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Quantitative profiling of glucosinolates by LC–MS analysis reveals several cultivars of cabbage and kale as promising sources of sulforaphane

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ABSTRACT

Sulforaphane is an isothiocyanate well known for its potential health benefits. With the aim of finding sulforaphane supply sources, its precursor, glucoraphanin, was widely searched for among *Brassica oleracea* varieties. Quantitative profiling of seven glucosinolates by LC–MS analysis was performed on 6 cultivars of broccoli, 32 of cabbage and 24 cultivars of kale. The glucoraphanin levels found in three cultivars of cabbage and six cultivars of kale were comparable with, or even higher than, the highest of broccoli (119.4 mg/100 g FW). The most promising group belonged to the black kale, *Cavolo nero*. Use of a C30 column and an ammonium formate buffer in LC–MS and a micro plate solid phase extraction technique was highly effective.

1. Introduction

Cole crops (*Brassica oleracea* L.) are important vegetables in many countries. One of the characteristic features of the crops is the presence of phytochemicals known as glucosinolates (GSLs, Fig. 1, [1]). They are precursors of isothiocyanates (Fig. 2), which not only add bitter and/or pungent taste to the vegetables but also are expected to exert multiple health benefits, e.g., inhibition of infectious *Helicobacter pylori* and a cancer preventive activity [2–5]. The most famous GSL, glucoraphanin (GR), produces an isothiocyanate called sulforaphane (SR, Fig. 2) by the catalytic action of myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1, Fig. 2). SR reportedly possesses numerous beneficial bioactivities [6–9]. The mechanism of action for isothiocyanates associated with anticarcinogenic activity has been reviewed elsewhere [2,10,11]. Prompted by the reports of potential beneficial functions, the occurrence of GR, the precursor of SR, was widely explored among Cole crops. The current consensus is that the flower buds and sprouts of broccoli are the best source of SR [12].

In view of the limited number of tested versus the vast numbers of un-tested Cole crops, we felt it worthwhile to carry out a further search for GR. The isothiocyanates can be conveniently evaluated by measuring the precursor GSLs, instead of the unstable intermediates produced during the conversion steps (Fig. 2), as proposed by [10,13]. To facilitate analysis of a large number of samples, we upgraded the clean-up procedure and analytical conditions of LC–MS. In addition to the potential health benefits of GSLs, attention was also paid to the contents of progoitrin, the precursor of the goiter-causing goitrin. Seeds and seedlings were obtained from established sources and grown under controlled conditions to minimize variation. Here we report quantitative GSL profiles in 6 cultivars of broccoli, 32 of cabbage and 24 of kale. From this, we identified several cultivars of cabbage and kale as promising sources of SR.

2. Materials and methods

2.1. Chemicals

All reagents used for HPLC were of chromatographic grade, purchased from Wako Pure Chemicals (Osaka, Japan). Sinigrin (SG) was purchased from Wako Pure Chemicals and the other five GSLs, glucoiberin (GI), progoitrin (PG), glucoraphanin (GR), glucoerucin (GE) and sinalbin were purchased from PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany). Glucobrassicin (GB), 4-methoxy GB (4mGB) and 1-methoxy GB (1mGB) were prepared at our laboratory from kale leaves and proven to be pure by spectrometry.

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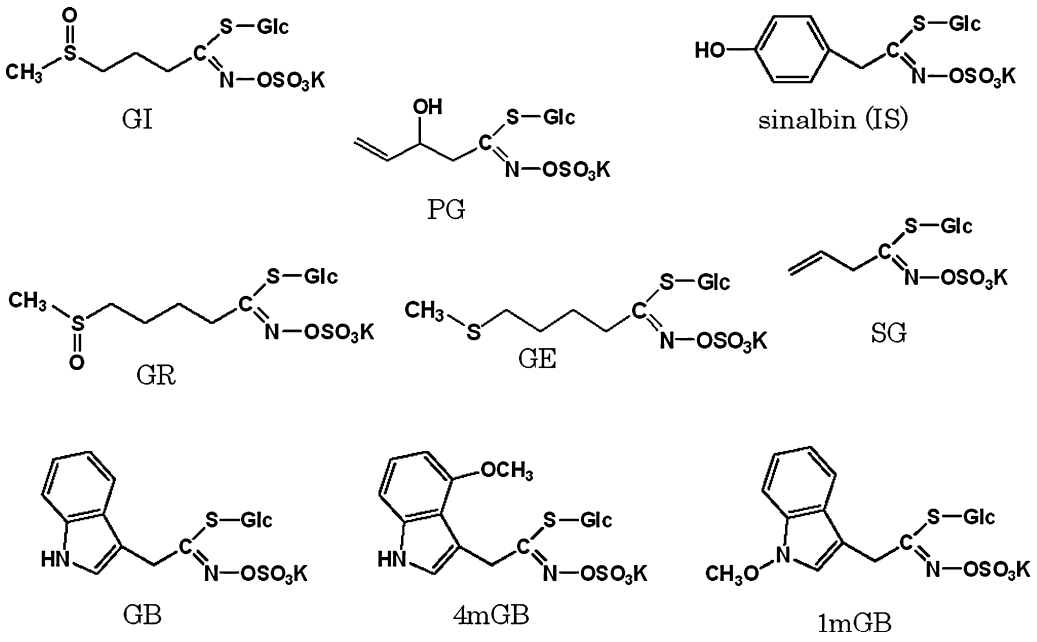

