

2. Materials and methods

2.1. Materials and samples

All chemicals, reagents and solvents were analytical grade purchased from Sigma Chemical Co. (St. Louis, MO, USA). The water was treated with a system of thermal mantles (Isopad Isomantle, Borehamwood, Hertz, England) and in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

B. oleracea seeds used belong to the following varieties: Broccoli (*B. oleracea* L. var. italica Plenck, cultivar calabrese), Galega kale (*B. oleracea* var. acephala DC), Portuguese Tronchuda cabbage (*B. oleracea* L. var. costata DC, landrace Penca da Póvoa) and Red cabbage (*B. oleracea* var. capitata f. rubra). Broccoli and Red cabbage seeds were purchased at Germisem-Sementes, Lda. while seeds of Penca cabbage and Galega kale were acquired from traditional farmers in the Póvoa do Varzim (Northwest of Portugal).

2.2. Sprouting method

Sprouting method was based on Martinez-Villaluenga et al. (2010) work with slight adjustments. Seeds were sanitized for 30 min in sodium hypochlorite (0.07%, v/v), rinsed with tap water and soaked for 12 h at room temperature, in darkness and with light agitation. Seedbed was made in polypropylene trays ($10 \times 15 \times 4$ cm) containing vermiculite and seeds for green sprouts (GS) production sprouted inside a growth chamber (Fitoclima 200) with controlled temperature (25 °C) and a photoperiod regime with cycles of 16 h light and 8 h darkness. White sprouts (WS) were grown in the same condition but in total darkness. Harvesting took place when sprouts reached commercial size at the 7th, 9th and 12th days after sowing. After harvesting, sprouts were frozen at −80 °C, freeze-dried (Scanlaf model 110-4 PRO), fine-ground in a mill (Retsch ZM 200) and kept in a desiccator protected from light until analysis.

2.3. Glucosinolate extraction and analysis

Glucosinolates (GLS) extraction was performed according to the methodology described by Pereira et al. (2002). Briefly, 0.2 mg of freeze dried sample was extracted with 3 mL of boiling methanol 90% (v/v) and homogenized for 2 min at 24,000 rpm (Ultra Turrax T_{25} equipped with a dispersing element S25N-10G). After 30 s from start boiling, 200 μL of glucotropaeolin (1 mg mL^{-1}), a benzyl GL used as internal standard, was added. The homogenized sample was centrifuged for 2 min at 5000 rpm (Kubota 2100). The extraction was repeated in the residue for 1 min with 2 mL of boiling methanol 70% (v/v) and the supernatants combined to a final volume of 10 mL with methanol 70%. Followed the purification and enzymatic desulfation of individual GLs in a small column of Sephadex DEAE A25 prepared in the laboratory (Rosa, 1978; Heaney and Fenwick, 1980). First, an aliquot of 2.5 mL of the extract was taken to dryness under air flow and resuspended in 2.5 mL of water. Meanwhile 0.5 mL of water was added to the Sephadex DEAE A25 small column and leave to drain. Then, 2×1 mL of resuspended extract was loaded in the Sephadex DEAE A25 column in order to trap the GLs in the Sephadex DEAE A25 resin. The resin was then washed with 2×1 mL of water followed by 2×0.5 mL of a 0.02 M pyridine buffer (C_5H_5N, K22146828, Merk). Finally the adsorbed GLs were desulfated by adding 75 μL of sulfatase, prepared accordingly to Aires (2004). The reaction time was of 18 h at 20–25 °C and after this; the column was washed with 3×0.5 mL water, being the eluted desulfated GLs collected in glass vials and preserved at −18 °C until HPLC analysis. The desulfo GLs were analyzed in an HPLC system (Gilson system, HPLC 712, Gilson) using a method described by Rosa et al. (2007). The compounds were separated in a C18 column (Spherisorb 5 μm ODS2, 250×4.6 mm i.d., Waters) and eluted with two solvents, being solvent A composed of ultra-pure water and solvent B by a solution of 20% of acetonitrile. Elution was performed at a flow rate of 1.5 mL min^{-1}. The chromatograms were recorded at 229 nm and GL peak identification and quantitative estimations were made using pure standard GL as internal standard (benzyl GL), and GLs response factor (Aires et al., 2012). All samples were analyzed in triplicate and the GLs content was expressed per 100 g (d.w.).

2.4. Myrosinase activity

The activity of the endogenous myrosinase of sprouts was measured by the extent of hydrolysis of a known amount of

