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Improvement of the nutraceutical quality of broccoli sprouts by elicitation

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ABSTRACT

Epidemiological studies show an inverse association between Brassica consumption and chronic diseases. Phytochemicals are thought to be beneficial for human health and therefore responsible for this protective effect. Increasing their levels into Brassica food is considered an expedient nutritional strategy that can be achieved through the manipulation of growth conditions by elicitors. In this work we systematically evaluated the influence of treatment with different elicitors (sucrose, mannitol, NaCl, 1-aminocyclopropane-l-carboxylic acid, salicylic acid and methyl jasmonate) on the phytochemical composition of broccoli sprouts. The content of total and single glucosinolates, total phenolic compounds, total flavonoids, total anthocyanins, vitamin C and E and β-carotene was assessed.

The exposure to different elicitors produced concentration- and elicitor-dependent specific changes in the content of all the phytochemicals considered. Sucrose, identified as the most effective elicitor by principal component analysis, induced a significant increase of total and specific glucosinolates, vitamin C, total anthocyanins and polyphenols. Sucrose is likely to represent an effective tool to increase the nutritional value of broccoli sprouts.

1. Introduction

Epidemiological evidence suggests that there is an inverse association between consumption of cruciferous vegetables (such as broccoli, brussel sprouts and cauliflower) and the risk of many types of cancer (Liu & Lv, 2013) and cardiovascular disease (Cornelis, El-Sohemy, & Campos, 2007). These vegetables contain numerous bioactive compounds (such as vitamins, minerals, glucosinolates and phenolic compounds) that are considered to be responsible for their health-promoting properties.

In particular, glucosinolates (GL), and their hydrolysis products isothiocyanates (ITC), are considered to have an important role in cancer chemoprevention. These compounds, in fact, are reported to have a chemo-preventative activity in a variety of cell and animal models, probably due to their capacity to induce phase 2 detoxification enzymes (Plumb et al., 1996). In addition, cruciferous vegetables are an excellent dietary source of other health promoting phytochemicals, such as vitamins (C and E), carotenoids and phenolic compounds (among them anthocyanins), having an established role in the prevention of several chronic diseases (Björkman et al., 2011).

According to these evidences, many studies have focused on strategies aimed to increase the content of bioactive compounds in cruciferous vegetables. Recently, young seedlings (sprouts) have attracted an increasing interest by nutritionists, as they possess a higher nutritional value than adult plants. Broccoli sprouts, in fact, have higher level of GLs (Pérez-Balibrea, Moreno, & García-Viguera, 2011), and phenolic compounds (Singh, Upadhyay, Prasad,

Abbreviations: ANOVA, one-way analysis of variance; ACC, 1-aminocyclopropane-l-carboxylic acid; ESI-MS, electrospray ionization source-mass spectrometry; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalents; GL, glucosinolate; HPLC, high performance liquid chromatography; ITC, isothiocyanates; MeJa, methyl jasmonate; PCA, principal component analysis; SA, salicylic acid; THF, tetrahydrofuran; TEAC, trolox equivalent antioxidant capacity.

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Bahadur, & Rai, 2007), and a higher phase 2 inducing activity (Fahey, Zhang, & Talalay, 1997) than adult plants. Sprouts can be easily grown at home and, irrespective of the season, they only need water and light for growth. Sprouts can be consumed raw, thus avoiding the unavoidable loss of bioactive molecules during cooking and/or processing. Therefore, sprouts could be used as a natural functional food.

Obviously, environmental growth conditions affect the synthesis and therefore the content of many of the health promoting bioactive molecules present in plants. Abiotic (temperature, light, radiation, water, exposure to salt or metals) and biotic (herbivory, fungal, bacterial and/or viral infection) stresses can affect both the content and profile of these phytochemicals (Björkman et al., 2011). Also, broccoli sprout composition is affected by environmental conditions and it has been demonstrated that light or sucrose treatment increase phytochemical composition in terms of vitamins, GLs and phenolic compounds (Guo, Yuan, & Wang, 2011b; Pérez-Balibrea et al., 2011).

To our knowledge, however, data currently available are scarce and fragmented. In fact, previous studies evaluated the effect of a limited number of inducers on few bioactive molecules, not considering the overall range of phytochemicals. Moreover, these studies are hardly comparable due to the wide different growth conditions taken in consideration (e.g. seedling age, treatment period).

The aim of this study was to systematically evaluate, in broccoli sprouts, the influence of different concentrations of many different elicitors (sucrose, mannitol, NaCl, 1-aminocyclopropane-l-carboxylic acid (ACC), salicylic acid (SA) and methyl jasmonate (MeJa)) on the content of many different bioactive molecules (total and individual glucosinolates, total phenolic compounds, total flavonoids, total anthocyanins, vitamin C and E and β-carotene). Moreover, taking into account the complexity of the interaction among these phytochemicals, we utilized principal component analysis (PCA) to assess the conditions (type of elicitor and dose) able to provide the most significant overall effect on phytochemical composition of broccoli sprouts.

