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Comparative analysis of phytochemicals and activity of endogenous enzymes associated with their stability, bioavailability and food quality in five Brassicaceae sprouts

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ABSTRACT

Five Brassicaceae sprouts (white cabbage, kale, broccoli, Chinese cabbage, arugula) were comparatively analyzed based on phytochemicals (polyphenols, glucosinolates, carotenoids, chlorophylls, ascorbic acid) content and accompanying enzymes associated with phytochemical stability and bioavailability (peroxidases, myrosinase, and polyphenol-oxidase) that consequently impact food quality. Significantly high content of polyphenols and glucosinolates, as well as a high antioxidant activity were found in white cabbage, followed by kale sprouts. In addition, white cabbage contained higher amount of fibers and lower polyphenol-oxidase activity which potentially indicates prevention of browning and consequently better sprout quality. Arugula and broccoli showed higher activity of myrosinase that may result in higher bioavailability of active glucosinolates forms. According to our data, sprouts are cheap, easy- and fast-growing source of phytochemicals but also they are characterized by different endogenous enzymes activity. Consequently, this parameter should also be taken into consideration in the studies related to the health benefits of the plant-based food.

1. Introduction

Cruciferous (Brassicaceae) vegetables include many species used in culinary and as traditional medicine. Due to the good environmental adaptation cruciferous vegetables have been grown and used by different cultures worldwide. They are recognized as a functional food because different epidemiological and meta-analysis suggested that consumption of cruciferous has preventive role against a variety of chronic disease, several cancers etc. (Šamec, Pavlović, & Salopek-Sondi, 2017). Beneficial effects include antioxidant, anti-inflammatory, gastro protective and anti-obesity activity associated with the presence of different phytochemicals such as glucosinolates, polyphenols, carotenoids etc. (Šamec et al., 2017). Cruciferous also can be used in different forms, as a salad, fresh or dried as a spice, cooked, fried, baked or fermented. In the last couple of years new culinary trend introduced cruciferous vegetable in a germinating stage, as sprouts. Consumption of such as vegetables provide unique taste, and additional health benefits due to the fact that Brassicaceae sprouts are rich in health-promoting phytochemicals, vitamins, amino acids, and minerals (Deng et al., 2017; Vale, Santos, Brito, Peixoto, et al., 2015; Vale, Santos, Melia, et al., 2015). During extensive period of growth and development, seedlings and young plantlets accumulate more phytochemicals (Šamec, Piljac-Žegarac, Bogović, Habjanič & Grúz, 2011), and, consequently, young seedlings or sprouts could contain from 2 to 10-fold more phytochemicals than vegetables in mature stage (Baenas, Gómez-Jodar, Morenoa, García-Viguera & Periago, 2017).

However, the bioaccessibility and bioavailability of each compound differs greatly. It is well known that endogenous plant enzymes may significantly influence postharvest stability of phytochemicals, food quality, consumer preferences and bioavailability (Martinez-Ballesta & Carvajal, 2015; Queiroz, Lopes, Filaho, & Valente-Mesquita, 2008; Toivonen & Sweeney, 1998). Antioxidant enzymes such as peroxidases are important in retention green color in vegetables and their activity are critical in controlling yellowing (Toivonen & Sweeney, 1998). Additionally, peroxidases could be an indicator of quality deterioration such as flavor loss and various biodegradation reactions. It is also relevant to enzymatic browning since diphenols may function as reducing substrate in the enzyme reaction and could promote darkening in fruit and vegetable products during processing and preservation (Jang & Moon, 2011). Although peroxidases are involved in browning, polyphenol oxidases (PPO) are the major cause of the brown coloration of many fruits and vegetables during ripening, handling, storage and

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processing. In the presence of oxygen and PPO, phenolic compounds present in plant tissue serve as precursors in the formation of quinones which consequently polymerize and form brown pigments (melanosis). Formed quinones can bind plant proteins reducing protein digestibility, amount of available polyphenols and nutritive value of the food. Therefore, PPO activity affects the nutritional quality, appearance and consumer's acceptability (Queiroz et al., 2008). Myrosinase is an enzyme found in all glucosinolate-containing vegetables from Brassicaceae family where catalyzes the hydrolysis of glucosinolates into D-glucose and an aglycone which may be spontaneously converted into isothiocyanates or indoles, the biologically active forms of glucosinolates associated with numerous health benefits. Therefore, myrosinase is the most important issue for glucosinolate turnover and its activity in plants substantially influences bioavailability and glucosinolates health benefits (Martinez-Ballesta, & Carvajal, 2015).

In recent years several studies reported content of health benefits compounds in Brassicaceae sprouts (Deng et al., 2017; Vale, Santos, Brito, Peixoto, et al., 2015; Vale, Santos, Melia, et al., 2015) although data which directly compare phytochemicals with endogenous enzymes in different cruciferous species are limited. Taking into consideration importance of endogenous enzyme activity in phytochemicals stability and bioavailability as well as in food quality, we aimed to study those parameters in five different Brassicaceae sprouts: white cabbage (*Brassica olearcea* var. *capitata*), kale (*B. oleracea* var. *acephala*), broccoli (*B. oleracea* var. *italic*), Chinese cabbage (*B. rapa* ssp. *pekinensis*), and arugula (*Eruca sativa*). In addition, we analyzed data using principal component analysis, a statistical tool which allows visualization of the interrelationships of the investigated parameters in the five different sprouts.

