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Original research article

Assessment of glucosinolates, antioxidative and antiproliferative activity of broccoli and collard extracts

Kristina Radošević[a], Višnja Gaurina Srček[a], Marina Cvjetko Bubalo[a], Suzana Rimac Brnčić[a,*], Krisztina Takács[b], Ivana Radojčić Redovniković[a]

[a] Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia
[b] National Agricultural Research and Innovation Centre, Food Science Research Institute, Budapest, Hungary

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ABSTRACT

Natural compounds isolated from vegetables have protective and disease-preventing potential for the development of functional food. This study investigates the *in vitro* antioxidant capacity, total polyphenol and total flavonoid content, glucosinolate and glucosinolate degradation products of broccoli and collard extracts. *In vitro* antitumor activities on two human tumour cell lines (MCF-7 and HeLa) were studied. Broccoli and collard extracts were extracted from fresh, blanched and boiled broccoli florets and collard leaves. The Oxygen Radical Absorbance Capacity (ORAC) assay revealed that collard extracts exhibited higher antioxidant capacity than broccoli extracts. Total polyphenol and total flavonoid contents were also higher in collard extracts. The extracts were compared for the contents of total glucosinolates, indol glucosinolates and aliphatic glucosinolates. Glucoraphanin was the predominant compound in total glucosinolate content, followed by glucobrassicin and neoglucobrassicin (95.97 μmol 100 g^{-1} fresh weight (fw), 85.42 μmol 100 g^{-1} fw and 82.12 μmol 100 g^{-1} fw, respectively) in broccoli extracts; while in collard extracts, the major glucosinolate was glucobrassicin (80.33 μmol 100 g^{-1} fw), followed by glucoiberin (67.50) μmol 100 g^{-1} fw and sinigrin (21.91 μmol 100 g^{-1} fw). The strongest antiproliferative effect was observed in extracts obtained by blanching. The present study established that the extracts that were examined possess antioxidant and promising antitumour activities.

1. Introduction

Epidemiological studies have indicated that consumption of *Brassica* vegetables in general, and broccoli in particular, is associated with reduced risk of many noncommunicable diseases. These health-promoting effects of *Brassica* vegetables are generally attributed to bioactive compounds such as glucosinolate (GSL), derived degradation products and phenolic compounds (Latte et al., 2011; Sikora and Bodziarczyk, 2012; Kumar and Andy, 2012; Ares et al., 2013). Glucosinolates are sulphur-containing secondary metabolites present in all members of the Brassicaceae family. In broccoli (*Brassica oleracea* L. var. *italic*) the most predominant GSL are aliphatic glucosinolate glucoraphanin (GRA) and indole glucosinolates such as glucobrassicin (GBS) and neoglucobrassicin (neoGBS) (Radojčić Redovniković et al., 2012; Latte et al., 2011).

To become biologically active molecules, the glucosinolates must be hydrolysed by the enzyme myrosinase (thioglucoside-glucohydrolase; EC: 3.2.3.1). Myrosinase always accompanies the glucosionolates present in vegetables, and within intact plant tissue they are localised in distinct compartments. When the tissues structure is damaged (during mastification, cutting or processing), myrosinase is released and comes into contact with glucosinolates, causing rapid hydrolysis which forms isothiocyanates, thiocyanates, nitrile, epithionitriles or oxazolidine-2-thiones. The nature of the hydrolysis product depends on the structure of the glucosionolates (i.e. side chain structure) and reaction conditions such as pH, the presents of ferrous ions and epithiospecific proteins (ESP) (Bones and Rossiter, 2006). The most interesting degradation products formed in broccoli are sulforaphane (SF) and indole-3-carbinol (I3C, the hydrolysis product derived from glucoraphanin and glucobrassicin, receptively), which are recognized as chemopreventive and anticancer compounds (Weng et al., 2008).

* Corresponding author.
E-mail address: srimac@pbf.hr (S. Rimac Brnčić).

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Collard (*Brassica oleracea* L. var. *acephala*) has not been studied as extensively as other *Brassica* vegetables (such as cabbage, Brussels sprouts, broccoli and cauliflower, for example). According to Delonga et al. (2007), indole glucosinolates in collard leaves were the most abundant, with GBS predominating followed by 4-hidroxyglucobrassicin (4-OHGBC), 4-methoxyglucobrassicin (4-OMeGBS), and neoGBC. Aliphatic glucosinolates glucoiberin (GBI) and sinigrin (SIN) were also identified.

Brassica species also contain phenols, a group of more than 8000 different natural compounds which are characterised by the presence of one or more hydroxylated benzene rings. Phenols in plant foods are important because of their antioxidant activity and free radical-scavenging activities and generally positive effects on human health. Among phenols, the flavonoids exhibit highest antioxidant activity and comprise the largest group of phenolic compounds (Balasundram et al., 2006). Furthermore, *Brassica* vegetables are widely considered to possess high levels of antioxidative activity, and their phenolic compounds are assumed to be the main dietary antioxidants (Roy et al., 2009).

The content of biologically active compounds is influenced not only by species, conditions of cultivation and climate factors, but also by post-harvest storage conditions such as pre-handling treatments, atmosphere control or packaging films (Deng et al., 2015). It is known that both the content and bioavailability of phytochemical compounds of vegetables are highly affected by culinary processes such as washing, cutting, blending, chopping, juicing, boiling, steaming, blanching or freezing/thawing. Broccoli and collard are usually consumed after thermal treatment such as steaming or boiling, which commonly causes thermal degradation and/or leaching of glucosinolates and phenolics. The amount of glucosinolates and phenolics that is lost depends on the cooking method (cooking time, cooking temperature, ratio vegetable/water, etc.) (Palermo et al., 2014). Cooking methods also affect myrosinase activity, which can partially or completely inhibit the formation of the beneficial glucosinolates degradation products.

