Radish sprouts – Characterization and elicitation of novel varieties rich in anthocyanins

Nieves Baenas, Federico Ferreres, Diego A. Moreno*, Cristina García-Viguera

CEBAS-CSIC, Phytochemistry Lab. Dept. of Food Science and Technology, P.O. Box 164, Espinardo, 30100, Murcia, Spain

*Corresponding author: Diego A. Moreno; Tel.: +34 968 396369 fax: +34 968 396213.
E-mail address: dmoreno@cebas.csic.es

Running title: Anthocyanins profiling by HPLC-DAD-ESI/MS^n in radish sprouts

Highlights

- *Raphanus sativus* edible sprouts rich in anthocyanins.
- Red radish anthocyanin profiling using HPLC-DAD-ESI/MS^n
- Elicitors enriched ready-to-eat sprouts in health-promoting anthocyanins
- Methyl jasmonate, glucose and sucrose were the most efficient elicitors.

Abstract

The anthocyanin profile of two varieties of red radish sprouts (*Raphanus sativus*), cv. China rose and Rambo, were studied using HPLC-DAD-ESI-MS^n and HPLC-DAD. The most abundant type of anthocyanins was cyanidin and its derivatives, with one or two acylated groups, with qualitative and quantitative differences among varieties. Some compounds were identified for the first time in both varieties, as we are concern. Radish sprouts were treated during germination (day 3 to 8) using methyl jasmonate, jasmonic acid, salicylic acid, sucrose and glucose as elicitors in order to enrich their total anthocyanins content (TAC). An increase in TAC was achieved by 50% in China rose radish sprouts and by 30% in Rambo red radish after glucose treatment. Methyl jasmonate and sucrose also contribute to enhance TAC. Enriching natural food in anthocyanins may contribute to sustain their regular intake with preventive and therapeutic roles in a number of human diseases.

Keywords: *Brassicaceae*, *Raphanus,* sprouts, elicitors, cyanidin.

1. Introduction

Promising results regarding nutrition and health benefits have been found when eating cruciferous sprouts containing significant greater concentrations of bioactive compounds (glucosinolates and phenolics) than mature plants (10-100 times) (Hanlon & Barnes, 2011; Moreno, Perez-Balibrea, & Garcia-Viguera, 2006). Even though cruciferous foods are recognized for their high content in glucosinolates, *Brassicaceae* foods are also rich in phenolic compounds (flavonols and anthocyanins), carotenoids, vitamins and minerals (Manchali, Chidambara Murthy, & Patil, 2012). Within the bioactive compounds classes, anthocyanins are water-soluble flavonoids that usually exist in plants in the form of glycosides, and their non-carbohydrate moieties (aglycones), called anthocyanidins. There are many types of anthocyanins, which are distinguished according to the number and position of the hydroxyl and methoxyl groups as substituent on the B ring, type and number of conjugated sugars, and the presence or absence of an acyl group. The six most important types are pelargonidin (Pg), cyanidin (Cy), delphinidin (Dp), peonidin (Pn), petunidin (Pt), and malvidin (Mv) (Jaakola, 2013). Cy and its derivatives, which possess two hydroxyl groups on the B-ring, are the most widely distributed, followed by Dp and its derivatives. They are not only responsible for red, blue and purple color of many fruits, vegetables, flowers and seeds, but also protect plants against various biotic and abiotic stresses (Harborne & Williams, 2000). In recent years, human intervention studies have focused on the preventive and suppressive effects of these compounds against obesity and diabetes, reducing inflammation associated with cancer pathogenesis, cardiovascular diseases, improvement of visual function, and the pro-effects of intake of anthocyanin-rich fruits on memory and on cognitive decline by delaying deterioration of neural function in aged individuals by inhibition of neuroinflammation (Pojer, Mattivi, Johnson, & Stockley, 2013).

The differences in the total anthocyanin content (TAC) among species are qualitative and quantitative, and also affected by environmental factors in different ways. Exogenous application of elicitors has been considered as a suitable strategy for the activation of secondary metabolites pathways in response to stress (Baenas, Garcia-Viguera, & Moreno, 2014a), the elicitors, methyl jasmonate (MeJA), jasmonic acid (JA), salicilyc acid (SA), sucrose and glucose have been selected as a successfully treatments for accumulation of anthocyanins.

In this work, two varieties of *Raphanus sativus* ready-to-eat sprouts (cv. China rose and Rambo), different in colour and visual appearance (white and rose hypocotyls and green cotyledons; and purple and deep red in hypocotyls and cotyledons, respectively), were selected in order to study their anthocyanin pigments, discussing their differences and investigating the potential for enrichment by elicitation the anthocyanin concentration, as natural healthy foods likely to be consumed by general population daily.

2. Material and methods

2.1. Plant material and germination conditions

China rose radish (*Raphanus sativus* var. *sativus*) and Rambo radish (*Raphanus sativus* cv. Rambo) seeds were provided by Intersemillas S.A (Valencia, Spain). Radish sprouts were grown according to Baenas, Garcia-Viguera, & Moreno, (2014b) with some modifications; sprouts were covered with perforated aluminum foil for increasing stem elongation in the environment chamber from day 0 to 3. Three replicates per treatment of radish sprouts were collected at day 8 after germination for analysis. All samples were frozen in liquid nitrogen, and stored at −80 ºC prior to analyses.

2.2. Treatments with elicitors

The phytohormones jasmonic acid (JA) (150 µM), methyl jasmonate (MeJA) (25 µM), salicylic acid (SA) (100 µM), and the oligosaccharides glucose (277 mM) and sucrose (176 mM) were selected as elicitors according to literature review (Baenas, Moreno, Garcia-Viguera, 2014a). JA (SIGMA-ALDRICH, Co., 3050 Spruce Street, St. Louis, MO. 63103, USA), MeJA (SAFC, 3050 Spruce Street, St. Louis, MO. 63103, USA), and SA (Panreac, S.A., Barcelona, Spain) were dissolved in 0.2 % ethanol in Milli-Q water. Sucrose and glucose (SIGMA CHEMICAL CO.14508, St. Louis, MO. 63178, USA) were also dissolved in Milli-Q water. Elicitors were applied as exogenous treatment (spraying) on the cotyledons with 30 mL of test solution per sample (10 mL per tray) from day 3 to day 7 of sprouting (5 days of treatment), using Milli-Q water as control.

2.3. Extraction and determination of anthocyanins

2.3.1. Sample extraction

Freeze-dried samples (100 mg) were extracted with 1.5 mL of methanol/water/formic acid (25:24:1, v/v/v), according to Moreno, Pérez-Balibrea, Ferreres, Gil-Izquierdo, & García-Viguera, (2010) with slight modifications. Briefly, samples were vortexed and extracted in an ultrasonic bath for 60 min at room temperature. The samples were kept at 4°C overnight and sonicated again for 60 min. A centrifugation (model EBA 21, Hettich Zentrifugen) step (9500 *xg*, 5 min) was used to separate the supernatant from the solid residue. This supernatant was filtered through a 0.22 µm (HPLC-DAD-ESI/MS^n) or 0.45 µm (HPLC-DAD) PVDF filter (Millex HV13, Millipore, Bedford, MA, USA) and stored at 4°C before the analyses were performed.

2.3.2. Identification of anthocyanins by HPLC-DAD-ESI-MS^n and quantification by HPLC-DAD

The chromatographic analyses with HPLC-DAD-ESI/MS^n for qualitative analysis were conducted as described by Moreno *et al*., (2010).

A HPLC-DAD system (Waters Cromatografía SA, Barcelona, Spain) was employed for the quantification, consisting of a W600E multisolvent delivery system, an in-line degasser, a W717Plus autosampler and a W2996 photodiode array detector set at 520nm. Anthocyanins were quantified using cyanidin 3-*O*-glucoside-β-glucopyranoside (Polyphenols, Norway) as external standard. Chromatograms were recorded at 520 nm. The retention time (Rt) of Tables 1 and 2 have different values than those of Table 3 and Figure 1 because the study of MS (Table 1 and 2) has been carried out in a different HPLC-equipment than the quantification UV study (Table 3 and Figure 1).

