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Corresponding Author: Dr. Dunja Samec, Ph.D.

Corresponding Author's Institution: Institute Ruđer Bošković

First Author: Dunja Samec, Ph.D.

Order of Authors: Dunja Samec, Ph.D.; Iva Pavlović; Ivana Radojčić-Redovniković; Branka Salopek-Sondi

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 caracterized 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.

***Highlights (for review)**

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Cruciferous vegetables are recognized as a functional food.

Endogenous enzymes may influence phytochemical stability, bioavaibility and food quality.

Cruciferous sprouts contain phytochemicals with health-promoting activity.

Cruciferous sprouts have different endogenous enzyme activity.

Sprouts are cheap, easy and fast-growing source of phytochemicals.

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

Dunja Šamec[1]*, Iva Pavlović[1], Ivana Radojčić-Redovniković[2], Branka Salopek-Sondi[1]

[1] Ruđer Bošković Institute, Department of Molecular Biology, Bijenička c. 54, 10000 Zagreb, Croatia

[2] Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia

*Corresponding author: Dunja Šamec

Department of Molecular Biology

Ruđer Bošković Institute,

Bijenička cesta 54, 10000 Zagreb, Croatia

tel. (385) 1 4561 002

fax. (385) 1 4561 177

e-mail: dsamec@irb.hr

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 caracterized 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.

Keywords: Brassicaceae sprouts, phytochemicals, antioxidant activity, myrosinase activity, polyphenol-oxidase

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 (Vale et al., 2015a; Vale et al., 2015b; Deng et al., 2017). 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 bioavaibility (Toivonen and Sweeney, 1998; Queiroz, Lopes, Filaho & Valente-Mesquita, 2008; Martinez-Ballesta and Carvajal, 2015). 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 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 (Vale et al., 2015a; Vale et al., 2015b; Deng et al., 2017) 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 bioavaibility 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, Maretić, Lugarić, Mešić, Salopek-Sondi & Duralija, 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 x 4.6 mm id; particle size 5 µm) using a Perkin-Elmer Series 200 HPLC system (Waltham, MA, USA) (Jakovljević el 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).

2.5. Enzymes activity

2.5.1. Antioxidant enzymes activity

Activity of antioxidant enzymes was determined in fresh frozen tissue. Plant material was grounded in mortar with pestle using liquid nitrogen prior analysis and around 130 mg of tissue was extracted with 1.5 mL of cold extraction buffer (100 mM potassium phosphate buffer pH 7.0, 0.1 mM EDTA) with addition of the insoluble polyvinylpirolidone (PVPP) and centrifuged for 20 min at 15000 g (at 4°C) (Salopek-Sondi et al., 2013).

Activity of guaiacol peroxidase (GPOD) was determined in 1 mL of total reaction volume containing 50 mM potassium buffer pH 7.0., 18 mM guaiacol and 5 mM H_2O_2. Reaction was started by adding 10 µL of extract and an increase in absorbance was monitored at 470 nm in linear range of reaction (Chance & Maehly, 1955). One unit of enzymatic activity was defined as 1 µmol of formed tetraguaiacol per minute calculated using molar extinction coefficient (26.6 mM^{-1} cm^{-1}). Results are shown as units per dry weight (U/mg dw).

Ascorbate peroxidase (APX) was determined according to the Nakano and Asada (1981) with modifications. Reaction mixture in total volume of 1 mL consisted of 50 mM potassium buffer pH 7.0, 0.1 mM EDTA and 0.1 mM ascorbic acid and 100 µL of extract. Reaction was started by adding 100 µL of H_2O_2 in final concentration of 0.6 mM and decrease in absorbance was monitored at 265 nm. Activity was calculated using the molar extinction coefficient for ascorbic acid (12.45 mM^{-1} cm^{-1}). One unit of enzymatic activity was defined

as 1 µmol of catalyzed ascorbic acid per minute. Results are shown as units per dry weight (U/mg dw).

Catalase activity (CAT) was determined in 1 mL reaction mixture containing 100 µL of extract and 50 mM potassium buffer pH 7.0 and 10 mM H_2O_2 (Aebi, 1984). Decrease in absorbance was monitored at 240 nm and activity was recalculated using extinction coefficient for hydrogen peroxide (40 mM^{-1} cm^{-1}). One unit of enzymatic activity was defined as 1 µmol of catalyzed hydrogen peroxide per minute. Results are shown as units per dry weight (U/mg dw).