sinigrin monohydrate (allyl GL), added to the incubated solution, during a short period of time (Oerlemans et al., 2006). Crude extracts from freeze dried sprouts were prepared by thoroughly mixing 500 mg of sprouts with 5 mL of ultrapure water, using a commercial blender (Ika, Ultra Turrax T_{18} basic, IKA-Labortechnik). Followed a centrifugation at 5000 rpm for 30 min and at 4 °C (Hettich 32R). The supernatant was collected and filtered (Whatman No. 1). Part of the filtrate was incubated for 1 h at 40 °C in a water bath, to allow for the myrosinase-catalyzed hydrolysis of all endogenous GLs, without affecting myrosinase activity. The other part of the filtrate was incubated for 15 min at 100 °C to inactivate the myrosinase and was used as negative control of the assay and for dilution purposes. Sinigrin (6 mM) was then added to the incubated crude extracts, in a proportion of 1 mL of sinigrin per 5.0 g of filtrate. The solution was mixed and incubated at 40 °C for 0, 5, 10, 20, 40 and 80 min with slow agitation. The reaction was stopped by adding methanol (9 ml mL^{-1}crude extract) and centrifugation at 5000*g* for 10 min. The extracts were then treated accordingly to Pérez-Balibrea et al. (2008) method with slight modifications. First, the extracts were heated at 70 °C for 30 min, with vigorous shaking every 5 min and centrifuged (17,500*g*, 30 min, 4 °C). The supernatants were evaporated under nitrogen flow, dissolved in 1 ml of ultrapure water and filtered through a 0.45 μm filter (Millipore) before injection into the HPLC system (Jasco LC-Net II/ADC) to determine the sinigrin content present in the samples. The HPLC system used comprised a pump (PU-2089 plus) with multisolvent delivery system and degasser, column oven CO-2060 Plus settled at 25 °C, autosampler AS-2057 Plus, a C18 column (C18 YMC-Pack ODS-AQ, 5 μm and pore size 120–200 Å, with a C18-YMC security guard, 4 mm × 3 mm) and a MD-2018 photodiode array detector set at 227 nm. An injection volume of 20 μL was used, with a mobile phase of 1 g L^{-1} of Trifluoro acetic acid (TFA) and an elution time of 15 min. All samples were analyzed in triplicate and the sinigrin content was expressed in mg per 100 g (d.w.).

2.5. Statistical analysis

For data interpretation an analysis of variance (ANOVA and univariate) was performed using the the SPSS 20.0 software (SPSS Inc., Chicago, Illinois, EUA) for Windows. Tukey's significant difference test was used to compare means. The significance of differences was compared using the least significant difference (LSD) at 95% confidence level. A principal component analysis was performed in order to understand the effect of light exposure on the overall glucosinolate profile of each brassica variety studied.

3. Results and discussion

3.1. Glucosinolates content

The GLs content of sprouts was monitored when they were ready for harvest, between the 7th and 12th day after sowing. Overall aliphatic GLs were the major GL group present in sprouts of all Brassica varieties during the three monitored stages (see Fig. 1). This tendency was already observed in red cabbage and broccoli sprouts (Bellostas et al., 2007a; Baenas et al., 2012), confirming that sprouts can be a better source of aliphatic GLs than mature brassicas. In mature broccoli and Galega kale plants indole GLs were predominant (Aires et al., 2012) reaching 60% and 65% of total GLs. The use of different photoperiods during sprouting had a significant effect on the production of GLs, having the sprouts produced under dark lower content of GLs (average level 4.7 ± 0.9 mmol 100 g^{-1} d.w.) than the produced under light/dark cycles (5.7 ± 1.4 mmol 100 g^{-1} d.w.). However, some exceptions were registered, namely for red cabbage and Penca cabbage sprouts with 7 days (see Fig. 1 A and C). Aliphatic GLs were the major GLs group in sprouts with higher expression in GS (88%), whereas WS had an average level of aliphatic GLs of 4.0 ± 0.9 mmol 100 g^{-1} d.w., which represented 84% of the total GL content. The opposite was seen regarding the influence of the photoperiod on the indole-GL content, as the absence of light led to a significant effect on these GLs content, having most of the samples grown under darkness a higher content of Indole-GL (see Fig. 1A, C and D), with exception of Galega kale sprouts in which levels were similar between GS and WS.

Galega kale produced under light conditions showed the highest aliphatic GLs content (5.8 ± 1.8 mmol 100 g^{-1} d.w.) followed by broccoli (5.7 ± 0.4 mmol 100 g^{-1} d.w.), red cabbage (5.2 ± 0.3