2.3.3. Statistical methods

All assays were conducted by triplicate. The data were processed using the SPSS 15.0 software package (LEAD Technologies, Inc., Chicago, USA). We carried out a multifactorial analysis of variance (ANOVA) and the Duncan's Multiple Range Test to determine significant differences at P values < 0.05.

3. Results and discussion

3.1. Qualitative and Quantitative Analysis of Anthocyanins

The identification of the anthocyanins was achieved by HPLC-DAD-ESI-MS^n analysis of the lyophilized radish sprouts extracts, according to our results, the most abundant anthocyanins were cyanidin derivatives, diglycosylated (dihexosydes) at C-3 and glycosylated (hexosides) at C-5 position, mainly with the presence of one or two cinnamoyl groups on the glycosylated fraction at 3 position (sinapoyl, feruloyl, *p*-coumaroyl, and caffeoyl) and malonyl at hexose in 5 position, according to the anthocyanins commonly described in *Brassicaceae*: cyanidin-3-*O*-sophoroside-5-*O*-glucoside derivatives (Andersen & Jordheim; 2006), with quantitative differences among species and crops (Park, *et al.*, 2014; Cartea, Francisco, Soengas, & Velasco, 2011; Giusti, Rodríguez-Saona, Griffin, & Wrolstad, 1999; Wu & Prior, 2005). Interpretation of mass spectra was based on previous observations that fragmentation of anthocyanins occurs almost exclusively at the glycosidic bonds, attached to hydroxyls, in 3 and/or 5 position, in addition to the possible loss of the carbonyl group (-44) or the malonyl radical (-86) (Giusti, *et al.*, 1999). Acylated groups were determined by calculating possible combinations of aliphatic and aromatic acids found in acylated anthocyanins (Wu & Prior, 2005).

Molecular ions of anthocyanins ($[M]^+$, *m/z*) and MS fragmentation are presented in tables 1 and 2 (tables have been prepared gathering compounds with similar structure and increasing Rt; the numbers assigned to compounds in Tables 1-2 are not comparable between them, being independent by variety).

The MS screening allowed the detection of 24 anthocyanins in China rose radish (Table 1) and 47 anthocyanins in Rambo red radish (Table 2) sprouts. A mass spectroscopic analysis is absolutely required for anthocyanin characterization, by the fact that compounds with similar UV spectral characteristics, can have similar retention time (Giusti, *et al.*, 1999). These pigments showed similar fragmentation patterns and their relative ion intensities according to their abundance are presented in Tables 1-2.

The anthocyanin composition of the varieties China rose and Rambo red radish sprouts are reported here for the first time. Some anthocyanins have been identified for the first time while others have been reported before in Sango red radish sprouts (Matera, *et al.*, 2012).

Radish cv. China rose showed only acylated anthocyanins: cinnamoyl, malonyl and cinnamoyl malonyl derivatives (Table 1). The glycosylation loss from C-5 was 162 [glycosyl]$^+$ (**5**, **6**, **11**) or 248 (162+86) [glycosyl-malonyl]$^+$ (**1-4**, **7-10**, **12-24**) to give rise the anthocyanidin ion bond to the glycosidic fraction in 3-position. Moreover, a diglucosyl loss (324) (**1**) with their corresponding cinnamoyl acid ([diglucosyl-acyl]$^+$) (**2-4**, **8**, **9, 10**, **12-15** and **20**) or [diglucosyl-acyl1-acyl2]$^+$ (**7**, **10**, **15-19** and **21-24**) was observed, giving rise to the anthocyanidin ion bonded to the glycosidic fraction in 5-position (*m/z* 535/519 in the malonyl derivatives, and 449/433 in the non malonated derivatives) (Table 1). Some cyanidin derivatives found were similar and coincident with previously published data on anthocyanins in *Brassicaceae* species (Matera, *et al*., 2012; Park, *et al*., 2014), nonetheless, we found and tentatively identified some new anthocyanins displayed [M]$^+$ at *m/z* 963 (Pelargonidin 3-*O*-(sinapoyl)sophoroside-5-*O*-glucoside) (**11**), 1065 (Cyanidin 3-*O*-(sinapoyl)sophoroside-5-*O*-(malonyl)glucoside) (**9** and **14**), 1227 (Cyanidin 3-*O*-(caffeoyl, sinapoyl)sophoroside-5-*O*-(malonyl)glucoside) (**7**, **15** and **22**), and 1271 (Cyanidin-3-*O*-(disinapoyl)sophoroside-5-*O*-(malonyl)glucoside) (**16**).

Red radish cv. Rambo sprouts exhibited a wide range of anthocyanins, which have been detected as cyanidin, being the predominant aglycone, and also peonidin and delphinidin in this cultivar. A particularity with respect to those described above is that several anthocyanins have been detected whose glycosylation on 5 position is dihexoside instead of glucoside that tentatively have been considered as sophoroside (**3, 11, 12, 18, 19, 23, 25, 26, 28, 29-33, 39, 40** and **44**), the fragmentation is similar to that described above. We observed in the malonyl-sophorosides (**11, 12, 18, 19, 23, 25, 28, 31, 32, 39, 40, 44**, except for **3**) the loss of the m/z 410 (324+86) due to fragmentation of the glycosydic fraction in 5-position ([diglucosyl-malonyl]$^-$), instead of the m/z 248 (162+86) ([glucosylmalonyl]$^{-}$) found in the malonyl-glucosides derivatives. We identified for the first time in red radish the following anthocyanins: The [M]$^{+}$ at *m/z* 757 (Pelargonidin-3-*O*-sophoroside-5-*O*-glucoside) (**2**), 859 (Cyanidin 3-*O*-sophoroside-5-*O*-(malonyl)glucoside) (**6**), 873 (Peonidin-3-*O*-sophoroside-5-*O*-(malonyl)glucoside) (**8**), 1065 (Cyanidin 3-*O*-(sinapoyl)sophoroside-5-*O*-(malonyl)glucoside) (**13**, **20** and **37**), 1181 (Cyanidin 3-*O*-(*p*-coumaryl,feruoyl)sophoroside-5-*O*-(malonyl)glucoside) (**46**), 1255 (Peonidin 3-*O*-(feruoyl,sinapoyl)sophoroside-5-*O*-(malonyl)glucoside) (**47**), 1227 (Cyanidin 3-*O*-(sinapoyl)sophoroside-5-*O*-(malonyl)sophoroside) (**11** and **18**), 1183 (Cyanidin 3-*O*-(caffeoyl)sophoroside-5-*O*-(malonyl)sophoroside) (**23**), 1167 (Cyanidin 3-*O*-(*p*-coumaric)sophoroside-5-*O*-(malonyl)sophoroside) (**31**), 1389 (Cyanidin 3-*O*-(caffeoyl, sinapoyl)sophoroside-5-*O*-(malonyl)sophoroside) (**25**) and 1359 (Cyanidin 3-*O*-(caffeoyl, feruoyl)sophoroside-5-*O*-(malonyl)sophoroside) (**28**) presented in Table 2.

Few published works showed that the characterization of anthocyanins in radish was dependent on the studied variety (Giusti & Wrolstad, 2003; Hanlon & Barnes, 2011). Hanlon and Barnes, (2011) showed a quantification of anthocyanins by classes (pelargonidin, cyanidin and delphinidin) in 8 different varieties of *Raphanus sativus* sprouts, finding large differences between them. Several research groups (Giusti & Wrolstad, 2003; Park, *et al.*, 2013; Wu & Prior, 2005) also found that the major anthocyanins in radish sprouts are acylated pelargonidins, such as Daikon cultivar (De Nicola, *et al.*, 2013), while others reported the isolation of cyanidin-based pigments in red radish (*R. sativus* L. var. Benikanmi) (Tatsuzawa, *et al.*, 2010), Purple Bordeaux radish (Lin, *et al.*, 2011), and radish cv. Sango sprouts (Matera, *et al.*, 2012).