2.5.2. Polyphenol oxidase activity

Extraction of polyphenol oxidase (PPO) enzymes was performed by overnight shaking (at 4 °C) of 100 mg grounded tissue with 1 mL of extraction buffer (50 mM sodium phosphate pH 7.0, 0.1% Triton x-100, 1 M NaCl) with subsequent centrifugation (15000 rpm, 4 °C). PPO activity was monitored in 1 mL of 50 mM sodium phosphate buffer pH 6.5 containing 50 mM catechol as a substrate. Reaction was started by addition of 20 µL of extract and monitored at 420 nm for 1 minute. One unit of PPO activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 per minute. Results are expressed as units per dry weight (U/mg dw).

2.5.3. Myrosinase activity

Approximately 100 mg of grounded frozen tissue was extracted with 1.5 mL of cold extraction buffer (0.2 M Tris-HCl, 10 mM EDTA, pH 5.5) with addition of the insoluble polyvinylpirolidone (PVPP) and centrifuged at 4 °C for 20 min at 15000 g. Removal of internal glucosinolates was performed as described by the Travers-Martin, Kuhlmann, and Müller (2008). Enzymatic reactions were performed in 0.5 mL of total reaction volume (50 mM Tris-HCl pH 5.5,) for 30 min at 37 °C using 100 µL of extract without (blanks) or with

substrate sinigrin (final conc. 0.2 mM). Reaction was stopped by boiling at 95 °C for 5 min and glucose level was determined by glucose assay kit (Glucose (GO) assay kit, Sigma, St. Louis, USA). One unit of myrosinase (MYR) activity corresponds to 1 μmol of produced glucose per minute. Results were expressed as units per dry weight (U/mg dw).

2.6. Statistical analysis

Statistical analysis was performed in Microsoft Excel 2010 upgraded with XLStat Premium (version 19.01). The data are presented as the mean ± standard deviations (SD). Values presented in tables and figures not sharing a common letter are significantly different at $p < 0.05$ by analysis performed using ANOVA and post hoc multiple mean comparison (Tukey's HSD test).

Since data were obtained using different methods before principle component analysis (PCA) we did mean-centering and auto scaling to put all parameters on an equal level. Standardized data contribute equally to the data set variance and to the principal component calculation.

3. Results and discussion

3.1. Dry weight, protein and dietary fiber content

In our experimental setup analyzed Brassicaceae sprouts contain approximately 12% of dry weight (dw) with slight differences depend on species/variety (Table 1). In order to make results comparable with literature data, we expressed all data per dry weight basis. Kale, arugula and broccoli contain 28-42% more proteins (43.88 – 46.24 mg/g dw) than Chinese and white cabbage (26.57 and 33.34 mg/g dw, respectively) (Table 1). Dietary fiber content of herein examined sprouts vary from 553.44 - 611.13 mg/g dw with higher content in white and Chinese cabbages in comparison to others (Table 1), and those results are comparable with total dietary fiber contents of cruciferous sprouts reported by Zielinski, Frias, Piskuła, Kozłowska and Vidal-Valverde (2005). Our results therefore support previous findings that

sprouts are an excellent and easy to prepare source of dietary fibers (Vale et al., 2015a). Total fiber is the sum of dietary fiber and functional fiber. Dietary fiber consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants, while functional fiber consists of isolated, nondigestible carbohydrates that have beneficial physiological effects in humans (USDA, 2015).

3.2. Phytochemical content and antioxidant activity

The main phytochemicals associated with health benefits in Brassicaceae species include polyphenols, carotenoids and glucosinolates which we evaluated in this study (Table 2 and 3). White cabbage sprouts showed the highest amount of total polyphenols (18.34 GAE/g dw, respectively) and total glucosinolates (81.67±4.90 µmol/g dw). Total polyphenolic content of white cabbage sprouts was around 80% higher than those, previously reported by our group, in two-month old white cabbage plants (around 10 mg GAE/g dw) (Šamec et al., 2014). Considering glucosinolates, we found that all examined sprouts contain higher amounts of aliphatic glucosinolates than indolic ones (Table 3). Among aliphatic glucosinolates the highest were sinirgin in white cabbage (59.93±4.38 µmol/g dw) and glucoraphanin in broccoli (60.04±2.39 µmol/g dw), similar as in previous studies where the same glucosinolates were reported as predominant ones in the mature white cabbage and broccoli (Cartea, Velasco, Obregon, Padilla & Haro, 2008; Radošević, Gaurina-Srček, Cvjetko-Bubalo, Rimac-Brnčić, Takács & Radojčić-Redovniković, 2017) indicating that presence of individual glucosinolates is genetically defined. Presence of sinirgin in food is associated with the anti-cancer, anti-inflammatory, antibacterial, antifungal, antioxidant, and wound healing effects (Mazumder, Dwivedi & Plessis, 2016). Glucoraphanin, detected in broccoli, is precursor for sulforaphane, an isothiocyanate with anticancerogenic activity (Atwell et al., 2015). Significantly higher total glucosinolates content we found in white cabbage and kale followed by commonly studeied broccoli. Total glucosinolates content in broccoli sprouts in