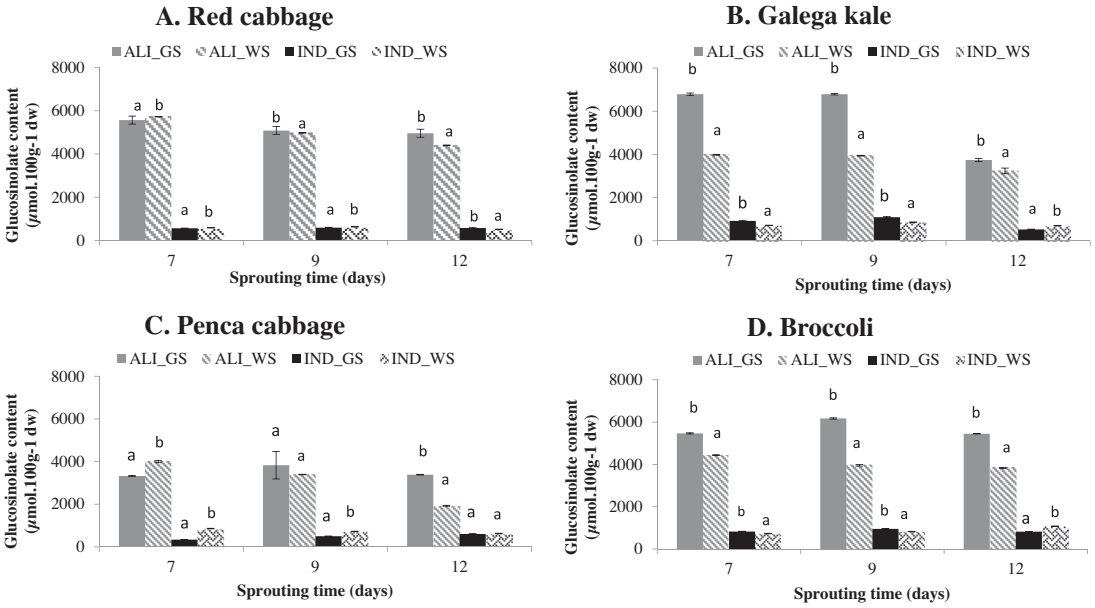

Fig. 1. Total aliphatic (ALI) and total indole glucosinolates (IND) of four Brassica sprouts at 3 different spouting times, under light (GS) and darkness (WS) conditions. Differences between WS and GS with the same letters are non-significant ($p < 0.05$).

mmol 100 g^{-1} d.w.) and Penca cabbage (3.5 ± 0.2 mmol 100 g^{-1} - d.w.). Aliphatic GLs content found in red cabbage GS were also higher than the values found in mature plants (3.8 mmol 100 g^{-1} - d.w.) reported by Volden et al. (2008). Under dark conditions the total aliphatic GLs content rank changed, being as follows: red cabbage > broccoli > Galega kale > Penca cabbage. Aliphatic GLs content under dark conditions was lower than under light in three of the studied varieties, corresponding to 83% in Galega kale (*vs* 87% in GS), 80% in Penca cabbage (*vs* 88% in GS) and 82% in broccoli (*vs* 86% in GS), whilst on red cabbage represented 89% for both GS and WS. The lowest content of indole GLs (0.5 ± 0.1 mmol 100 g^{-1} - d.w.) were registered on Penca cabbage GS, whilst sprouts from broccoli were the better source of indole GL (average content of 0.9 ± 0.2 mmol 100 g^{-1} d.w. for both GS and WS), followed by Galega kale GS and WS (0.8 ± 0.1 mmol 100 g^{-1} d.w.), Penca cabbage WS (0.7 ± 0.05 mmol 100 g^{-1} d.w.) and red cabbage WS and GS (0.6 mmol 100 g^{-1} d.w.).

The time of sprouting was also an important factor to benefit from higher concentrations of aliphatic GL. Longer sprouting times showed a reduction in aliphatic GLs concentration, especially after 12 days of sprouting. Overall sprouts with 7 days of germination had a significantly ($p < 0.05$) higher content of total and of the aliphatic GLs (see Fig. 1). However, in relation to indole GLs content, the highest values were reached for sprouts grown during 9 days. Some exceptions were registered, namely in Galega kale sprouts, where no differences were detected between 7th and 9th day of sprouting and between GS and WS aliphatic GLs content. Another exception was seen in broccoli sprouts GLs content, where GS with 9 days had a higher aliphatic GLs content, and WS with 12 days showed a higher indole GLs content. The decreasing of GL total level in sprouts with longer sprouting period was also recorded for red cabbage and broccoli by Baenas et al. (2012).

The composition of glucosinolates profile is important as the beneficial effects resulting from the presence of glucosinolates depend on the nature of the breakdown products, after degradation and absorption. The GLs profile of the four brassica varieties is presented in Fig. 2. Regarding the individual aliphatic GLs of all the sprouts studied, sinigrin was main GL in GS (7.4 ± 1.1 mmol 100 g^{-1} d.w.), followed by glucoraphanin (4.6 ± 1.3 mmol 100 g^{-1} d.w.) and glucoiberin (3.8 ± 0.3 mmol 100 g^{-1} d.w.). Sprouts produced under dark conditions showed a 21% reduction in sinigrin level, 22% in glucoraphanin and 8% in glucoiberin. The GLs profile of sprouts was an intermediate between the one found in seeds and the described for mature tissues (Bellostas et al., 2007b; Brown et al., 2003), which explains the predominance of aliphatic GL rather than the indole GL found by several researchers in mature vegetables (Fahey et al., 1997; Brown et al., 2003; Volden et al., 2008; Aires et al., 2012). The predominance of indole GL in mature plants was related to "*de novo synthesis*" of this group of GL during growth (Chen and Andreasson, 2001). These differences increase the nutritional importance of the sprouts, particularly Galega kale GS and Penca Cabbage, both with a high content of sinigrin, an aliphatic-GL, that together with glucoraphanin (only in Penca cabbage) are known as an important source of isothiocyanates, a potent inducer of phase II enzymes, with proved action in cancer prevention (Fahey et al., 1997; Barillari et al., 2005). In individual indole-GLs the highest concentration was observed in WS with 1.7 ± 0.1 mmol 100 g^{-1} d.w. of 4-methoxyglucobrasscin followed by 0.5 ± 0.02 mmol 100 g^{-1} d.w. of neoglucobrassicin and 0.5 ± 0.02 mmol.100 g^{-1} d.w. of 4-hydroxyglucobrassicin. Individually, red cabbage sprouts showed a greater diversity in GLs profile, containing ten different GLs. Progoitrin, glucoraphanin and sinigrin represented the major GLs present in Red cabbage, accounting for 33%, 21% and 18% of total GLs in GS and 25%, 21% and 19% in WS. Under light conditions sprouts were significantly richer in progoitrin than the produced under darkness. Progoitrin is considered an antinutrient that can cause goitrogenic effects, and whose presence as one of the major GLs of red cabbage sprouts