The anthocyanins tentatively identified were then quantified in HPLC-DAD (Figure 1) by comparing their retention times and spectra to those of compounds found in the mass spectra experiments, using peak spectral characteristic and the absorption at 520nm.

The total anthocyanin content (TAC) on China rose radish sprouts was 15.8 mg·100g Fresh weight (F.W.), and in the red radish sprouts was >10 fold more (180 mg·100g F.W.) (Figure 2). China rose radish showed its most abundant anthocyanin at minute 32.6, comprising the elution of three compounds with $[M]^+$ at *m/z* 1005 (**12**, Cyanidin 3-*O*-(*p*-coumaroyl)sophoroside-5-*O*-(malonyl)glucoside), 1035 (**13**, Cyanidin 3-*O*-(feruloyl)sophoroside-5-*O*-(malonyl)glucoside), 1065 (**14**, Cyanidin 3-*O*-(sinapoyl)sophoroside-5-*O*-(malonyl)glucoside) (Table 4a), and representing 7.4 mg·100g F.W. from the total (15.8 mg·100g F.W.); (Figure 2). These anthocyanins presented three different aromatic groups (*p*-coumaroyl, feruloyl and sinapoyl) in C-3 diglycosidic substituent while one aliphatic group (malonic acid) in sugar of C5, as have been described before in red cabbage (Park, *et al.,* 2014) and Sango radish sprouts (Matera, *et al.,* 2012). The relevant anthocyanins in Rambo red radish sprouts showed $[M]^+$ at *m/z* 1065 (**37**, Cyanidin 3-*O*-(sinapoyl)sophoroside-5-*O*-(malonyl)glucoside) and 1035 (**38**, Cyanidin 3-*O*-(feruoyl)sophoroside-5-*O*-(malonyl)glucoside) (Rt 32.5) (Table 4c), representing almost 30% of the total anthocyanins (181.5 mg·100g F.W.) (Figure 2). Also compounds with $[M]^+$ at *m/z* 1005 (**35**, Cyanidin 3-*O*-(*p*-coumaryl)sophoroside-5-*O*-(malonyl)glucoside) and 1155 (**36**, Cyanidin 3-*O*-(feruloyl, sinapoyl)sophoroside-5-*O*-glucoside) (co-eluting at Rt 32.0); 1373 (**40**, Cyanidin 3-*O*-(*p*-coumaric, sinapoyl)sophoroside-5-*O*-(malonyl)sophoroside) and 1241 (**41**, Cyanidin 3-*O*-(feruoyl, sinapoyl)sophoroside-5-*O*-(malonyl)glucoside) were abundant in this sample, representing each one a 14% from the total amount of anthocyanins.

Compared to other plants studied for their TAC, China rose radish sprouts might be comparable to the values found in strawberry (19 - 55 mg·100g F.W.), plum (10 - 25 mg·100g F.W.) and gooseberry (2 - 40 mg·100g F.W.), while Rambo red radish was found comparable to red cabbage (50 - 300 mg·100g F.W.) (Zabaras, *et al.,* 2013), black currant (130 - 476 mg·100g F.W.), and blackberry (83 - 326 mg·100g F.W.) (De Pascual-Teresa & Sanchez-Ballesta, 2008). Anthocyanin compounds have interesting biological activities connected to cancer prevention, oxidative damage and cardiovascular protection (Pojer, *et al.*, 2013). The results obtained in this work showed that radish sprouts are rich sources of anthocyanins, especially in the red radish Rambo variety.

3.2. Elicitors Enhance Anthocyanin Content in Radish Sprouts

The roles of spray treatments of elicitors as appropriate tool for enhance production of anthocyanins in radish sprouts was studied in this work. The effects were determined in 8-days-old sprouts, after exposure to elicitors for 5-days.

The signalling molecules salicylic acid (SA), methyl jasmonate (MeJA) and jasmonic acid (JA) play an important role in plant defense signal transduction pathways, through expression of defense related genes, leading to the biosynthesis of secondary metabolites from the stimulation of the phenylpropanoids pathway (Tovar, Romero, Girona, & Motilva, 2002). MeJA elicitor (25 µM) showed higher effects in radish sprouts, increasing by 23% and 11% the TAC in China rose (19.45 mg·100g F.W.) and Rambo radish sprouts (201.4 mg·100g F.W.), respectively. By contrast, TAC in radish sprouts were no affected by JA elicitor (Figure 2), however, SA treatment increased the TAC by 21% and 7% in China rose and Rambo radish, respectively. The activity of MeJA as up-regulator of PAL was determined by Kim, Park, & Lim (2011), who showed that MeJA-treated buckwheat sprouts had about twice as high as activity that of the control, with an increase of total phenolic compounds. Few results have been found about phytohormone treatments over *Brassicaceae* species, such as the study done by Park, *et al.*, (2013), where the mRNA transcript levels of genes involved in anthocyanin biosynthesis (RsMYB) were higher in MeJA treated radish sprouts than in the untreated control.

Glucose (277mM) and sucrose (176mM) effectively enhanced TAC in China rose radish sprouts, by 57 and 20%, and, in red radish sprouts, by 33 and 8%, respectively (Figure 2). In previous studies, sugar-regulated plant secondary metabolite production was observed in broccoli sprouts treated with 88 and 176mM of sucrose which increased total anthocyanins by 26 and 44%, respectively (Guo, Yuan & Wang, 2011a), being the transcription level of PAL treated by sucrose much higher than control (Guo, Yuan & Wang, 2011b). Hara, Oki, Hoshino, & Kuboi, (2004) found an induction of expression of the chalcone synthase gene and the dihydroflavonol reductase and anthocyanin synthase gene by sucrose treatment, increasing as the TAC (7-fold) in red radish sprouts (cv. Comet) after 6 days of sucrose optimized treatment (175mM). Wei *et al.*, (2011), observed an increased TAC by 101%, 120%, and 83% in radish, Chinese kale and pak choi sprouts, respectively, after a 5% glucose solution treatment. Sugars are an important source of energy and carbon for plant development (Loreti, *et al.*, 2008). In addition, the mechanism of sucrose and glucose specific induction of anthocyanin biosynthesis gene expression was demonstrated in *Arabidopsis* seedlings (Solfanelli, *et al.*, 2006).

4. Conclusions

The results supported the hypothesis that anthocyanin synthesis may allow the plant to develop resistance to a number of elicitor treatments by stimulation of the PAL pathway. All individual anthocyanins identified were increased by elicitor treatments, leading to the observed increase of TAC. Sugars elicitors were considered the most effective elicitors. The selection of ready-to-eat cruciferous sprouts rich in anthocyanins, as well as the appropriate elicitor treatment is a candidate strategy to develop novel plant foods with beneficial nutritional and health properties.

ACKNOWLEDGEMENTS

This work was supported by the Spanish Ministerio de Economía y Competitividad (MINECO) CICYT (AGL2012-40175-C02-01) and by the Seneca Foundation-Regional Agency for Science and Technology of the Murcia Region (CARM; Project Ref. 08753/PI/08, and the Excellence in research Grant 04486/GERM/06). N. Baenas was funded by a FPU (Formación Profesorado Universitario) grant of the Fellowship Programme from the Spanish Ministry of Education.

REFERENCES

Andersen, O. M. & Jordheim, M. (2006). The anthocyanins. In Andersen, O.M., Markham, K. (Eds.), Flavonoids: Chemistry, biochemistry and applications. (pp. 471-551). Taylor & Francis, Boca Raton (USA).