our experiment was 65.40±2.62 µmol/g dw, comparable with previous reports on broccoli sprouts (Pereira, Rosa, Fahey, Stephenson, Carvalh & Aires, A, 2002). In our experiments analyzed samples showed total ascorbic acid (vitamine C) content in all examined sprouts around 1 mg/g with the exception of Chinese cabbage which had significantly lower ascorbic acid content. High amount of ascorbic acid was detected in kale sprouts which contained, in addition, the highest glucobrassicin content. Hydrolysis product of glucosinolate glucobrassicin, indole-3-carbinol, received considerable interest as cancer chemoprotective agent and has been studied *in vitro* and *in vivo*. In the presence of ascorbic acid, dependent on the pH and temperature, indole-3-carbinol may be converted to ascorbigen, another glucosinolates derivatives associated with anticancerogenic effects (Wagner & Rimbach, 2009). Therefore, considering presence of glucobrassicin and ascorbic acid, kale is potent source of ascorbigen. Total carotenoids content in analyzed sprouts did not show significantly different value among different sprouts and was, in average, lower than total carotenoid content in four-week old white cabbage plants as reported in previous study (Šamec et al., 2014). Arugula and kale, showed significantly higher total chlorophylls content what is evident in their more intensive green color.

In parallel with the high content of polyphenolic compounds, known for their antioxidant activity, white cabbage shown the highest DPPH radical scavenging activity (Figure 1a) and ferric reducing antioxidant capacity (FRAP activity) (Figure 1b). These findings once more confirmed previous observation that polyphenolic compounds (Vale, Cidade, Pinto, Beatriz & Oliveira, 2014; Šamec et al., 2014) are the main compounds with antioxidant activity in Brassicaceae. Interestingly, broccoli is, so far, the most commonly studied Brassicaceae regarding health benefits recognized as a vegetable with high antioxidant capacity. However, our data confirmed that, regarding sprouts, also other Brassicaceae species could be good candidates as a source of health-promoting compounds and surely they deserve more

scientific attention. This is supported by recent paper that compared antioxidative and antiproliferative activity in mature collard and broccoli and authors found that both plants possess promising antitumor activities (Radošević et al., 2017).

3.3. Enzymes activity

Activities of the enzymes associated with stability and bioavaibility of phytochemicals and consequently food quality of five Brassicaceae sprouts are shown in Figure 2 a-e. Green vegetables such as sprouts are attractive and eye-catching to a large degree because of the richness of chlorophyll pigments that they contain. Green color of vegetables is associated with freshness and quality, and consequently, preservation of chlorophyll is of vital importance to maintain quality. Two groups of enzymes which can influence loss of green color and/or browning are peroxidase and polyphenol oxidase. Peroxidases are one of the key antioxidant enzymes, widely distributed in nature and catalyze oxidation of various electron donor substrates concomitant with the decomposition of H_2O_2. The plant peroxidases are involved in various essential physiological processes of plant growth and development as well as biotic and abiotic stress responses (Pandey, Awasthi, Singh, Tiwari and Dwivedl, 2017). In plant based food, peroxidase activity in plant tissue can produce free radicals which react with food components (ascorbic acid, carotenoids and fatty acids) leading to the loss of nutrients and the development of damage symptoms including deterioration in flavor, color, and nutritional quality (Jang & Moon, 2011). In order to monitor a peroxidase activity in cruciferous sprouts, activities of GPOD and APX were measured. As is evident from the Figure 2a and 2c, Chinese cabbage showed significantly highest level GPOD, and together with white cabbage high APX activity in comparison to other sprouts. High APX activity in Chinese cabbage may be consequently connected with lower AA level when compared with other varieties (Fig, 2c, Table 2). Singh, Sharma and Singh (2010) measured antioxidant enzymes activity in 36 diverse white cabbage genotypes and found that peroxidase together

with catalase activity varied significantly among genotypes. Catalase (CAT) is another important antioxidant enzyme for plants growth but according to the Toivonen and Sweeney (1998) it is less important for food quality regarding chlorophyll loss than peroxidase. In our study, catalase just showed significantly different activity between arugula and kale (Figure 2b). In kale, that in our study showed the lowest CAT and APX activity, according to the literature data antioxidant enzyme activity depend on the cultivar and maturity stage (Korus, 2011).

