Journal of Food Composition and Analysis 61 (2017) 40–46

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original research article

Quantification and bioaccessibility of intact glucosinolates in broccoli 'Parthenon' and Savoy cabbage 'Dama'

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ARTICLE INFO

Article history:
Received 26 May 2016
Received in revised form 13 October 2016
Accepted 20 November 2016
Available online 21 November 2016

Keywords:
Brassicas
Food analysis
Food composition
Health-promoting compounds
Glucosinolates
In vitro
Gastrointestinal digestion
Bioaccessibility

ABSTRACT

Brassica has demonstrated to exhibit health benefits due to their content in bioactive compounds, such as glucosinolates (GS) among others. The aim of this work was to identify and quantify the intact GS of two brassicas, broccoli 'Parthenon' and Savoy cabbage 'Dama'. Additionally, the bioaccessibility of these compounds was evaluated by *in vitro* digestion procedure simulating gastric and small intestinal digestion. Glucobrassicin and glucobrassicanapin were the most abundant GS in both brassicas. The aromatic GS gluconasturtiin was only detected in broccoli. After *in vitro* gastrointestinal digestion, losses in GS content were found, 63% for broccoli and 69% for Savoy cabbage. The highest percentage of bioaccessibility was for the GS glucobrassicin in both brassicas (~42%). In general, GS bioaccessibility was higher in broccoli 'Parthenon' than in Savoy cabbage 'Dama'.

1. Introduction

The genus Brassica (*Brassicaceae* or *Cruciferae* family) includes a large number of cultivated plants of great agricultural interest, among them the most relevant is the *Brassica oleracea* species. Broccoli, cabbage, cauliflower, kohlrabi and mustard are the most known members of this family.

These vegetables are a good source of health-promoting compounds and potentially protective phytochemicals, including vitamins C and E, carotenoids, chlorophylls, phenolic compounds and isothiociantes (Kumar and Andy, 2012). All these compounds are characterized by their high antioxidant activity, as they may act to reduce reactive oxygen species (Podsedek, 2007). Many factors are the responsible for antioxidant activity variability of Brassica vegetables such as the cultivar, maturity at harvest, growing conditions, and post-harvest storage conditions (Singh et al., 2007).

Among the most consumed vegetables, broccoli appears to be one of the healthier, as well as different cultivated types of cabbage, because they are associated with the reduced risk of developing various chronic diseases (Dominguez-Perles et al., 2011; Temple, 2000). In addition, different studies have shown an inverse association between cruciferous vegetable intake and cancer risk in many tissues including lung, bladder and prostate (Steinbrecher et al., 2009; Tang et al., 2010).

Glucosinolates are sulfur-based compounds that consist of β-D-thioglycoside *N*-hydroxysulfates with various side chains and a sulfur-linked β-D-glycopyranose moiety. Glucosinolates are not always bioactive compounds in cruciferous vegetables; their hydrolysis products, isothiocyanates, are the bioactive compounds (Fahey et al., 2001) as they have shown protective effects against cancer (Fahey et al., 2001; Keck et al., 2002; Keck and Finley, 2004; Barba et al., 2016). Some of them were: sulforaphane derived from glucoraphanin, phenethyl isothiocyanate derived from gluconasturtiin, allyl isothiozante from sinigrin, indole-3-carbinol from glucobrassicin and crambene from progoitrin (Clarke et al., 2011;

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http://dx.doi.org/10.1016/j.jfca.2016.11.010
0889-1575/

Glade and Meguid, 2015; Keck and Finley, 2004; Schreiner et al., 2006, 2007).

Within cruciferous vegetables, there are different glucosinolates (Fernández-León et al., 2011, 2012, 2014), each yielding a different isothiocyanates (Tian et al., 2005). The glucosinolates hydrolysis is dependent upon a β-thioglucoside glucohydrolase enzyme called myrosinase (Barba et al., 2016). Clarke et al. (2011) reported that when humans consume Brassica vegetables the only sources of myrosinase activity are endogenous plant enzymes and intestinal microbiota; no evidence that mammalian cells are able to metabolize glucosinolates.

In most humans, the extent of activation of myrosinase within the human oral cavity and the rapid release of the isothiocyanates determine its overall bioavailability from brassicas (Oliviero et al., 2014). Some isothiocyanates have been investigated for their anticancer properties and there is a preponderance of evidence from *in vitro* studies in cell culture over *in vivo* studies, with a major number of *in vitro* studies, because of being a faster method. An important area of research about brassicas and cancer prevention is a better understanding of the bioavailability of bioactive isothiocyanates after human consumption of glucosinolates (Clarke et al., 2011). The concept of a compound bioaccessibility has been defined as the fraction released from the food matrix in the gastrointestinal tract that becomes available for absorption (Carbonell-Capella et al., 2014).

This research was designed to identify and quantify the glucosinolates of two brassicas, broccoli 'Parthenon' and Savoy cabbage 'Dama'. In addition, a comparison study was completed to assess the bioaccessibility of these compounds after the process of *in vitro* gastrointestinal digestion. Having precise information about the bioaccessibility of glucosinolates will be useful to a better understanding about the beneficial properties of these vegetables, as consumers demand food products with high nutritional value, health benefits, and high quality standards.