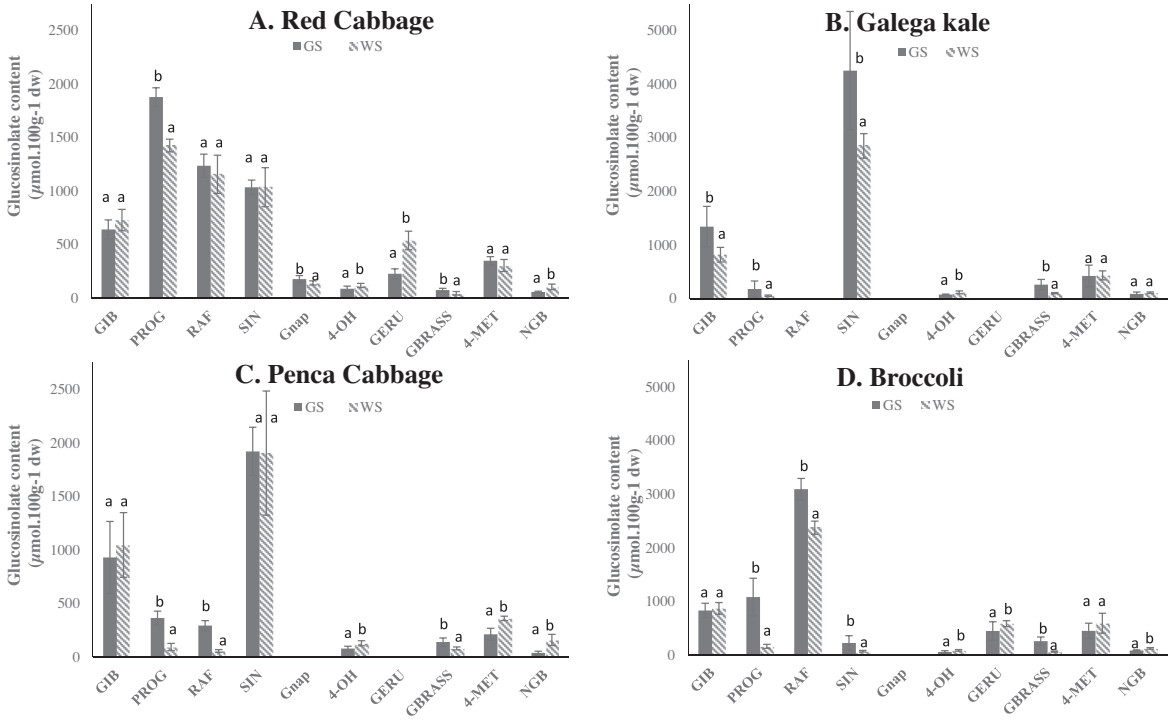

Fig. 2. Glucosinolate profile identified in sprouts of red cabbage, Galega kale, Penca cabbage and broccoli between the 7th and 12th day of sprouting (mean value ± standard deviation, $n = 9$, expressed in μmol 100 g^{-1} d.w.; differences between white sprouts (WS) and green sprouts (GS) with the same letters are non-significant ($p < 0.05$)). Abbreviations: GIB, glucoiberin; PROG, progoitrin; RAF, Glucoraphanin; SIN, sinigrin; Gnap, gluconapin; 4-OH, 4-hydroxyglucobrassicin; GERU, glucoerucin; GBRASS, Glucobrassicin; 4-MET, 4-methoxyglucobrassicin; NGB, neoglucobrassicin.