One of the most important and widely studied enzyme for food quality is polyphenol oxidase (PPO) (Figure 2e) which mediate first step in undesirable reaction of enzymatic browning and lead to brown, and, even in some cases reddish-brown, blue-gray and even black discolorations. Some cultivars can have higher PPO activity and/or high concentration or types of phenolic PPO substrates which, under appropriate conditions lead to a higher tendency to brown (Queiroz et al., 2008). Herein, sprout samples did not show significant variations in PPO activity, expect white cabbage which has significantly lower activity in comparison to broccoli and kale. PPO activity depends on the cultivar, growing location and maturity stage (Korus, 2011). Since all sprouts in herein reported experiment were grown under identical growing condition, variations in PPO activities are more probably determined by genetic background. Interesting, Korus (2011) reported that PPO activity in kale significantly increase with the age of the plant which may indicate that PPO is less likely to influence food quality in plants at younger age, such as sprouts.

Brassicaceae vegetables are known to contain specialized metabolites glucosinolates whose degradation products, isothiocyanates, have been widely identified as beneficial compounds to human diet (Martinez-Ballesta & Carvajal, 2015). The enzymes myrosinase plays an important role in the glucosinolate turnover to active products, and therefore it plays an important role in Brassicaceae health benefits. Results presented on Figure 2d shows the

294 highest myrosinase activity in arugula and broccoli sprouts. Broccoli sprouts contain
295 glucosinolate glucoraphanin (Table 3) which could be hydrolyzed by an endogenous plant
296 myrosinase to the potent chemopreventive agent sulforaphane (Liang & Yuan, 2012).
297 Presence of active myrosynase in food matrix is of the great importance for sulforaphane
298 bioavailability (Atwell et al., 2015). For example, the study reported by Fahey, Holtzclaw,
299 Wehage, Wade, Stephenson and Talalay (2015) showed that when broccoli sprouts are
300 administered directly to subjects without prior extraction, and consequent inactivation of
301 endogenous myrosinase, the sulforaphane is 3- to 4-fold more bioavailable than sulforaphane
302 from glucoraphanin delivered without active plant myrosinase.

303 **3.4. Relationship between phytochemicals and enzymes activity**

304 Recent studies have demonstrated the effectiveness of PCA plotting using phytochemical
305 parameters in different food quality studies (Šamec et al. 2014, Šamec et al., 2016) including
306 studies on vegetable sprouts (Vale et al., 2015c, Viacava & Roura, 2015; Raimondi,
307 Rouphael, Kyriacou, Stasio, Barbieri & Pascale, 2017). In order to visualized relationship
308 between phytochemical content and enzyme activity we performed principle component
309 analysis (PCA), where first two principle components explained 82.65% variability. Bi-plot
310 at Figure 3 represents the observations and variables simultaneously in the new space whose
311 analysis and examination of the component loadings by extracting eigenvectors enabled as an
312 assessment of which individual parameters were associated with samples differences. On the
313 separation in first principle component (F1) the great influence had antioxidant activity
314 (DPPH and FRAP) and content of polyphenol and glucosinolate (TF, TGL, TFL, TPA)
315 compounds. It can be seen the strong positive loadings of the white cabbage which had high
316 amounts of those phytochemicals. Kale, in addition to white cabbage showed high content of
317 phytochemicals, but due to the higher content of PRAN, TF and AA which caused separation
318 in second principle component (F2), it is located in upper part of bi-plot. Chinese cabbage

319 which shows the highest catalase (CAT) and peroxidase activities (GPOD and APX) is
320 located in the lower part of the bi-plot. Arugula is positioned on the left side of the bi-plot
321 due to the highest amount of chlorophylls and carotenoids.

322 **4. Conclusions**

323 Obtained results showed that all five analyzed sprouts contain phytochemicals with health-
324 promoting benefits. Significantly high content of polyphenols and glucosinolates, and
325 antioxidant activity were found in white cabbage sprouts, followed by kale sprouts. Both
326 varieties have not so far been widely used for food as sprouts. Another advantage of white
327 cabbage is lower PPO activity which potentially indicates phytochemical stability, and
328 consequently, better food quality. Based on presented results, examined Brassicaceae sprouts,
329 with particular focus to white cabbage deserve more scientific attention as a cheap source of
330 phytochemicals with health-promoting benefits.