2. Material and methods

2.1. Plant material and sprouting conditions

Seeds of Brassicaceae species were purchased from the specialized seeds producers as listed: broccoli (*Brassica oleracea* var. *italica* cv. Corveti F1) and white cabbage (*Brassica oleracea* var. *capitata* cv. Varaždinski) from Semenarna Ljubljana, Chinese cabbage (*Brassica rapa* var. *pekinensis* cv. Lour) from International Seeds Processing GmbH Germany and arugula (*Eruca sativa* cv. Riga) from Vita Bella Italy. Kale seeds (*Brassica oleracea* var. *acephala*) were obtained from the local grower. Prior sprouting, seeds were washed several times with distilled water and placed on the 1% agar plates at 4 °C on 24 h hydration. Afterwards, seeds were transferred to plates containing cotton wool covered with filter paper and set in the growing chamber at 22 °C, and photoperiod 16/8 h (light/dark). To obtain moisture during whole sprouting process plates were supplied with distilled water. Ten days after germination started, sprouts were collected and immediately frozen using liquid nitrogen. For the enzymatic assays, quickly frozen tissue was stored at −80 °C until analysis. Samples for phytochemical analysis were freeze-dried and stored in dark and dry place until use.

2.2. Dietary fibers and proteins

Dietary fiber content was determined using the Total dietary fiber assay kit (Megazyme International Ireland, Bray, Ireland). Total soluble proteins were isolated in 100 mM potassium phosphate buffer (pH 7.0, 0.1 mM EDTA) with addition of the insoluble polyvinylpirolidone (PVPP). Protein content was determined according to the Bradford (1976).

2.3. Phytochemicals analysis

2.3.1. Total ascorbic acid

Levels of total ascorbic acid were determined using the dinitrophenylhydrazine (DNPH) method adapted to small scale analysis as we reported earlier (Šamec et al., 2016.)

2.3.2. Polyphenolic compounds

Extractions were carried out in a Mixer Mill MM 400 (Retsch, Haan, Germany) for 5 min at 30 Hz using 60 mg of freeze-dried tissue in 2 mL of 80% methanol, followed by 10 min sonication and 1 h mixing at 15 rpm on tube rotator. Extracts were centrifuged and supernatants recovered for the analysis. All extractions were performed in triplicates (Šamec et al., 2011.). The total polyphenol content (TP) was determined according to the Folin–Ciocalteu method (Singleton & Rossi, 1965) adapted to small volumes and results were expressed as equivalents of gallic acid per dry weight (mg GAE/g dw). The total flavonoids (TF) were analyzed using the $AlCl_3$ method adapted to small scale (Šamec et al., 2011) and presented as catechin equivalents per dry weight (mg CE/g dw). The total flavanols (TFL) were determined using the *p*-dimethylaminocinnamaldehyde (DMACA) reagent and proanthocyanidins (PRAN) were determined using the vanillin-HCl method (Šamec, Bogović, Vincek, Martinčić & Salopek-Sondi, 2014) and expressed as catechin equivalents per dry weight (mg CE/g dw). Total phenolic acids (TPA) were measured according to the European Pharmacopoeia (2004) and shown as caffeic acid equivalents per dry weight (mg CAE/g dw).

2.3.3. Glucosinolates

The extraction, isolation and desulphation of glucosinolates were carried out according to the ISO method 10633-1 (1995) with modifications. In brief, triplicates of lyophilized tissue (30 mg) were extracted twice with 900 μL of 70% methanol at 70 °C for 15 min by addition of an internal standard glucotropeolin (20 μL of 5 mM glucotropeolin). After centrifugation, recovered extracts were passed through an ion-exchange resin Fast DEAE Sepharose CL-6B microcolumn for desulphation with purified sulphatase (from Helix pomatia) and left overnight at the room temperature. Desulphoglucosinolates were eluted with 1.5 mL of deionized water and separated on a ZORBAX C18 column (250 mm × 4.6 mm id; particle size 5 μm) using a Perkin-Elmer Series 200 HPLC system (Waltham, MA, USA) (Jakovljević et al., 2013). A two-component solvent system consisting of water (A) and 20% acetonitrile in water (B) was used. A constant flow rate of 1 mL min^{-1} was employed with gradient elution: 0–1 min 100% A, 1–30 min linear gradient change to 100% B, 30–35 min linear gradient change to 100% A and 35–40 min 100% A. Detection was performed with a UV-Diode Array Detector at 229 nm. Positive identification of desulphglucosinolates was accomplished by comparing elution order with the retention time of a sinigrin and internal standard glucotropeolin based on ISO standard method for determination of glucosinolates content (ISO, 10633-1:1995) and UV-DAD peak spectral analyses. Individual glucosinolates were recalculated from HPLC peak areas using the response factors to correct the absorbance differences between the internal standard (glucotropeolin) and other identified glucosinolates (ISO, 10633-1:1995). Results are expressed as μmol/g dw (dry weight).

2.3.4. Pigments

Plant pigments (chlorophylls and carotenoids) were extracted and quantified according to the Lichtenthaler and Buschmann (2001) with modification (Šamec et al., 2014). Results are expressed as mg/g dw (dry weight).

2.4. Antioxidant capacity

Methanol extracts used for spectrophotometric polyphenols analysis were used for determination of antioxidant capacity of samples by DPPH radical scavenging capacity assay (Brand-Williams, Cuvelier, & Berset, 1995) and ferric reducing/antioxidant power assay (FRAP) as reported by Benzie and Strain (1999).