Baenas, N., Garcia-Viguera, C. & Moreno, D. A. (2014a). Elicitation: A tool for enriching the bioactive composition of foods. *Molecules, 19*(9), 13541-13563.

Baenas, N., Garcia-Viguera, C. & Moreno, D. A. (2014b). Biotic elicitors effectively increase the glucosinolates content in *Brassicaceae* sprouts. *Journal of Agricultural and Food Chemistry*, *62*(8), 1881-1889.

Cartea, M. E., Francisco, M., Soengas, P. & Velasco, P. (2011). Phenolic compounds in *Brassica* vegetables. *Molecules*, *16*, 251-280.

Cominelli, E., Gusmaroli, G., Allegra, D., Galbiati, M., Wade, H. K., Jenkins, G. I. & Tonelli, C. (2008). Expression analysis of anthocyanin regulatory genes in response to different light qualities in *Arabidopsis thaliana*. *Journal of Plant Physiology*, 165, 886-894.

De Nicola, G. R., Bagatta, M., Pagnotta, E., Angelino, D., Gennari, L., Ninfali, P., Rollin, P. & Iori, R. (2013). Comparison of bioactive phytochemical content and release of isothiocyanates in selected brassica sprouts. *Food Chemistry*, *141*, 297-303.

De Pascual-Teresa, S. & Sanchez-Ballesta, M. T. (2008). Anthocyanins: from plant to health. *Phytochemistry Reviews*, *7*, 281-299.

Giusti, M. M., Rodríguez-Saona, L. E., Griffin, D. & Wrolstad, R. E. (1999). Electrospray and tandem mass spectroscopy as tools for anthocyanin characterization. *Journal of Agricultural and Food Chemistry*, *47*, 4657-4664.

Giusti, M. M. & Wrolstad, R. E. (2003). Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal*, *14*, 217-225.

Guo, R., Yuan, G. & Wang, Q. (2011a). Sucrose enhances the accumulation of anthocyanins and glucosinolates in broccoli sprouts. *Food Chemistry*, *129*, 1080-1087.

Guo, R., Yuan, G. & Wang, Q. (2011b). Effect of sucrose and mannitol on the accumulation of health-promoting compounds and the activity of metabolic enzymes in broccoli sprouts. *Scientia Horticulturae*, *128*, 159-165.

Hanlon, P. R. & Barnes, D. M. (2011). Phytochemical composition and biological activity of 8 varieties of radish (*Raphanus sativus* L.) sprouts and mature taproots. *Journal of Food Science*, *76*, C185-C192.

Hara, M., Oki, K., Hoshino, K. & Kuboi, T. (2004). Effects of sucrose on anthocyanin production in hypocotyl of two radish (*Raphanus sativus*) varieties. *Plant Biotechnology Journal*, *21*, 401-405.

Harborne, J. B. & Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry*, *55*, 481-504.

Kim, H. J., Park, K. J. & Lim, J. H. (2011). Metabolomic analysis of phenolic compounds in buckwheat (*Fagopyrum esculentum* M.) sprouts treated with methyl jasmonate. *Journal of Agricultural and Food Chemistry, 59*, 5707-5713.

Lin, L. Z., Sun, J., Chen, P. & Harnly, J. A. (2011). LC-PDA-ESI/MS(n) identification of new anthocyanins in purple Bordeaux radish (*Raphanus sativus* L. variety). *Journal of Agricultural and Food Chemistry*, *59*, 6616-6627.

Loreti, E., Povero, G., Novi, G., Solfanelli, C., Alpi, A. & Perata, P. (2008). Gibberellins, jasmonate and abscisic acid modulate the sucrose-induced expression of anthocyanin biosynthetic genes in *Arabidopsis*. *New Phytologist*, *179*, 1004-1016.

Manchali, S., Chidambara Murthy, K. N. & Patil, B. S. (2012) Crucial facts about health benefits of popular cruciferous vegetables. *Journal of Functional Foods*, *4*, 94-106.

Matera, R., Gabbanini, S., De Nicola, G. R., Iori, R., Petrillo, G. & Valgimigli, L. (2012). Identification and analysis of isothiocyanates and new acylated anthocyanins in the juice of *Raphanus sativus* cv. Sango sprouts. *Food Chemistry*, *133*, 563-572.

Moreno, D. A., Pérez-Balibrea, S. & García-Viguera, C. (2006). Phytochemical quality and bioactiviy of edible sprouts. *Natural Product Communications*, *1*, 1037-1048.

Moreno, D. A., Pérez-Balibrea, S., Ferreres, F., Gil-Izquierdo, A. & García-Viguera, C. (2010). Acylated anthocyanins in broccoli sprouts. *Food Chemistry*, *123*, 358-363.

Park, W. T., Kim, Y. B., Seo, J. M., Kim, S. J., Chung, E., Lee, J. H. & Park, S. U. (2013). Accumulation of anthocyanin and associated gene expression in radish sprouts exposed to light and methyl jasmonate. *Journal of Agricultural and Food Chemistry*, *61*, 4127-4132.

Park, S., Valan Arasu, M., Lee, M.-K., Chun, J.-H., Seo, J. M., Lee, S.-W., Al-Dhabi, N. A. & Kim, S.-J. (2014). Quantification of glucosinolates, anthocyanins, free amino acids, and vitamin C in inbred lines of cabbage (*Brassica oleracea* L.). *Food Chemistry*, *145*, 77-85.

Pojer, E., Mattivi, F., Johnson, D. & Stockley, C. S. (2013). The case for anthocyanin consumption to promote human health: A review. *Comprehensive Reviews in Food Science and Food Safety*, *12*, 483-508.

Solfanelli, C., Poggi, A., Loreti, E., Alpi, A. & Perata, P. (2006). Sucrose-specific induction of the anthocyanin biosynthetic pathway in Arabidopsis. Plant Physiology, *140*, 637-646.

Tatsuzawa, F., Saito, N., Toki, K., Shinoda, K., Shigihara, A. & Honda, T. (2010). Acylated cyanidin 3-sophoroside-5-glucosides from the purple roots of red radish (*Raphanus sativus* L.). *Journal of the Japanese Society for Horticultural*, *79*, 103-107.

Tovar, M. J., Romero, M. P., Girona, J. & Motilva, M. J. (2002). L-Phenylalanine ammonia-lyase activity and concentration of phenolics in developing olive (*Olea europaea* L cv Arbequina) fruit grown under different irrigation regimes. *Journal of the Science of Food and Agriculture*, *82*, 892-898.

Wie, J., Miao, H.Y., Wang, Q.M. (2011). Effect of glucose on glucosinolates, antioxidants and metabolic enzymes in Brassica sprouts. *Scientia Horticulturae, 129*, 535–540.

Wu, X. & Prior, R. L. (2005). Identification and characterization of anthocyanins by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry in common foods in the United States: vegetables, nuts, and grains. *Journal of Agricultural and Food Chemistry, 53*, 3101-13.

Zabaras, D., Roohani, M., Krishnamurthy, R., Cochet, M. & Delahunty, C. M. (2013). Characterisation of taste-active extracts from raw *Brassica oleracea* vegetables. *Food & Function*, *4*, 592-601.

FIGURE CAPTION

Figure 1. Chromatogram profile (520 nm) by HPLC-DAD of quantified anthocyanin in the radish species under study. (legend of compounds)

Figure 2. Total anthocyanin contents (TAC) in radish sprouts after elicitor treatments.