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Figure 1. Antioxidant capacities of five Brassicaceae sprouts measured by DPPH (a) and FRAP (b). Values with different superscript letters in the same column differ significantly at $p < 0.05$.

Figure 2. Activity of enzymes in five *Brassicaceae* sprouts: a) guaiacol peroxidase, GPOD; b) catalase, CAT; c) ascorbate peroxidase, APX; d) myrosinase, MYR and e) polyphenol oxidase, PPO. Values with different superscript letters in the same column differ significantly at $p < 0.05$.

Figure 3. The principal component analysis (PCA) bi-plot performed on the correlation matrix of average values of phytochemicals content (Total polyphenols, TP; total flavonoids, TF; total flavanols, TFL; proanthocyanidins, PRAN; total phenolic acids, TPA; total ascorbic acid, AA; total glucosinolates, TGL; chlorophyll a, Chl a; chlorophyll b, Chl b; total chlorophylls, TChls; carotenoids, Car), antioxidant capacity (DPPH, FRAP) and measured enzymes activity (catalase, CAT; ascorbate peroxidase, APX; guaiacol peroxidase, GPOD; polyphenol oxidase, PPO; myrosinase, MYR) of five analysed Brassicacea sprouts.

***Tables**

Table 1. Common name, Latin name, dry weight and content of proteins and total dietary fibers in five analysed Brassicacea species

Common name	Latin name	Dry weight (%)	Proteins (mg/g dw)	Total dietary fiber (mg/g dw)
kale	*Brassica oleracea* var. *acephala*	11.53±0.25	43.88±2.05	547.40±6.45
arugula	*Eruca sativa*	12.05± 0.39	46.24±1.88	557.33±14.96
Chinese cabbage	*Brassica rapa*	12.03± 1.42	26.57±3.51	577.57±29.71
white cabbage	*Brassica oleracea* var. *capitata*	12.58± 0.46	33.34±3.96	611.13±1.39
broccoli	*Brassica oleracea* var. *italica*	12.07± 0.68	43.88±1.36	553.44±10.74

Table2. Total polyphenols (TP), total flavonoids (TF), total flavanols (TFL), proanthocyanidins (PRAN), total phenolic acids (TPA), total ascorbic acid, chlorophyll a, chlorophyll b, total chlorophylls and carotenoids content of five analysed Brassicacea species

	kale	arugula	Chinese cabbage	white cabbage	broccoli
TP (mg GAE/g dw)	15.13±0.43^{b}	13.48±0.39^{c}	13.20±0.63^{c}	18.34±0.50^{a}	15.02±0.25^{b}
TF (mg CE/g dw)	3.67±0.19^{a}	2.71±0.23^{c}	2.57±0.07^{c}	3.58±0.17^{ab}	3.18±0.06^{b}
TFL (mg CE/g dw)	0.20±0.01^{a}	0.09±0.01^{c}	0.15±0.01^{b}	0.21±0.00^{a}	0.19±0.01^{a}
PRAN (mg CE/g dw)	1.89±0.11^{a}	1.94±0.11^{a}	0.98±0.16^{c}	1.39±0.18^{b}	1.25±0.09^{bc}
TPA (mg CAE/ g dw)	4.44±0.14^{a}	3.33±0.15^{c}	3.21±0.21^{c}	4.77±0.21^{a}	3.89±0.03^{b}
AA (mg/g dw)	1.09±0.01^{a}	0.92±0.15^{ab}	0.78±0.04^{c}	0.91±0.10^{ab}	0.95±0.08^{ab}
chlorophyll a (mg/g dw)	2.27±0.11^{ab}	2.38±0.21^{a}	1.88±0.12^{bc}	1.30±0.12^{d}	1.77±0.18^{c}
chlorophyll b (mg/g dw)	1.19±0.09^{ab}	1.29±0.16^{a}	0.85±0.20^{bc}	0.53±0.10^{c}	0.74±0.09^{c}
total chlorophylls (mg/g dw)	3.46±0.09^{a}	3.67±0.14^{a}	2.73±0.32^{b}	1.86±0.23^{c}	2.51±0.26^{b}
Carotenoids (mg/g dw)	0.53±0.08^{a}	0.55±0.11^{a}	0.38±0.05^{a}	0.37±0.01^{a}	0.46±0.02^{a}

Values with different superscript letters in the same row differ significantly at $p < 0.05$.