Fig. 1. Structures of GSLs detected in *B. oleracea* crops and sinalbin used as an internal standard (IS). GI: glucoiberin, PG: progoitrin, SG: sinigrin, GR: glucoraphanin, GE: glucoerucin, GB: glucobrassicin, 4mGB: 4-methoxyglucobrassicin, and 1mGB: 1-methoxyglucobrassicin.

2.2. Samples and extraction procedure

2.2.1. Samples

Seeds of 62 commercial cultivars of *B. oleracea* crops were purchased from seed companies in Japan (Sakata Seed, Yokohama; Takii, Kyoto; Tohoku Seed, Utsunomiya), UK (Thompson & Morgan, Suffolk; Chilternseeds, Cumbria; Franchi Seeds, London Borough of Harrow) and France (B & T World Seeds, Aigues-Vives). Traditional Japanese cultivars of kale and cabbage (non F_1 hybrid) were obtained from the National Institute of Agrobiological Sciences (Tsukuba, Japan). Seeds were sown in pots filled with compost for vegetables and five plants of each cultivar were grown in a green house under natural daylight at 23 °C, controlled by air-conditioners. All lines were cultivated in the same environment with consistent agronomic measurements. Three months after germination, fully developed young leaves were sampled, between 13:00 and 15:00 pm. Each sample was weighed and immediately blanched using 0.1% (v/v) formic acid in 80% (v/v) MeOH aq. to extract GSLs. Cultivars were kept for further growth after sampling and used to verify the characteristic traits of the cultivar.

2.2.2. Extraction procedure

After addition of 995 μL of 0.1% (v/v) formic acid in 80% (v/v) MeOH aq. to a sample tube containing a weighed sample of fresh leaves (50–150 mg) and a zirconia bead (Ø 5 mm), the mixture was milled using a mixer mill, 25 times/s for 5 min (Retsch MM301, Retsch GmbH, Haan, Germany). Then, 5 μL of sinalbin (15 μg/μL) was added as an internal standard (IS, Fig. 1). After centrifugation (10,000 × *g*, RT, 5 min), ion exchange solid phase extraction (SPE) was performed. An aliquot of 100 μL of the supernatant was loaded onto an Oasis WAX 96-well plate, particle size: 30 μm, weight: 30 mg (Waters, Milford, MA, USA) which had been previously washed with 1 mL of MeOH and activated with 2% (v/v) formic acid in water. The wells were washed with 1 mL of 2% formic acid in water and 1 mL of MeOH. The GSL fraction was finally recovered from the resin with 900 μL of a freshly prepared 5% (v/v) solution of concentrated NH_4OH aq. in MeOH. Then, 500 μL of the fraction was dried using a centrifugal evaporator (EZ-2 plus, Genevac, Gardiner NY, USA) and dissolved in the same volume of 0.1% (v/v) formic acid in water. An aliquot of 10 μL was subjected to LC–ESI-MS analysis. Each analysis was carried out in triplicate.