Figure 1. Chromatogram profile (520 nm) by HPLC-DAD of quantified anthocyanin in the radish species under study. (legend of compounds)

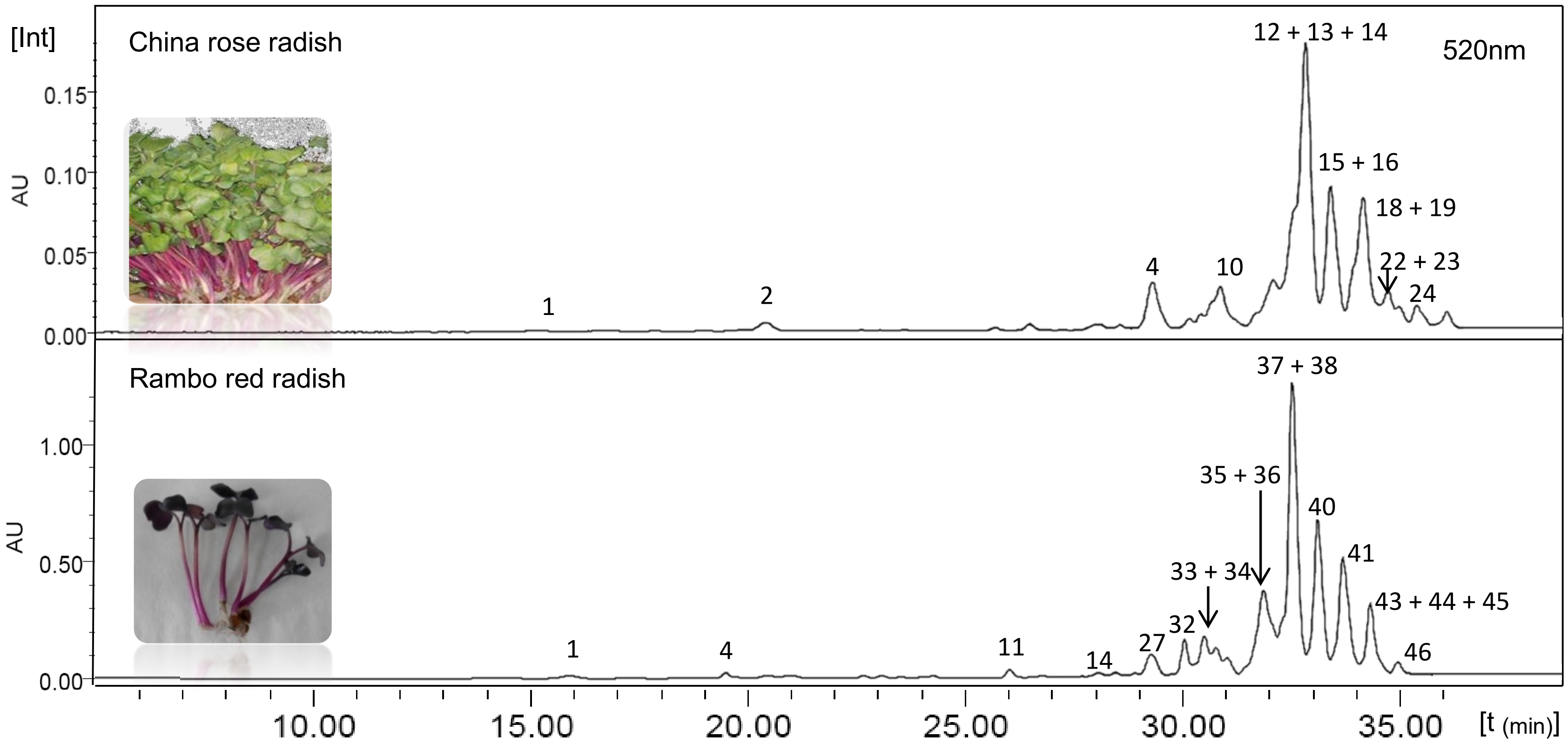

Figure 2. Total anthocyanin contents (TAC) in radish sprouts after elicitor treatments.

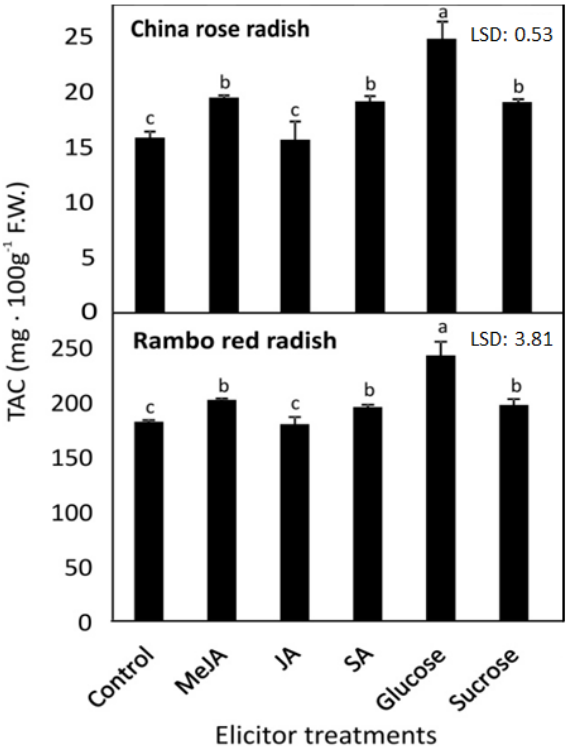

Table 1. Anthocyanins in China rose radish sprouts.[1]

Anthocyanin 3-*O*-(cinnamoyl)sophoroside-5-*O*-glucoside derivatives									
			MS2 $[M]^+$, *m/z* (%)					MS3 $[M-162]^+$, *m/z* (%)	Compound
Peak	Rt (min)	$[M]^+$*m/z*	-162			-(324 + acyl)	Aglc		
5	26.8	979	817 (100)			449 (7)	287 (17)	287 (100)	Cyanidin 3-*O*-(sinapoyl)sophoroside-5-*O*-glucoside
6	26.8	949	787 (100)			449 (20)	287 (21)	287 (100)	Cyanidin 3-*O*-(feruloyl)sophoroside-5-*O*-glucoside
11	28.4	963	801 (100)			433 (7)	271 (8)	271 (100)	Pelargonidin 3-*O*-(sinapoyl)sophoroside-5-*O*-glucoside
Anthocyanin 3-*O*-sophoroside-5-*O*-(malonyl)glucoside derivatives									
								MS3 $[M-324]^+$, *m/z* (%)	
			-44	-248	-324				
1	17.2	859	-	611 (15)	535 (100)	449 (16)	287 (49)	449 (16), 490 (13), 287 (70)	Cyanidin 3-*O*-sophoroside-5-*O*-(malonyl)glucoside
Anthocyanin 3-*O*-(cinnamoyl)sophoroside-5-*O*-(malonyl)glucoside/sophoroside derivatives									
								MS3$[535/519]^+$, *m/z* (%)	
					-(324 + acyl)	-(324+acyl+44)			
2	24.0	1021	977 (20)	773 (68)	535 (100)	491 (15)	287 (28)	287 (100)	Cyanidin 3-*O*-sophoroside-5-*O*-(malonyl)sophoroside
3	24.9	1035	991 (48)	787 (67)	535 (100)	491 (46)	287 (7)	287 (100)	Cyanidin 3-*O*-(feruloyl)sophoroside-5-*O*-(malonyl)glucosid
4	26.0	1021	977 (20)	773 (93)	535 (100)	491(31)	287 (17)	517 (29), 449 (22), 287 (100)	Cyanidin 3-*O*-(caffeoyl)sophoroside-5-*O*-(malonyl)glucosid
8	27.6	1035	991 (8)	787 (76)	535 (100)	491 (20)	287 (46)	449 (55), 287 (100)	Cyanidin 3-*O*-(feruloyl)sophoroside-5-*O*-(malonyl)glucosid
9	27.6	1065	1021 (5)	817 (73)	535 (100)	491 (17)	-	287 (100)	Cyanidin 3-*O*-(sinapoyl)sophoroside-5-*O*-(malonyl)glucosid
12	29.2	1005	961 (15)	757 (52)	535 (100)	491 (24)	287 (36)	287 (100)	Cyanidin 3-*O*-(*p*-coumaroyl)sophoroside-5-*O*-(malonyl)glucoside
13	29.4	1035	991 (7)	787 (43)	535 (100)	491 (10)	287 (11)	287 (100)	Cyanidin 3-*O*-(feruloyl)sophoroside-5-*O*-(malonyl)glucosid
14	29.3	1065	1021 (8)	817 (71)	535 (100)	491 (1)	-	287 (100)	Cyanidin 3-*O*-(sinapoyl)sophoroside-5-O-(malonyl)glucosid
20	31.2	1019	975 (13)	771(68)	519 (100)	475(15)	-	433 (100), 271(52)	Pelargonidin-3-*O*-(feruloyl)sophoroside-5-*O*-(malonyl)glucoside