Table 3. Glucosinolates content (µmol/g dw) in analysed *Brassicacea* species

		kale	arugula	Chinese cabbage	White cabbage	broccoli
aliphatic	glucoiberin	24.41±0.70	0.88±0.07	n.d.	6.37±0.49	2.43±0.23
	progoitrin	9.69±0.98	n.d.	15.43±1.44	6.86±0.28	n.d.
	sinigrin	30.65±1.72	n.d.	0.23±0.03	59.93±4.38	n.d.
	glucoraphanin	2.70±0.45	5.10±0.54	nd	0.77±0.08	60.04±2.39
	glucoalyssin	0.66±0.03	1.42±0.05	0.16±0.01	nd	nd
	gluconapin	nd	nd	1.8 ±0.10	nd	0.22±0.01
	glucobrassicanapin	6.15±0.76	6.00±0.37	4.92±0.26	1.92±0.11	n.d.
indolic	4-hydroxyglucobrassicin	0.63±0.13	0.08±0.01	0.24±0.01	0.23±0.03	0.13±0.01
	glucobrassicin	2.55±0.50	1.81±0.06	0.43±0.03	1.19±0.02	1.07±0.16
	4-methoxyglucobrassicin	1.32±0.04	3.36±0.13	0.65±0.02	1.95±0.42	0.12±0.02
	neoglucobrasscin	1.58±0.18	1.95±0.51	0.97±0.10	2.46±0.06	1.39±0.13
total		80.33±2.04^{a}	20.59±0.90^{c}	24.90±1.68^{c}	81.67±4.90^{a}	65.40±2.62^{b}

nd, not detected

Values with different superscript letters in the same row differ significantly at $p < 0.05$.

Figure 1

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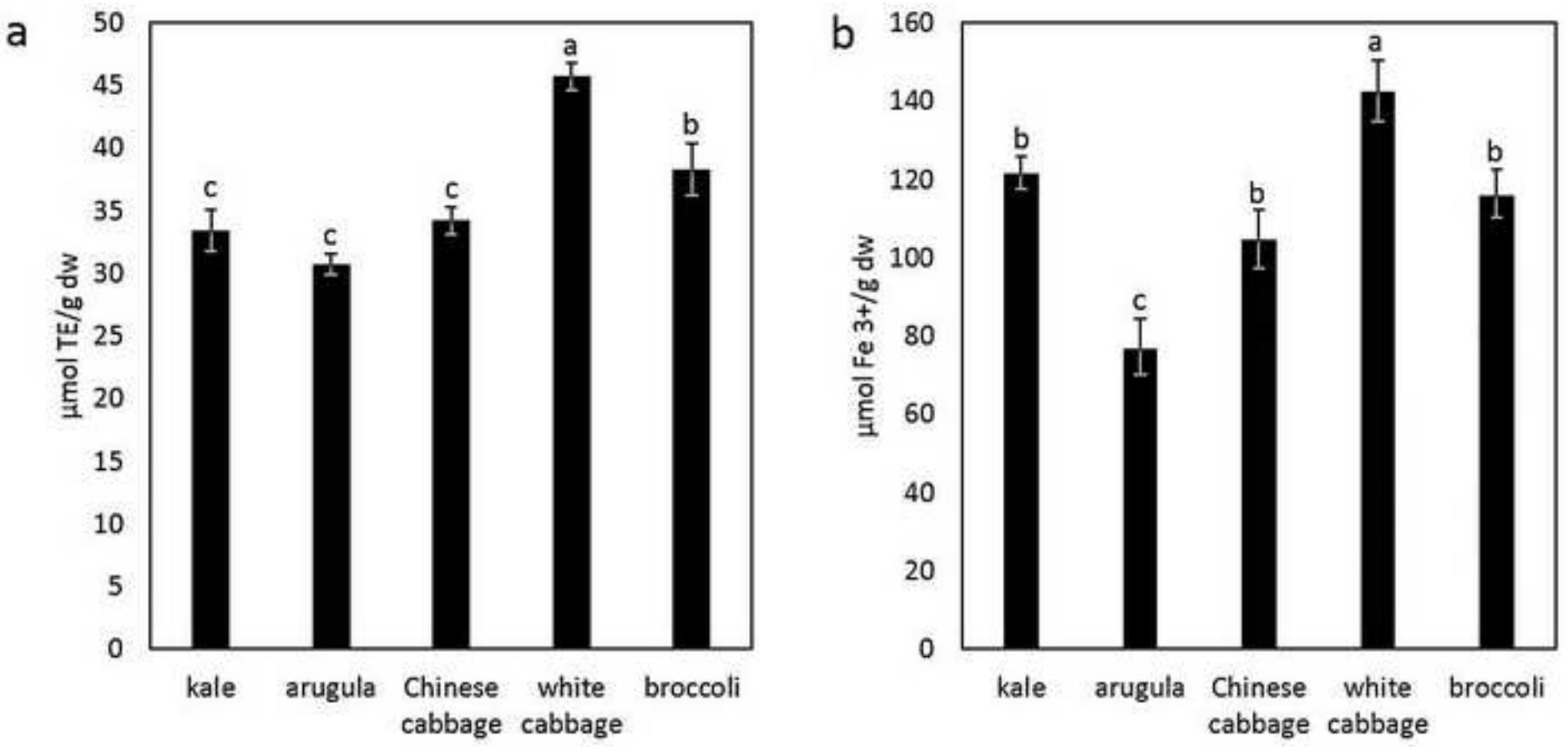