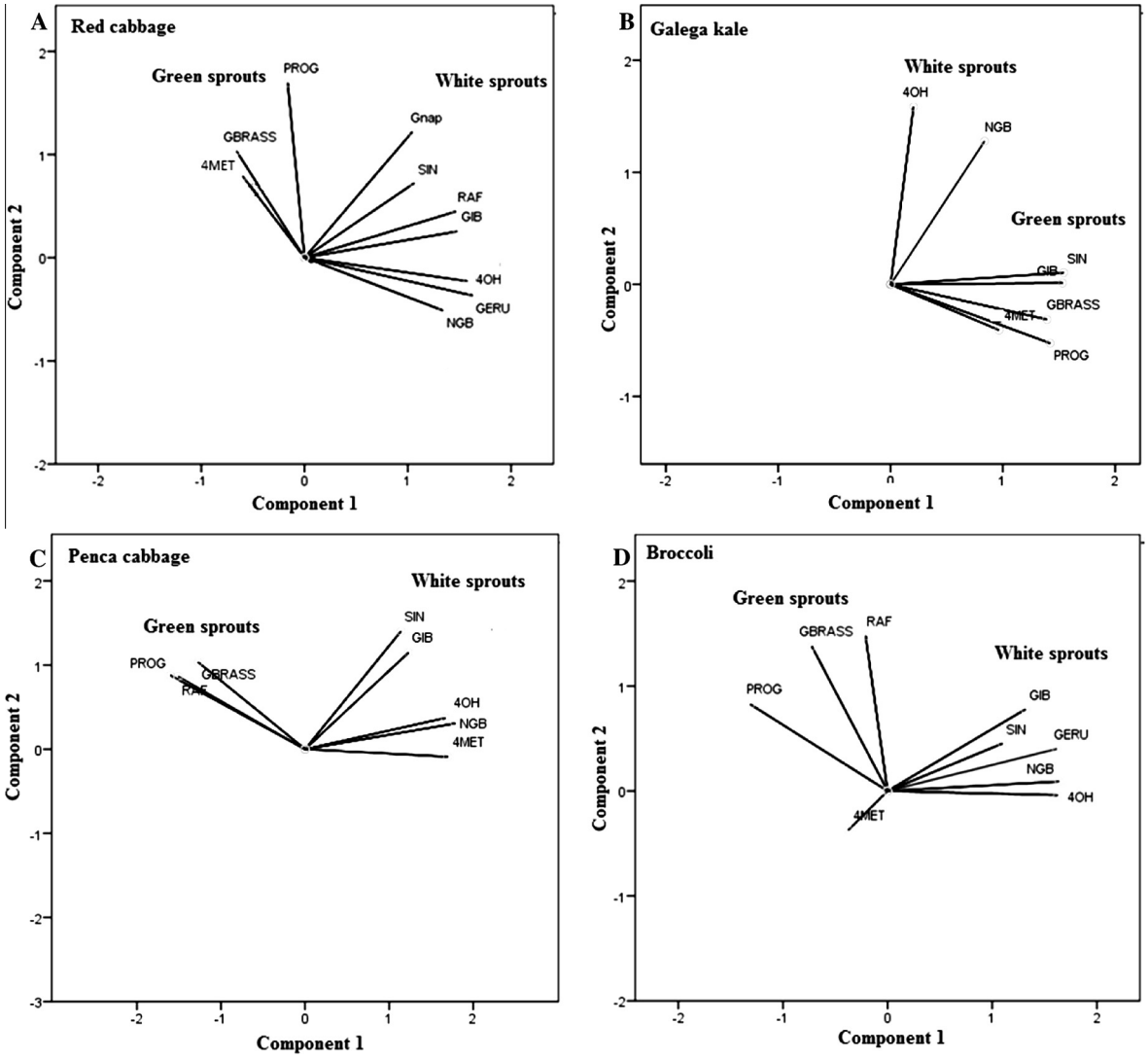

Fig. 3. Principal component analysis (PCA) results, aggregating glucosinolates in white sprouts (WS) and green spouts (GS). (For abbreviations see Fig. 2 legend).

and mature plants was also reported by Baenas et al. (2012) and Ciska et al. (2000), respectively. Glucoraphanin, sinigrin and glucobrassicin were also described as major GLs in the profile of red cabbage mature plants (Meyer and Adam, 2008). The levels of the indole glucobrassicin were significantly lower in sprouts, representing only 1.4% of the total GLs content in GS and 0.8% in WS. Gluconapin, an aliphatic GLs, was exclusive of red cabbage sprouts, but the mean concentration encountered (0.16 ± 0.03 mmol 100 g^{-1} d.w.) was lower than the levels found in mature plants (Meyer and Adam, 2008; Volden et al., 2008). The same happened in the 4-methoxyglucobrassicin content, described as a major indole GLs of red cabbage mature plants (Volden et al., 2008), but that only represented 6% of total GLs in red cabbage sprouts.

The Portuguese varieties, Galega kale and Penca cabbage, presented a similar GL profile, with exception for glucoraphanin that was not detected in Galega kale sprouts. Glucoraphanin is considered an important and desirable GL since sulphoraphane, the isothiocyanate (ITC) from glucoraphanin, is considered the most potent inducer of phase II enzymes (Fahey and Talalay, 1999; Bellostas et al., 2007a), representing one of the nutritional advantages of Penca cabbage sprouts. The Penca cabbage GS showed also a significantly ($p < 0.05$) higher glucoraphanin content (0.30 ± 0.04 mmol 100 g^{-1} d.w.) than the sprouts produced under darkness (0.06 ± 0.01 mmol 100 g^{-1} d.w.). The major GLs of the studied Portuguese varieties were sinigrin and glucoiberin, whose degradation products are prop-2-enyl ITC and iberin, respectively. These compounds are also inducers of phase II enzymes and have antiproliferative activity (Wallig et al., 1998; Canistro et al., 2004). Sinigrin and glucoiberin accounted respectively for 64% and 19% of the total GLs in Galega kale and 48% and 25% in Penca cabbage. Sprout production under light cycles resulted in an increased level of sinigrin in Galega kale sprouts, having WS showed losses of 33% of this compound. The same behavior was observed in relation to glucoiberin, since the absence of light resulted in sprouts with 39% less glucoiberin. The photoperiod effect in Penca cabbage sprouts was different having the absence of light resulted in 0.8% losses of sinigrin and in an increased level of glucoiberin (11%). The third most abundant GLs in these sprouts was 4-methoxyglucobrassicin, having the WS a significantly higher ($p < 0.05$) content than GS and accounting for 9% of the total GLs. The 4-methoxyglucobrassicin levels found represented less 30% than the proportion in Portuguese cabbage mature plants (Aires et al., 2012), nevertheless the content was higher than the related for cabbage sprouts (Kestwal et al., 2011).