								MS3 [M-248]$^+$, *m/z* (%)	
					-(324+acyl1)	-(324+acyl1 +acyl2)	[535-44]$^-$		
7	27.6	1227	1183 (2)	979 (100)	-	535 (39)	-	797 (13), 703 (14), 287 (100)	Cyanidin 3-*O*-(caffeoyl, sinapoyl)sophoroside-5-*O*-(malonyl)glucoside
10	28.3	1197	1153 (18)	949 (100)	697 (49)	535 (43)	491 (14)	287 (100)	Cyanidin 3-*O*-(caffeoyl, feruloyl)sophoroside-5-*O*-(malonyl)glucoside
15	30.0	1227	1183 (12)	979 (100)	679 (6)	535 (90)	491 (8)	662 (6), 287 (100)	Cyanidin 3-*O*-(caffeoyl, sinapoyl)sophoroside-5-*O*-(malonyl)glucoside
17	30.3	1197	1153 (17)	949 (100)	-	535 (53)	491 (16)	287 (100)	Cyanidin 3-*O*-(caffeoyl, feruloyl)sophoroside-5-*O*-(malonyl)glucoside
22	31.9	1227	1183 (23)	979 (100)	-	535 (90)	491 (27)	491(100), 287 (81)	Cyanidin 3-*O*-(caffeoyl, sinapoyl)sophoroside-5-*O*-(malonyl)glucoside
23	31.9	1197	1153 (14)	949 (74)	-	535 (100)	-	287 (100)	Cyanidin 3-*O*-(caffeoyl, feruloyl)sophoroside-5-*O*-(malonyl)glucoside
								MS3 [535]$^+$, *m/z* (%)	
16	30.3	1271	1227 (8)	1023 (88)	-	535 (100)	491 (11)	287 (100)	Cyanidin 3-*O*-(disinapoyl)sophoroside-5-*O*-(malonyl)glucoside
18	30.7	1241	1197 (11)	993 (66)	-	535 (100)	-	287 (100)	Cyanidin 3-*O*-(sinapoyl,feruloyl)sophoroside-5-*O*-(malonyl)glucoside
19	31.1	1241	1197 (12)	993 (65)	-	535 (100)	-	287 (100)	Cyanidin 3-*O*-(sinapoyl,feruloyl)sophoroside-5-*O*-(malonyl)glucoside
21	31.5	1211	-	-	963 (100)	535 (43)	491 (14)	287 (100)	Cyanidin 3-*O*-(*p*-coumaroyl, sinapoyl)sophoroside-5-*O*-(malonyl)glucoside or Cyanidin 3-*O*-(di-feruloyl)sophoroside-5-*O*-(malonyl)glucoside
24	32.5	1211	1167 (11)	-	963 (37)	535 (100)	491 (9)	287 (100)	Cyanidin 3-*O*-(*p*-coumaroyl, sinapoyl)sophoroside-5-*O*-(malonyl)glucoside or Cyanidin 3-*O*-(di-feruloyl)sophoroside-5-*O*-(malonyl)glucoside

[1] Main observed fragments. Other ions were found but they have not been included.

Table 2. Anthocyanins in Rambo red radish sprouts.[1]

			MS2 $[M]^+$(m/z)			MS3 $[M-162]^+$(m/z)	Compound
Peak	Rt (min)	$[M]^+$(m/z)	-162	-324	Aglc		
							Non-acylated anthocyanins
1	12.1	773	611 (58)	449 (100)	287 (89)	287 (100)	Cyanidin 3-*O*-sophoroside-5-*O*-glucoside
2	13.5	757	595 (100)	433 (53)	271 (39)	271(100)	Pelargonidin-3-O-sophoroside-5-O-glucoside
7	18.9	773	611 (100)	-	303 (11)	465 (100), 303 (11)	Delphinidin- 3- rutinoside-5-glucoside
17	22.5	611	449 (100)	-	303 (31)	368 (5), 303 (100)	Delphinidin- 3- rutinoside
							Anthocyanin 3-*O*-sophoroside-5-*O*-(malonyl)glucoside/sophoroside derivatives
3	16.2	1021	-	697 (100)	287 (6)	287 (100)	Cyanidin 3-*O*-sophoroside-5-*O*-(malonyl)sophoroside
6	17.0	859	-	535 (100)	287 (21)	287 (100)	Cyanidin-3-*O*-(caffeoyl)sophoroside-5-*O*-(malonyl)glucoside
8	19.2	873	-	549 (100)	-	301 (100)	Peonidin-3-*O*-sophoroside-5-*O*-(malonyl)glucoside
							Anthocyanin 3-*O*-(cinnamoyl)glucoside derivatives
10	19.8	611	465 (100)	-	303 (35)	303 (100)	Delphinidin-3-*O*-(*p*-coumaroyl)glucoside
							Anthocyanin 3-*O*-(cinnamoyl)sophoroside-5-*O*-glucoside/sophoroside derivatives
				-(324 + acyl)			
4	16.6	979	817 (100)	449 (70)	287 (29)	287 (100)	Cyanidin 3-*O*-(sinapoyl)sophoroside-5-*O*-glucoside
5	17.0	949	787 (100)	449 (58)	287 (20)	287 (100)	Cyanidin 3-*O*-(feruloyl)sophoroside-5-*O*-glucoside
9	19.5	993	831 (100)	463 (93)	301 (45)	301 (100)	Peonidin-3-*O*-(sinapoyl)sophoroside -5-*O*-glucoside
14	20.6	979	817 (100)	449 (46)	287 (34)	287 (100)	Cyanidin 3-*O*-(sinapoyl)sophoroside-5-*O*-glucoside
15	20.7	949	787 (100)	449 (71)	287 (37)	287 (100)	Cyanidin 3-*O*-(feruloyl)sophoroside-5-*O*-glucoside
				-(324+acyl1+acyl2)			
22	25.2	1141	979 (100)		-	773 (100), 449 (16), 287 (38)	Cyanidin-3-*O*-(caffeoyl, sinapoyl)sophoroside -5-*O*-glucoside
27	27.5	1155	993 (100)	449 (4)	-	678 (25), 287 (100)	Cyanidin-3-*O*-(feruloyl, sinapoyl)sophoroside -5-*O*-glucoside
36	28.9	1155	993 (100)	449 (5)	-	678 (31), 287 (100)	Cyanidin-3-*O*-(feruloyl sinapoyl)sophoroside -5-*O*-glucoside

			-324			MS3 [M-324]$^{+}$, *m/z* (%)	
26	27.0	1317	993 (100)	611 (2)	-	287 (100)	Cyanidin-3-*O*-(feruloyl, sinapoyl)sophoroside-5-*O*-sophoroside
29	27.5	1287	963 (100)	-	-	287 (100)	Cyanidin-3-*O*-(di-feruloyl)sophoroside-5-*O*-sophoroside *or* Cyanidin-3-O-(*p*-coumaroyl, feruloyl)sophoroside -5-*O*-sophoroside
30	27.7	1287	963 (100)	611 (27)	-	287 (100)	Cyanidin-3-*O*-(di-feruloyl)sophoroside-5-*O*-sophoroside *or* Cyanidin-3-O-(*p*-coumaroyl, feruloyl)sophoroside-5-*O*-sophoroside
33	28.5	1317	993 (100)	611 (7)	-	287 (100)	Cyanidin-3-*O*-(feruloyl, sinapoyl)sophoroside-5-*O*-sophoroside