2. Materials and methods

2.1. Materials

Solvents and HPLC grade methanol used for extraction were of high purity (Carlo Erba, Milano, Italy). Acetonitrile and formic acid were from Sigma–Aldrich Chemical Company (St Louis, MO). HPLC grade water (18 mΩ) was prepared using a Millipore (Bedford, MA, USA) Milli-Q purification system. Glucobrassicin potassium salt, glucoraphanin potassium salt, glucoiberin potassium salt, glucoerucin potassium salt, progoitrin potassium salt, gluconapin potassium salt, sinigrin potassium salt and glucocheirolin potassium salt were purchased from PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany). Folin & Ciocalteau's phenol reagent, gallic acid, (+) catechin, aluminium chloride, sodium nitrite were from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Growth condition of broccoli sprouts

Broccoli seeds (*Brassica oleracea* L. var. *botrytis* subvar. *cymosa*) were purchased from SUBA&UNICO (Longiano, FC, Italy). Seeds were surface sterilized by incubating for 15 min in 40% bleach (2% sodium hypochlorite) with shaking, than drained and rinsed 10 times with distilled water. After soaking, in distilled water, for 16–18 h (overnight) at 21 °C, seeds were rinsed in distilled water and transferred in the germination cylinder, Vitaseed sprouter (SUBA&UNICO, Longiano, FC, Italy). Sprouts were grown at 21 °C in a plant growth chamber (Weiss Gallenkamp, Loughborough, United Kingdom) equipped with PHILIPS Master TL-D 36W/840 cool-white fluorescent tubes providing a photosynthetic photon flux density of 110 mmol m^{-2} s^{-1}, with a 16 h light/8 h dark photoperiod and subjected to different stressors.

Mannitol, sucrose, NaCl, ACC, SA and MeJa were selected as elicitors, for their known activity as stress inductors or signal molecules: mannitol is an inductor of osmotic stress, NaCl is an inductor of salt stress, SA is a plant hormone regulating resistance to fungal, bacteria and viral pathogen, ACC is a precursor of ethylene and a signal molecule, MeJa is a plant hormone regulating resistance against insect, and sucrose is a carbon source, an inductor of osmotic stress and a modulator of developmental and metabolic process (Baenas, García-Viguera, & Moreno, 2014a). Each elicitor was used at different concentrations that were selected according to previous studies. Mannitol and sucrose were dissolved in water at 88 and 176 mM concentrations. NaCl was dissolved in water at 10, 50 and 100 mM concentrations. SA was dissolved in water at 250 and 750 μM concentrations. ACC was dissolved in ethanol and added at 50 and 500 μM final concentrations. MeJa was dissolved in ethanol and added at 30 and 300 μM final concentrations. All solutions were freshly prepared before application and used to replace water in the sprouter 3 days after sowing. The sprouts were grown in the presence of the elicitors for 2 more days, for a total of 5-day germination. This time point was selected as five day-old sprouts still possess both a suitable biomass and a high phytochemical content; it is in fact known that, in the course of the germination, there is a steady decline of the major bioactive molecules (Maldini, Baima, Morelli, Scaccini, & Natella, 2012). Treated and control 5-day-old sprouts samples were rapidly and gently collected from the surface of the germination cylinder at midday, weighed (fresh mass) and immediately frozen in liquid nitrogen and stored at −80 °C for further analysis. Frozen sprouts were ground to a fine powder in a Waring blender, cooled with liquid nitrogen and aliquots of sprouts powder were used for humidity content determination and further analysis.

2.3. GLs determination

For GLs measure, each sample of broccoli sprouts was extracted with methanol:water (70:30 v/v; sample to solvent ratio 1:25 w/v) at 70 °C for 30 min under vortex mixing to facilitate the extraction. The samples were successively centrifuged (4000 rpm, 30 min, 4 °C), the supernatants were collected and the solvent was completely removed, using a rotary evaporato, under vacuum at 40 °C. The dried samples were dissolved in ultrapure water with the same volume of extraction and filtered through 0.20 mm syringe PVDF filters (Whatmann International Ltd., UK).

GLs analyses were performed as previously described (Maldini et al., 2012), using an HPLC system (Perkin–Elmer, USA) interfaced to an Applied Biosystems (Foster City, CA, USA) API3200 Q-Trap spectrometer and GLs were analyzed. Briefly, quantitative on-line HPLC–ESI-MS/MS analyses were performed using the mass spectrometer in MRM mode. Samples were injected (10 μl) into a Luna C_{18} column (Phenomenex, USA) (150 × 2.1 mm i.d., 5 μm d) and eluted at flow rate of 0.3 ml min^{-1}. Mobile phase A was H_2O containing 0.1% formic acid while mobile phase B was acetonitrile containing 0.1% formic acid. Elution gradient was: 0′ 6% B, 0.1–15 min → 12% B, 15.1–21 min → 25% B, 21.1–30 min → 60%B, 30.1–31 min → 100% B, 31.1–38 min 100% B and finally for re-equilibration: 38.1–39 min → 6% B, 39.1–45 min 6% B. The column was kept at 25 °C, using a Peltier Column Oven Series 200 (Perkin Elmer). The source temperature was held at 450 °C, and MS parameters were those optimised for the ESI-MS and ESI-MS/MS analyses with ion spray voltage at −4300 V. For all compounds, dwell time