The glucosinolate profile of broccoli GS sprouts showed glucoraphanin as the most prominent GL in both GS and WS broccoli sprouts (represented 48% of total GLs), which is in agreement with the results of most broccoli sprouts varieties (Charron et al., 2005;

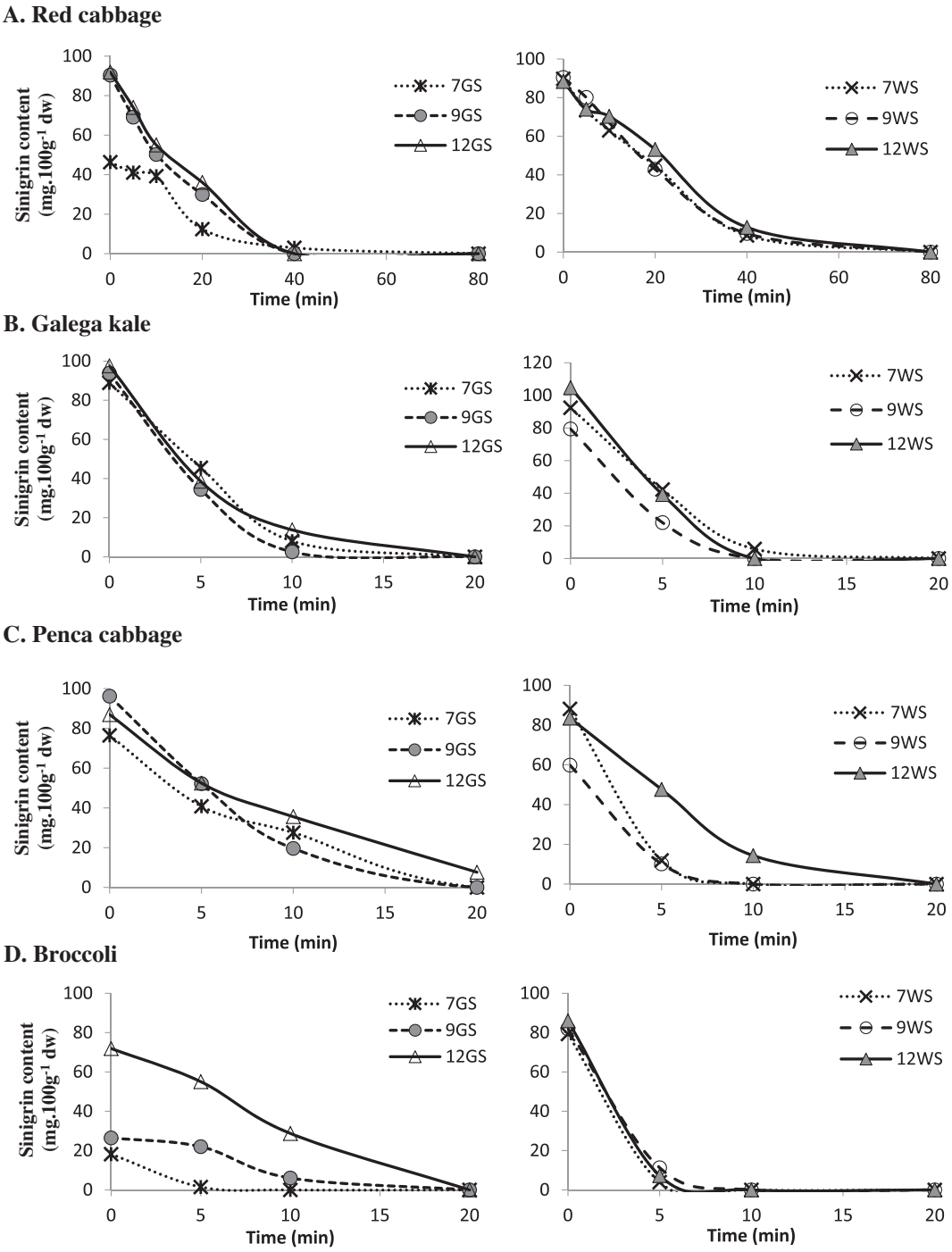

Fig. 4. Residual evolution of exogenous sinigrin during a maximum time of 80 min in red cabbage sprouts and 20 min in the other *Brassica* varieties. Sprouts with different sprouting times and different photoperiod were analyzed: 7GS – 7 days sprouting time under light, 7WS – 7 days sprouting time under darkness, 9GS – 9 days sprouting time under light, 9WS – 9 days sprouting time under darkness, 12GS – 12 days sprouting time under light, 12WS – 12 days sprouting time under darkness.