Anthocyanin 3-*O*-(cinnamoyl)sophoroside-5-*O*-(malonyl)glucoside/sophoroside derivatives

			-44	-248	-(324 + acyl1)	MS2 [535-44]$^{+}$	Aglc	MS3 [M-(324+acyl)]+m/z (%)	
13	20.4	1065	-	817 (7)	535 (100)	-	-	287 (100)	Cyanidin-3-*O*-(sinapoyl)sophoroside-5-*O*-(malonyl)glucoside
16	21.0	1035	-	787 (26)	535 (100)	491 (20)	-	491 (10), 287 (100)	Cyanidin-3-*O*-(feruoyl)sophoroside-5-*O*-(malonyl)glucoside
20	24.0	1065	-	817 (53)	535 (100)	491 (9)	-	491 (7),287 (100)	Cyanidin-3-*O*-(sinapoyl)sophoroside-5-*O*-(malonyl)glucoside
21	24.0	1035	-	787 (84)	535 (100)	491 (22)	287 (12)	491 (10), 287 (100)	Cyanidin-3-*O*-(feruoyl)sophoroside-5-*O*-(malonyl)glucoside
24	25.8	1021	977 (20)	773 (60)	535 (100)	-	-	491 (31), 287 (100)	Cyanidin-3-*O*-(caffeoyl)sophoroside-5-*O*-(malonyl)glucoside
34	28.6	1005	961 (44)	757 (100)	535 (16)	491 (7)	-	287 (100)	Cyanidin-3-*O*-(*p*-coumaryl)sophoroside-5-*O*-(malonyl)glucoside
35	28.9	1005	961 (44)	757 (100)	535 (100)	491 (7)	-	287 (100)	Cyanidin-3-*O*-(*p*-coumaryl)sophoroside-5-*O*-(malonyl)glucoside
37	29.2	1065	1021 (5)	817 (59)	535 (100)	-	-	287 (100)	Cyanidin-3-*O*-(sinapoyl sophoroside-5-*O*-(malonyl)glucoside
38	29.5	1035	-	787 (100)	535 (79)	-	-	287 (100)	Cyanidin-3-*O*-(feruoyl)sophoroside-5-*O*-(malonyl)glucoside
41	30.6	1241	1197 (44)	993 (28)	535 (100)	491 (10)	-	287 (100)	Cyanidin-3-*O*-(feruoyl,sinapoyl)sophoroside-5-*O*-(malonyl)glucoside
42	31.0	1019	975 (6)	771 (72)	519 (100)	475 (23)	-	475 (12), 271 (100)	Pelargonidin-3-O-(feruoyl)sophoroside-5-*O*-(malonyl)glucoside
43	31.3	1211	1167 (16)	963 (100)	535 (74)	-	-	287 (100)	Cyanidin-3-*O*-(p-coumaryl,sinapoyl)sophoroside-5-*O*-(malonyl)glucoside
45	31.5	1241	1197 (10)	993 (70)	535 (100)	491 (9)	-	491 (97), 287 (100)	Cyanidin-3-*O*-(feruoyl, sinapoyl)sophoroside-5-*O*-(malonyl)glucoside
46	32.5	1181	1137 (33)	933 (98)	535 (100)	-	-	287 (100)	Cyanidin-3-*O*-(p-coumaryl, feruoyl)sophoroside-5-*O*-(malonyl)glucoside
47	34.3	1255	1211 (13)	1007 (74)	549 (100)	-	-	409 (18), 301 (45), 201 (100)	Peonidin-3-O-(feruoyl, sinapoyl)sophoroside-5-*O*-(malonyl)glucoside

							MS3 [M-(324 + acyl1)]$^+$,m/z (%)	
			-44	-248	-410	-(324 + acyl1)		
11	19.8	1227	1183 (4)	979 (9)	817 (22)	697 (100)	610 (13), 287 (100)	Cyanidin 3-*O*-(sinapoyl)sophoroside-5-*O*-(malonyl)sophoroside
12	20.3	1197	1153 (10)	-	787 (19)	697 (100)	553 (7), 473 (100), 287 (70)	Cyanidin 3-*O*-(feruoyl)sophoroside-5-*O*-(malonyl)sophoroside
18	22.7	1227	1183 (1)	-	817 (29)	697 (100)	287 (100)	Cyanidin 3-*O*-(sinapoyl)sophoroside-5-*O*-(malonyl)sophoroside
19	23.5	1197	1153 (24)	-	787 (53)	697 (100)	653 (28), 455 (13), 287 (100)	Cyanidin 3-*O*-(feruoyl)sophoroside-5-*O*-(malonyl)sophoroside
23	25.2	1183	1139 (15)	-	773 (49)	697 (100)	653 (20), 455 (8), 287 (100)	Cyanidin 3-*O*-(caffeoyl)sophoroside-5-*O*-(malonyl)sophoroside
31	27.8	1167	1123 (13)	-	757 (43)	697 (100)	653 (26), 619 (10), 515 (8), 287 (100)	Cyanidin-3-*O*-(*p*-coumaric)sophoroside-5-*O*-(malonyl)sophoroside
32	28.1	1197	1153 (8)	949 (23)	787 (97)	697 (100)	611 (52), 287 (100)	Cyanidin-3-*O*-(feruloyl)sophoroside-5-*O*-(malonyl)sophoroside

						MS3 [M-410]+m/z (%)	
			-44	-410	-(324 + acyl1)		
25	26.9	1398	1345 (8)	979 (100)	697 (69)	773 (100), 287 (40)	Cyanidin-3-*O*-(caffeoyl, sinapoyl)sophoroside-5-*O*-(malonyl)sophoroside
28	27.5	1359	1315 (8)	949 (100)	697 (98)	773 (100), 287 (35)	Cyanidin-3-*O*-(caffeoyl, feruoyl)sophoroside-5-*O*-(malonyl)sophoroside
39	29.6	1373	1329 (3)	963 (100)	697 (61)	595 (100), 287 (46)	Cyanidin-3-*O*-(*p*-coumaric, sinapoyl)sophoroside-5-*O*-(malonyl)sophoroside *or* Cyanidin-3-*O*-(diferuoyl)sophoroside-5-*O*-(malonyl)sophoroside
40	30.2	1373	1329 (8)	963 (94)	697 (100)	287 (100)	Cyanidin-3-*O*-(*p*-coumaric, sinapoyl)sophoroside-5-O-(malonyl)sophoroside *or* Cyanidin-3-*O*-(diferuoyl)sophoroside-5-O-(malonyl)sophoroside
44	31.3	1373	1329 (2)	963 (63)	697 (100)	654 (17), 455 (73), 287 (100)	Cyanidin-3-*O*-(*p*-coumaric, sinapoyl)sophoroside-5-*O*-(malonyl)sophoroside *or* Cyanidin-3-*O*-(diferuoyl)sophoroside-5-*O*-(malonyl)sophoroside

[1] Main observed fragments.

[2] Other ions were observed in MS2 (-248) of compound **6** (611 (9)) and compound **8** (625 (24)).

1

Table 3. List of individual anthocyanins (mg · 100g^{-1} F.W.) tentatively identified and quantified in China rose radish and Rambo red radish sprouts by HPLC-DAD.