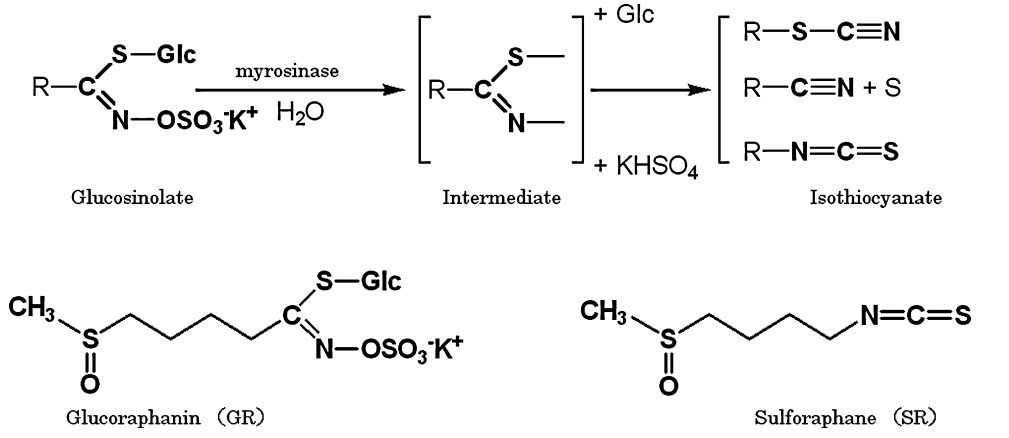

Fig. 2. Structure of sulforaphane derived from glucoraphanin, which is catalyzed by myrosinase.

2.3. Instruments

High resolution time of flight mass spectrometry (HR-TOF-MS) spectra were measured on an Agilent G1969A (Agilent Technologies, Santa Clara, CA, USA) operating in the infusion mode to detect the negative ion. ^{1}H NMR spectra were generated on a Bruker AMX-400 (400 MHz, Bruker BioSpin, Billerica, MA, USA) at 17 °C in D_2O.

2.4. HPLC

2.4.1. LC–ESI-MS

The LC–ESI-MS system consisted of a Shimadzu (Kyoto, Japan) LCMS-2010 EV mass spectrometer, two Shimadzu LC-20AD pumps and the Shimadzu SPD-M20A photodiode array detection (DAD) system coupled to FUJITSU FMV ESPRIMO personal computer to regulate the system using LCMS solution ver. 1.0.0.1 (Shimadzu). The wavelengths were set at 200–400 nm, mainly to monitor the gradient system. The conditions of the LC system were as follows: the samples were loaded onto the reversed phase HPLC system with a C30 column (Develosil RPAQUEOUS-AR-3, 3 μm, 150 mm × 2.0 mm, Nomura Chemical, Seto, Japan). Two solvents were used for the elution: solvent A was 10 mM ammonium formate in water (pH 3.75) and solvent B was 10 mM ammonium formate in acetonitrile:water at a 50:50 ratio (v/v, pH 3.75). GSLs were eluted using a linear gradient system from 0% to 60% (v/v) of solvent B for 0–10 min, 60% (v/v) to 100% of solvent B for 10.1–15 min and 0% of solvent B for 15.1–25 min at the flow rate of 0.2 mL/min. The temperature of the column oven was 40 °C. The mass instrument was regulated by Shimadzu LCMS solution software, as mentioned above. Auto tuning set all parameters. Mass data were obtained from *m/z* 300 to 500 in the scan mode (negative ion).

2.4.2. Preparative HPLC to purify GB, 4mGB and 1mGB

Three indole GSLs, glucobrassicin (GB), 4-methoxyGB (4mGB) and 1-methoxyGB (1mGB) were not commercially sold and thus were purified from kale leaves (var. *acephala* DC). The leaves (1 kg, FW) were autoclaved (100 °C, 10 min) to inactivate myrosinase and the other enzymes for blanching, and then boiling water was added to make up to 3 L and the samples were homogenized using a juicer mixer. After centrifugation (1460 × *g*, 10 min), the supernatant was taken and made up to 3 L with boiling water. Aliquots of 15 mL were loaded onto Waters Sep-Pak Vac amino propyl cartridges (10 g) and subjected to ion exchange purification, as described in the former section. A 500 μL aliquot of the GSL fraction was injected into a HPLC column (XBridge Shield RP_{18} 5 μm, 250 mm × 10 mm, Waters). The HPLC system consisted of two Hitachi L-7000 pumps and a Hitachi L-7455 DAD system coupled to a Hitachi Flora Mobile Intel Pentium personal computer regulating the system using both D-7000 HSM and D-7000 MSM applications. Solvent A was 10 mM ammonium hydrogen carbonate in water (pH 10.0) and solvent B was 10 mM ammonium hydrogen carbonate in acetonitrile:water at a 50:50 ratio (v/v, pH 10.0). Indole GSLs were eluted using a linear gradient system from 10% (v/v) to 100% of solvent B for 0–20 min, 100% of solvent B for 20.1–30 min, 10% (v/v) of solvent B for 30.1–40 min at the flow rate of 2.0 mL/min, monitoring at 280 nm to specifically detect each indole moiety. The temperature of the column oven was 40 °C. Ammonium hydrogen carbonate underwent thermal decomposition to CO_2, NH_3 and H_2O during evaporation. The identity and purity of individual GSLs were confirmed by HR-TOF-MS analysis and measurements of ^{1}H NMR spectra.