Tian et al., 2005). Broccoli sprouts are normally considered a better source of glucoraphanin than mature plants (Meyer and Adam, 2008), that showed as the most bioactive isothiocyanates, the sulforaphane (derived from glucoraphanin), the allyl isothiocyanate (derived from sinigrin) and indole-3-carbinol (derived from glucobrassicin) (Jones et al., 2006). In broccoli sprouts, in addition to glucoraphanin, the progoitrin (17%) and glucoiberin (13%) in GS, and glucoiberin (18%) and the 4-methoxyglucobrassicin (12%) in WS, were the major GLs presented. The absence light during sprouting had an effect on the GLs profile of broccoli sprouts, causing a 13% decreased in the progoitrin levels in WS. That effect was also seen in sinigrin levels, were it represented 4% to the total GLs in GS and 2% in WS. Regarding glucoraphanin, the same effect was observed, since the absence of light resulted in 23% decrease in the level of glucoraphanin, being GS a better source of the most potent inducer of the enzymes of phase II. The sprouts produced under darkness were significantly richer in glucoerucin and 4-methoxyglucobrasscin (more 5%) than the GS.

To better understand the influence of the photoperiod on the glucosinolate profile of each brassica variety, a multivariate analysis of the data was performed. The principal component analysis (PCA) was applied to each variety as a way to understand the

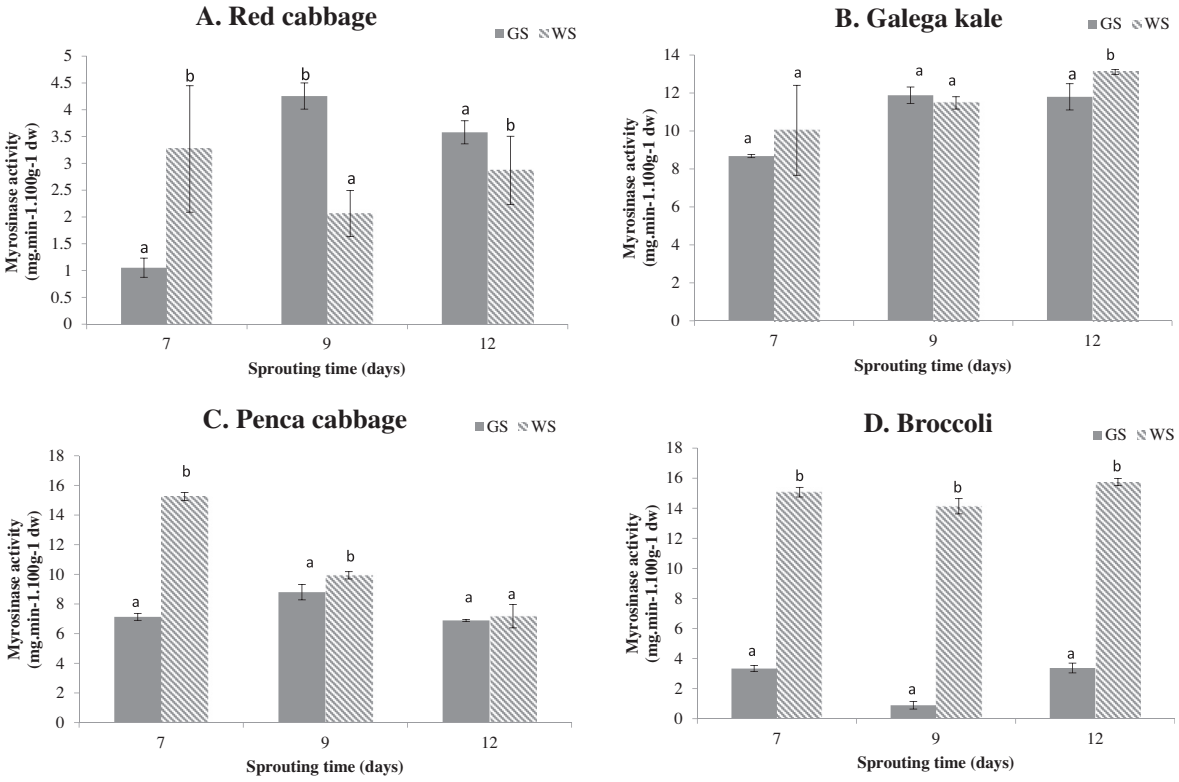

Fig. 5. Myrosinase activity (mg min^{-1} 100 g^{-1} d.w.), based on the residual evolution of exogenous sinigrin between zero and five minutes, in sprouts of four *Brassica* varieties. Abbreviations: GS – green spouts, WS – white sprouts. Differences between WS and GS with the same letters are non-significant ($p < 0.05$).

influence of light exposure during sprouting in GLs composition (Fig. 3). These analysis allowed for the representation of GS and WS in two different principal components that contain more than 80% of the total variance of the original variables. In red cabbage, broccoli and Penca cabbage, GS were mainly represented by component 2. Progoitrin, glucobrassicin and glucoraphanin in broccoli and Penca cabbage and progoitrin, glucobrassicin and 4-methoxyglucobrassicin in red cabbage, were the main differentiating GLs between GS and WS. Galega kale GLs results presented a different behavior, being the component 2 represented mainly WS, with 4-hydroxyglucobrassicin and neoglucobrassicin as the main GLs responsible for the differentiation between WS and GS.