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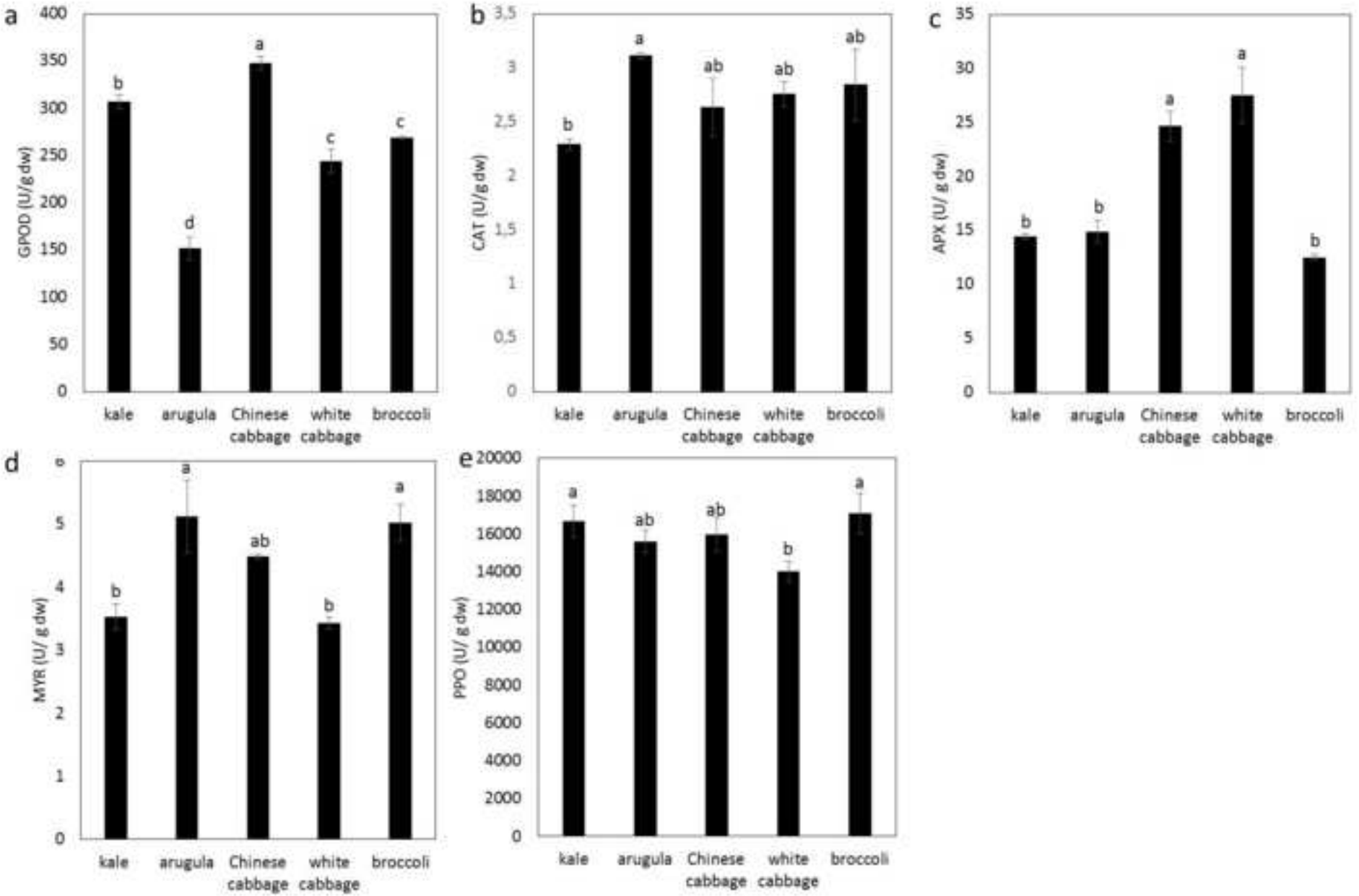