3. Results and discussion

3.1. Preparation of reference GSL

From the leaves of kale (3 kg), GB (8.7 mg), 4mGB (3.6 mg) and 1mGB (6.8 mg) were isolated as ammonium salts. The HR-TOF-MS data agreed well with the molecular formula of the respective compounds: GB, *m/z* 447.0528, calculated for $[C_{17}H_{21}N_2O_{10}S_2]^-$, *m/z* 477.0533, 4mGB, *m/z* 477.0633, calculated for $[C_{17}H_{21}N_2O_{10}S_2]^-$, *m/z* 477.0638 and 1mGB, *m/z* 477.0633, calculated for $[C_{17}H_{21}N_2O_{10}S_2]^-$, *m/z* 477.0638. The ^{1}H NMR spectra agreed well with those reported for GB [14], 4mGB [15] and 1mGB [16]. Thus, both the HR-MS and ^{1}H NMR spectra proved the identity of the three GSLs isolated.

3.2. Separation and detection

Fig. 3 shows a typical chromatographic profile of GSLs isolated from *B. oleracea*. Of more than 200 reported GSLs [1], seven GSLs (GI, PG, SG, GR, GB, 4mGB and 1mGB) are known to be dominant in the Cole crops, whereas GE is detectable only in seeds and sprouts.

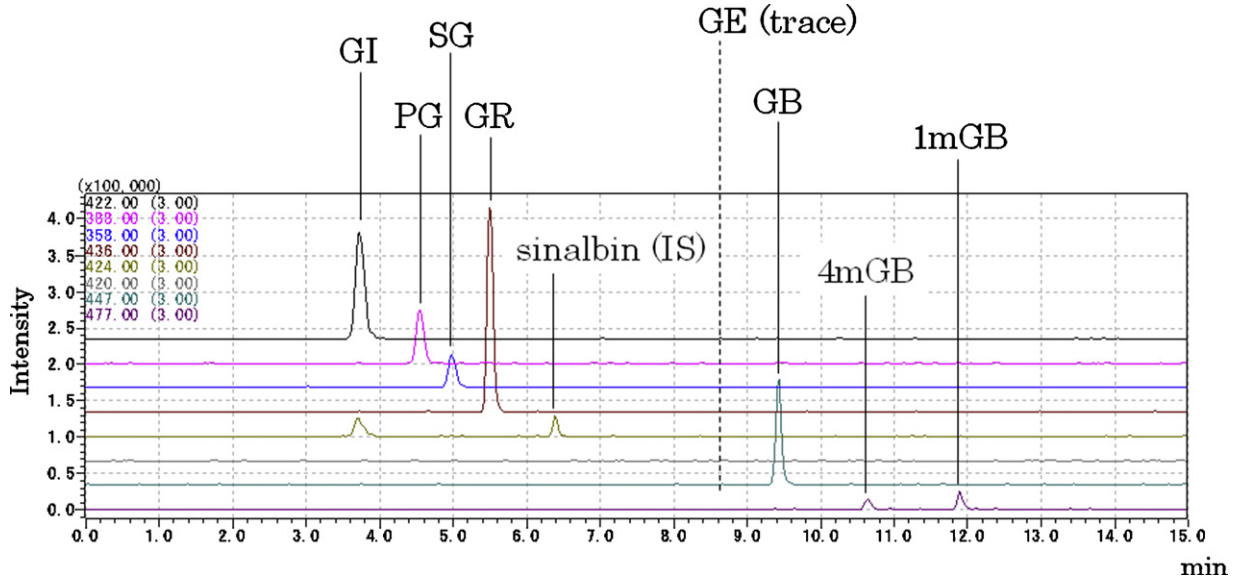

Fig. 3. Typical mass chromatograms of GSLs contained in *B. oleracea* using LC–ESI-MS (negative ion mode).

Table 1
Glucosinolate profiles of different cultivars of the broccoli variety expressed as mg/100 g[a] fresh leaves.