3.2. Myrosinase activity

The results regarding the activity of myronase activity on brassica sprouts are presented in Figs. 4 and 5. Myrosinase activity of sprouts was monitored for 80 min, however no sinigrin was detected after 40 min of incubation, revealing that was completely hydrolyzed by myrosinase (see Fig. 4). Broccoli WS showed the most intense myrosinase activity, whilst red cabbage showed the lowest myrosinase activity, despite some enzymatic activity after 40 min of incubation. In broccoli sprouts almost all sinigrin was hydrolyzed after 5 min incubation in WS when compared to the results obtained in the other varieties (see Fig. 4D). Galega kale and Penca cabbage sprouts were similar ($p > 0.05$) on its capacity to breakdown sinigrin showing enzymatic activity in most GS until 10 min of incubation (Fig. 4B and C). Glucosinolates are essentially hydrolyzed by plant myrosinase in the small intestine and in the mouth, playing an essential role in the conversion of glucosinolates in humans. The inactivation of plant myrosinase will consequently affect the dietary absorption of bioactive compounds, therefore the preservation of plant myrosinase intact as much as possible represents a health advantage that comes from the consumption of *Brassica* vegetables (Aires et al., 2012). *Brassica* plants with high myrosinase activity can exhibit a high rate of natural GLs losses (Aires et al., 2012), thus sprouting conditions and handling should be optimize to improve the potential nutritional benefits of sprouts.

Most of the sprouts produced under darkness showed an intense myrosinase activity, with no exogenous sinigrin detected after 10 min incubation (Fig. 4B–D). Exceptions were registered in Red cabbage (Fig. 4A), Galega kale WS with 7 days sprouting and Penca cabbage with 12 days. In order to better understand the behavior of sprouts capacity to breakdown sinigrin, the lower common incubation time (5 min) was selected to evaluate the activity of myrosinase per minute (mg min^{-1} 100 g^{-1} d.w.) (Fig. 5). In general, the presence of light reduced myrosinase activity, since at 5 min incubation it was possible to verify that GS samples presented higher level of sinigrin as a result of lower myrosinase activity (see Fig. 5A, C and D). Besides light exposure, the sprouting time was also determinant for the capacity of sprouts to hydrolyze sinigrin. Young sprouts were more susceptible to losses of GLs since sprouts with 7 days showed lower levels of exogenous sinigrin, whilst the GLs degradation capacity tends to be reduced with sprouting time. Although it was observed that the majority of sprouts produced under darkness had higher myrosinase activity, red cabbage WS with 9 days sprouting where an exception. Broccoli GS were the most affected by light exposure showing an enzymatic activity 83% lower than the registered in WS (15 mg min^{-1} 100 g^{-1} d.w.) which may indicate also a higher rate of natural degradation of GLs in sprouts produced under dark conditions (Fig. 5D). Galega kale sprouts showed similar myrosinase activity between GS and WS, until the 12 days of sprouting, when WS revealed a higher activity. Penca cabbage revealed the opposite behavior, with the myrosinase activity decreasing over the sprouting time, especially in WS. Sprouts are normally consumed in a fresh state, preserving the myrosinase activity. In

this way, sprouts harvest with a lower myrosinase activity can preserve more their GL content. The 7 days sprouts grown under light can benefit from lower myrosinase activity in all varieties, except in broccoli since the lowest activity of the enzyme was found at 9 days sprouting (see Fig. 5). Nevertheless the myrosinase activity in broccoli under light was always very low relatively to the other varieties. Penca cabbage showed an intermediate myrosinase activity, being higher than in red cabbage sprouts and lower than in Galega kale sprouts. Intact glucosinolates have limited biological activity and their potency arises when glucosinolates comes into contact with myrosinase, therefore sprouts produced in darkness have high potential for breakdown glucosinolates fostering their biological activity. Nevertheless the handling of sprouts after harvesting should be done carefully in order to avoid disruption of the tissues and cells as it will contributed to higher losses in GLs content.

4. Conclusions

The glucosinolate content of brassica vegetables is recognized as one of their main nutritional advantages, gaining even more relevance in sprouts as they are consumed in a raw state, preserving their natural glucosinolate content. Within the studied sprouts varieties Galega kale stood out by their higher glucosinolate content, especially when sprouted under light and darkness cycles. Galega kale is a traditionally consumed brassica plant in Portugal and its sprouts showed to be an important source of aliphatic GLs. The consumption of Galega kale sprouts, with sprouting times between 7 and 9 days, can be a healthy component of the diet able to supply inducers of phase II enzymes. The preservation of these autochthonous varieties can be made by diversifying gastronomic recipes with introduction of sprouts as a healthy food and as an alternative to typical Galega kale. However, a special attention on handling sprouts is necessary since plant myrosinase should be kept intact as much as possible to optimize their health benefits. Myrosinase activity in Galega kale sprouts was very high when compared with the other varieties, only exceed by the one found in broccoli sprouts grown under dark conditions. However, sprouting under light/darkness cycles with shorter sprouting phases is recommended to promote a higher GL content, but it also requires that harvesting and handling until consumption must be performed with care to avoid GLs degradation due to the higher myrosinase activity of these sprouts. Sprouts produced under darkness, had a lower GL content and higher myrosinase activity that could compromise their GL content during shelf life due to the necessary handling procedures.

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