Figure 3

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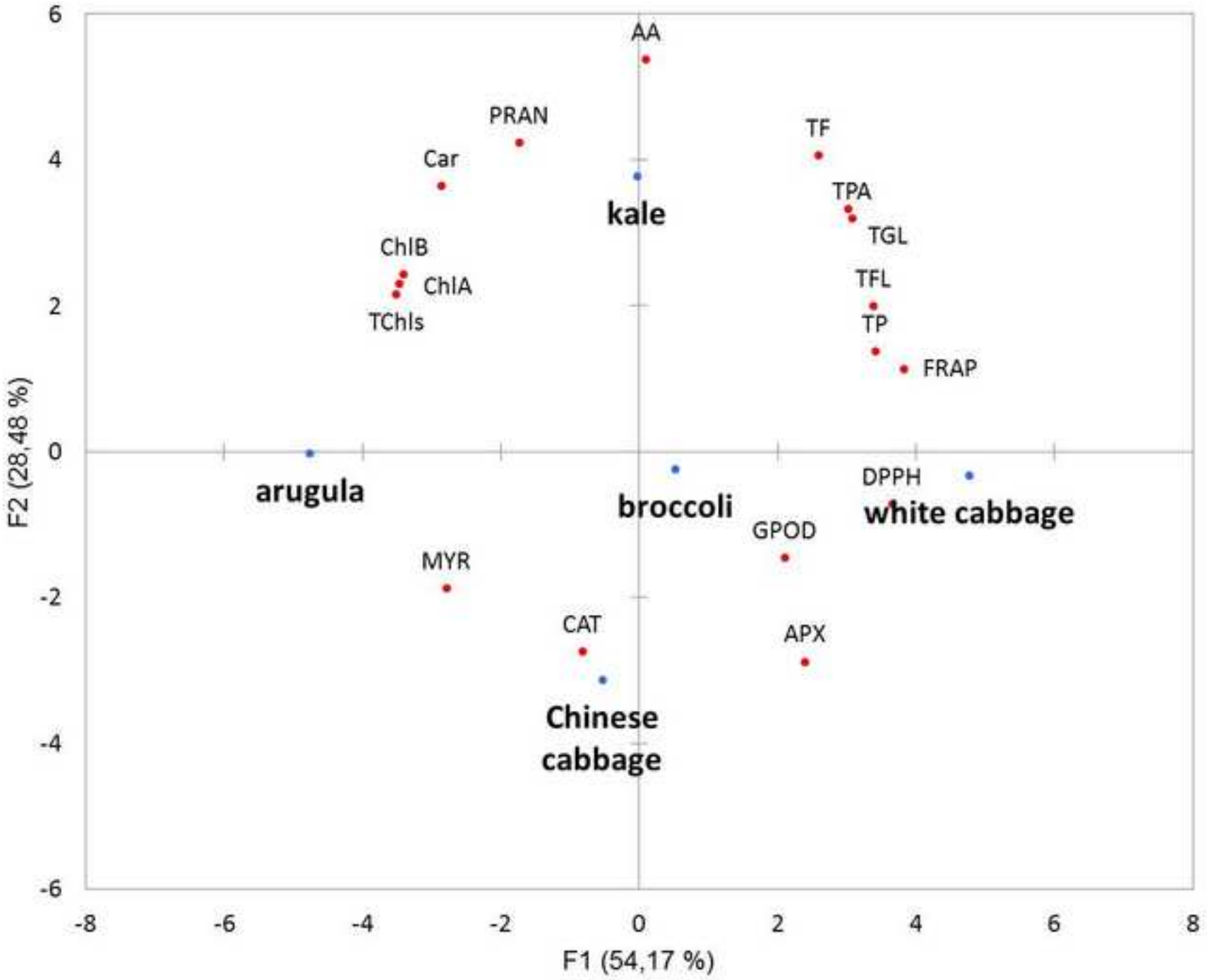

Supplementary Material

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