2. Materials and methods

2.1. Plant material

Broccoli (*Brassica oleracea* L. var. *italica* Plenck) 'Parthenon' and Savoy cabbage (*Brassica oleracea* L. var. *sabauda*) 'Dama' were used in this study as they had shown the best agronomic, nutritional and functional characteristics in previous studies (Fernández-León et al., 2012, 2014). A total of 6 fresh head samples were analyzed for each cultivar of broccoli and Savoy cabbage. The plants were purchased from a local producer and rapidly transported to the laboratory after harvest. Savoy cabbage leaves were randomly selected, external, middle and internal leaves from the cabbage heads. Both broccoli and Savoy cabbage were processed separately, performing on the same day the *in vitro* digestion of both brassicas.

2.2. Intact glucosinolates determination

Intact glucosinolates were extracted following Kiddle and Vallejo slightly modified methods (Kiddle et al., 2001; Vallejo et al., 2003). The extraction was performed with methanol:water (70:30, v:v) and heating at 70 °C for 15 min to inactivate the enzyme myrosinase and to preserve intact glucosinolates during extraction process (Moreno et al., 2006).

A high-performance liquid chromatography instrument coupled to an Ion Trap mass spectrometer (Varian 500-MS, Varian Ibérica S.L., Madrid, Spain) was used for the intact glucosinolates separation and determination from the crude and digested sample extracts.

The chromatographic separation was performed on a 150 mm × 2.0 mm (3 μm) Pursuit C18 reversed-phase column with a Varian security guard column. The column oven temperature was set at 40 °C and the injected volume was 10 μL. The composition of the mobile phase varied linearly from 100% A (0.1 mL formic acid/100 mL) to 15% B (methanol MS-MS) in 15 min, to 40% B in 5 min, to 50% B in 5 min, and returned to 100% A in 5 min at a flow rate of 200 μL/min. The mass spectrometer was tuned by direct infusion of sinigrin standard solution producing maximum abundant precursor ion m/z 358 ($[M\text{-}H]^-$) and fragment ion m/z 97 ($[SO_3H]^-$) signals during MS/MS (Tian et al., 2005). The following transitions were used to assay 11 individual glucosinolates (Fig. 1): glucoraphanin (436 > 97), glucoiberin (422 > 97), sinigrin (358 > 97), progoitrin (388 > 97), glucobrassicin (447 > 97), glucoalysin (450 > 97), gluconasturtiin (422 > 97), 4-methoxyglucobrassicin (477 > 97), neoglucobrassicin (477 > 97), gluconapin (372 > 97) and glucobrassicanapin (386 > 97). These glucosinolates were quantified using the calibration curve of sinigrin (Sigma-Aldrich) as an external standard and expressed as mg sinigrin equivalent/100 g of fresh weight (Fernández-León et al., 2011). Sinigrin standard was purchased from Sigma-Aldrich SA. (Madrid, Spain).

2.3. Simulated gastrointestinal digestion

In order to assess the bioaccessibility of glucosinolates, 6 samples of broccoli and Savoy cabbage were subjected to *in vitro* digestion process, thus obtaining 6 independent extracts for each digested brassica, n = 6.

The employed method simulates the gastric and intestinal phases of the human gastrointestinal digestion process. The *in vitro*

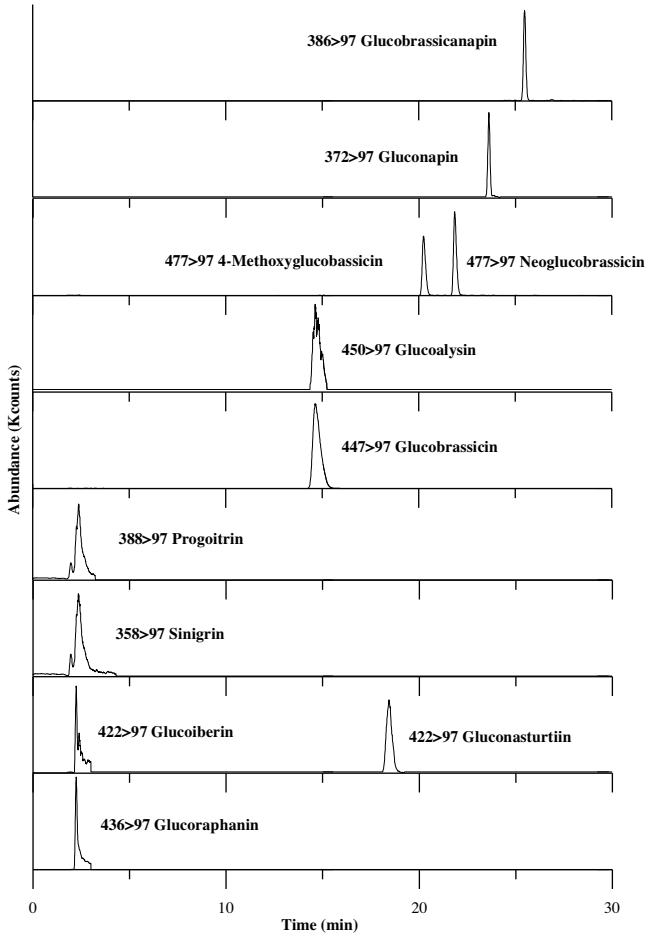

Fig. 1. Mass chromatograms of glucosinolates in broccoli using negative ion LC–ESI/MS–MS.