Peak LC-MS	[M]$^+$*m/z*	Rt HPLC -DAD	Compound	4.a. China rose radish							
				Control	MeJA	JA	SA	Glucose	Sucrose	Methionine	LSD$_{0.05}$
1	859	15.5	Cyanidin 3-*O*-sophoroside-5-*O*-(malonyl)glucoside	0.03b	0.03ab	0.02b	0.03b	0.04a	0.03b	0.02b	0.004 n.d.
2	1021	20.2	Cyanidin 3-*O*-sophoroside-5-*O*-(malonyl)sophoroside	0.16cd	0.25a	0.17cd	0.21b	0.21ab	0.20bc	0.15d	0.12^{**}
4	1021	29.0	Cyanidin 3-*O*-(caffeoyl)sophoroside-5-*O*-(malonyl)glucoside	0.95de	1.08cd	0.60f	1.10c	1.29b	1.52a	0.84e	0.04^{**}
10	1197	30.8	Cyanidin 3-*O*-(caffeoyl, feruloyl)sophoroside-5-*O*-(malonyl)glucoside	0.99cd	1.00d	0.88de	1.24b	1.52a	1.20c	0.85e	0.04^{***}
12 + 13 + 14	1005 + 1035 + 1065	32.6	Cyanidin 3-*O*-(*p*-coumaroyl)sophoroside-5-*O*-(malonyl)glucoside + Cyanidin 3-*O*-(feruloyl)sophoroside-5-*O*-(malonyl)glucoside + Cyanidin 3-O-(sinapoyl)sophoroside-5-O-(malonyl)glucoside	7.43c	10.7a	5.89d	8.48b	10.32a	9.72a	6.91c	0.33^{***}
16 + 17	1271 + 1197	33.8	Cyanidin 3-O-(disinapoyl)sophoroside-5-O-(malonyl)glucoside + Cyanidin 3-*O*-(caffeoyl, eruloyl)sophoroside-5-*O*-(malonyl)glucoside	1.65c	1.84c	2.29b	2.48b	3.35a	1.74c	1.73c	0.13^{***}
19 + 20	1241 + 1019	34.0	Cyanidin 3-O-(feruoyl, sinapoyl)sophoroside-5-O-(malonyl)glucoside + Pelargonidin-3-*O*-(feruloyl)sophoroside-5-*O*-(malonyl)glucoside	2.49c	2.71b	2.58c	3.13c	3.77a	2.66c	2.17d	0.10^{***}
22 + 23	1227 + 1197	34.5	Cyanidin 3-O-(caffeoyl, sinapoyl)sophoroside-5-O-(malonyl)glucoside + Cyanidin 3-O-(caffeoyl, feruoyl)sophoroside-5-O-(malonyl)glucoside	0.79e	0.9cd	1.11b	0.94c	1.22a	0.83de	0.65f	0.03^{***}
24	1211	35.2	Cyanidin 3-O-(*p*-coumaroyl, sinapoyl)sophoroside-5-O-(malonyl)glucoside or Cyanidin 3-O-(diferuloyl)sophoroside-5-O-(malonyl)glucoside	0.35cd	0.38bc	0.42ab	0.45a	0.44a	0.37c	0.3d	0.02^{**}
			Unidentified anthocyanins	0.99de	0.56e	1.64b	1.04c	2.63a	0.76cde	0.61de	0.12^{***}

				4.b. Rambo red radish							
				Control	MeJA	JA	SA	Glucose	Sucrose	Methionine	LSD$_{0.05}$
1	773	16.0	Cyanidin 3-*O*-sophoroside-5-*O*-glucoside	0.62b	0.52bc	0.6bc	0.79a	0.80a	0.56bc	0.49c	0.04^{***}
4	979	19.5	Cyanidin 3-*O*-(sinapoyl)sophoroside-5-*O*-glucoside	1.1bc	1.03c	0.98c	1.40ab	1.57a	1.14bc	0.90c	0.09^{**}
11	1227	26.0	Cyanidin 3-O-(sinapoyl)sophoroside-5-*O*-(malonyl)sophoroside	0.75d	0.79cd	0.95bc	1.37a	1.48a	0.99b	0.75d	0.06^{***}
14	979	28.9	Cyanidin 3-*O*-(sinapoyl)sophoroside-5-*O*-glucoside	5.38cde	6.26b	5.11de	5.65bcd	8.35a	5.91bc	4.75e	0.21^{***}
27	1155	29.5	Cyanidin 3-*O*-(feruloyl,sinapoyl)diglucosido-5-*O*-glucoside	5.3cd	5.53bc	4.75de	5.97b	7.61a	5.68bc	4.67e	0.18^{***}
32	1197	30.1	Cyanidin 3-*O*-(feruoyl)sophoroside-5-*O*-(malonyl)sophoroside	11.27bc	11.29bc	10.77cd	12.5b	15.84a	11.61bc	9.63d	0.39^{***}
33 + 34	1317 + 1005	30.7	Cyanidin 3-*O*-(feruloyl, sinapoyl)sophoroside-5-*O*-sophoroside + Cyanidin-3-*O*-(*p*-coumaryl)sophoroside-5-*O*-(malonyl)glucoside	2.98c	2.98b	2.42d	3.28b	3.84a	3.15b	2.48c	0.09^{***}

35 + 36	1005 + 1155	32.0	Cyanidin-3-*O*-(*p*-coumaryl)sophoroside-5-*O*-(malonyl)glucoside + Cyanidin-3-*O*-(feruloyl, sinapoyl)diglucosido-5-*O*-glucoside	23.5bc	24.04b	23.35bc	23.3bc	35.5a	24.82b	19.47c	1.37^{***}
37 + 38	1065 + 1035	32.5	Cyanidin-3-*O*-(sinapoyl)sophoroside-5-*O*-(malonyl)glucoside +Cyanidin-3-*O*-(feruoyl)sophoroside-5-*O*-(malonyl)glucoside	50.37e	65.17b	53.49de	56.67cd	68.37a	57.53c	44.54f	1.05^{***}
40	1373	33.6	Cyanidin 3-*O*-(*p*-coumaroyl, sinapoyl)sophoroside-5-*O*-(malonyl)sophoroside or Cyanidin 3-*O*-(feruoyl, feruoyl)sophoroside-5-*O*-(malonyl)sophoroside	27.29c	28.82bc	22.93d	29.17bc	35.62a	30.50b	23.31d	0.59^{***}
41	1241	33.8	Cyanidin 3-*O*-(feruoyl, sinapoyl)sophoroside-5-*O*-(malonyl)glucoside	26.27c	28.96b	25.20c	25.19c	31.28a	26.4c	22.88d	0.66^{***}
43 + 44 + 45	1211 + 1371 + 1241	34.2	Cyanidin 3-*O*-(*p*-coumaroyl, sinapoyl)sophoroside-5-*O*-(malonyl)glucoside + Cyanidin 3-*O*-(*p*-coumaroyl, sinapoyl)sophoroside-5-*O*-(malonyl)sophoroside or Cyanidin 3-*O*-(p-coumaroyl,sinapoyl)sophoroside-5-*O*-(malonyl)sophoroside + Cyanidin 3-*O*-(feruoyl, sinapoyl)sophoroside-5-*O*-(malonyl)glucoside	15.19bc	15.87ab	13.22d	14.60bcd	17.34a	16.79a	14.16cd	0.47^{***}
46	1181	35.0	Cyanidin 3-*O*-(*p*-coumaroyl, sinapoyl)sophoroside-5-*O*-(malonyl)glucoside	3.41bc	3.35bc	2.78d	3.76b	4.39a	3.68b	2.98cd	0.18^{***}
			Unidentified anthocyanins	8.1c	6.79d	12.64a	7.96c	9.78a	8.10c	10.24b	0.34^{***}

[A] Mean values (n=3). a-d, Different lowercase-letters mean statistically significant differences between treatments.

[B], Least Dignificant Difference (LSD) for separating means in the respective column. The LSD was computed only after analysis of variance indicated a significant ($p<0.05$) entry effect. Anova p value, * $p<0.05$; ** $p<0.01$; *** $p<0.001$; n.s. $p>0.05$