Fortunately, glucosinolate hydrolysis may occur in gastrointestinal tract under the action of the colonic tract microflora (Bones and Rossiter, 2006). Furthermore, the health effects of phytochemicals depend on that amount of bioactive compounds that can reach the systemic circulation. The bioavailability of bioactive compounds depends on numerous parameters (chemical structure, molecular weight, hydrophobicity, site of metabolism, pH, intestinal fermentations, biliary excretion, transit time, microbiota) (Holstand Williamson, 2004; Gawlik-Dziki et al., 2012).

Taking all of these factors into consideration, the objective of this study was to prepare extracts of broccoli florets and collard leaves after different cooking treatments in order to monitor the differences and/or changes in the amount of different biologically active compounds (glucosinolate and its degradation products, total phenolic compounds, total flavonoids). Valorisation of the prepared extracts was carried out by determining antioxidant capacity with the Oxygen Radical Absorbance Capacity (ORAC) assay, and biological activity by testing their antiproliferative effect on two human tumour cell lines (MCF-7 and HeLa).

2. Materials and methods

2.1. Chemicals

Methanol, acetonitrile and Folin–Ciocalteu reagent (109001) were obtained from Merck (Darmstadt, Germany). Quercetin-3-rutinoside, gallic acid, sinigrin standard (85440) (≥99%), thioglucosidase from Sinapis alba (T4528), sulphatase from Helix pomatia (S9626) ≥300 unit/mg solid β-glucuronidase, Fast DEAE Sepharose CL-6B, sulforaphane,indole-3-carbinol, indole-3-acetonitrile, 3,3′-diindolylmethane, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH) and fluorescein (FL) were purchased from Sigma–Aldrich Chemical Co (St. Louis, MO, USA). All chemicals were of analytical grade.

2.2. Plant material

Freshly harvested bunches of the wild-type collard (*Brassica oleracea var. acephala L.)* and broccoli florets were obtained from local markets in Zagreb (Croatia) and taken to the laboratory for immediate analysis. Random representative vegetables were selected. Five collard heads were trimmed of their outer leaves and stem. Vegetables were cleaned in tap water, then cut into small pieces (3–4 cm) of leaves and florets, respectively, to prepare for culinary heat treatments and extractions.

2.3. Culinary processes and extract preparation

Broccoli florets and collard leaves were homogenised using a laboratory blender (Moulinex, model DJ2000) for 1 min.

2.3.1. Blanching and boiling procedures

Cut broccoli florets (60 g) and collard leaves (60 g) were weighed out, respectively, and then submitted to blanching in 300 mL tap water for 5 min at 60 °C (samples abbr. B60, C60). Furthermore, cut broccoli florets (60 g) and collard leaves (60 g) were boiled, respectively, in 300 mL tap water for 5 min at 100 °C (samples abbr. B100, C100). All samples were drained after both blanching and boiling and left to cool on ice. The control samples were 60 g of untreated (fresh/raw) cut broccoli florets and collard leaves (samples abbr. B0, C0).

2.3.2. Extraction procedures

First, 60 g of both vegetables were extracted respectively in 60–60 mL PBS buffer (0.01 M, pH 7.4) for 2.5 h at room temperature (samples abbr. B0-P, B60-P, B100-P, C0-P, C60-P, C100-P). Extracts were filtered and the filtered extracts were stored at −18 °C.

Also, 4.5 g un-treated (fresh/raw) cut broccoli florets and collard leaves were extracted in 10 mL 70% methanol at 70 °C for 15 min in two steps, respectively. Extracts were filtered and the filtered supernatants from the two-step extraction were combined, and then filled up to 20 mL with 70% methanol, and then kept in the refrigerator at −18 °C. These samples (samples abbr. B0-M, C0-M) were used as classical extracted control samples.

An aliquot of all prepared extracts (10 mL) was extracted twice with 20 mL of dichloromethane. Supernatants were combined, salted using sodium sulfate (5 g), dried using a rotary evaporator (37 °C) and redissolved in acetonitrile prior analysis of glucosinolate degradation products. All extract were prepared in triplicate and were used for total phenolic content, total flavonoid, glucosinolates analysis and antioxidant capacity measurements.

2.4. Determination of glucosinolates

Determination of glucosionolates were carried out with high-performance liquid chromatography (HPLC) according to the ISO method (ISO, 1995) with some modification. One mL of vegetable extract was passed through an ion-exchange resin Fast DEAE Sepharose CL-6B microcolumn for desulphation with purified sulphatase (EC 3.1.6.1, type H-1, from *Helix pomatia*, Sigma, St. Louis, MO, USA). Desulphoglucosinolates (DSGSL) were eluted with 1.5 mL of deionised water. Desulphoglucosinolate extracts were separated on Supelcosil C18 reversed-phase column (150 × 4.6 mm I.D., 5 μm, Supelco Park, Belafonte, USA) using a Varian LC Star System equipped with a Star Solvent Delivery System 9010, Injector Rheodine 7125, Polychrom 9065 (Palo Alto, CA, USA). The