Cultivar	GI	PG	SG	GR	GB	4mGB	1mGB
B1	ND	ND	ND	119.4 (26.6)	22.9 (3.6)	25.0 (2.9)	31.1 (6.6)
B2	27.9 (23.3)	ND	ND	73.2 (53.2)	7.3 (4.7)	15.3 (12.6)	23.5 (38.2)
B3	12.1 (6.5)	ND	ND	42.2 (36.1)	13.9 (11.4)	12.6 (6.6)	16.0 (8.8)
B4	ND	ND	ND	86.3 (12.0)	14.9 (3.7)	12.9 (1.8)	10.3 (5.0)
B5	50.9 (25.0)	ND	3.2 (7.2)	12.2 (11.7)	9.7 (5.5)	8.8 (6.9)	10.3 (10.0)
B6	67.4 (26.0)	ND	ND	23.7 (26.9)	15.0 (3.9)	6.8 (1.9)	3.1 (1.8)

ND denotes "Not Detected".
[a] Average of five samples. Figures in parenthesis denote standard deviation (SD).

Therefore, the seven references should be enough to evaluate the GSL profiles of the cultivars tested.

A linear regression of the calibration curves of individual standards was obtained for the eight GSLs, from 20 to 1200 ng, with a regression coefficient (R^2) greater than 0.99. For all of them, the calculated LOD (S/N = 3) was 5.0 ng/10 μL and LOQ (S/N = 10) was 16.4 ng/10 μL.

Sinalbin was selected as the internal standard (IS) (Fig. 1), because it was present only in a trace amount in samples tested and showed good recovery (85–105%) when spiked. The Oasis WAX SPE micro plates, which operate in a mixed mode of ion exchange and reversed phase, ensured rapid and effective clean-up of GSLs which possess a sulfate moiety. Using eleven 96-well plates, 1000 samples could be handled within 2 h, efficiency unmatched by the cartridge method. The separation efficiency was unaffected by the flow-through of air.

Compounds containing ionized sulfate moieties are difficult to quantify, as they tend to drift during HPLC separation [17,18]. Intact GSLs possessing sulfate can be analyzed by reversed phase HPLC employing TFA [19]. Agerbirk et al. reported that ammonium formate was effective for ion pairing with sulfate moieties in LC–ESI-MS [20,21]. This buffer was also superior to TFA with respect to peak shape [20,21]. Use of the ammonium formate buffer (pH 3.75) in combination with a C30 column proved to be effective. The retention times were reproducible on a C30 column (within ±0.02 min, $n = 50$) but fluctuated on a C18 column. Replacing the ammonium formate buffer with formic acid resulted in disfiguration or drift of peaks. Reproducible retention times enabled us to detect and quantify individual GSL automatically. The ammonium formate buffer (pH 3.75) is benign to both the column and operating persons. The sulfate moiety helped detection of GSLs at high sensitivity in ESI-MS in a negative ion mode.

3.3. GSL profiles of individual Cole crops

Tables 1–3 show the GSL profiles of broccoli, cabbage and kale respectively. The GSL contents varied widely despite care being taken to keep the conditions homogeneous. Nevertheless, it was possible to compare the profile and amount of GSLs among different cultivars. The broccoli variety tended to have a high GR content (Table 1). Some cultivars contained glucoiberin (GI, Fig. 1), which has a side chain one methylene shorter than GR, and which was

Table 2
Glucosinolate profiles of different cultivars of the cabbage variety expressed as mg/100 g[a] fresh leaves.

Cultivar	GI	PG	SG	GR	GB	4mGB	1mGB
C1	46.8 (12.9)	52.4 (45.3)	56.2 (20.4)	54.4 (26.1)	9.3 (2.8)	5.4 (3.5)	ND
C2	63.5 (18.8)	85.9 (33.6)	82.9 (24.9)	74.4 (32.0)	21.5 (1.8)	9.5 (5.4)	ND
C3	28.0 (14.1)	12.7 (11.6)	11.0 (7.9)	80.7 (61.7)	67.0 (49.5)	5.8 (0.7)	11.3 (5.3)
C4	20.8 (22.2)	0.5 (0.3)	1.8 (1.7)	10.7 (11.0)	26.7 (23.5)	1.1 (2.4)	4.2 (4.9)
C5	49.1 (4.3)	21.8 (21.9)	16.9 (15.4)	75.9 (34.0)	52.8 (34.6)	12.5 (6.8)	3.7 (2.1)
C6	36.2 (5.1)	1.1 (0.5)	17.6 (6.5)	3.5 (0.9)	35.8 (15.8)	ND	2.6 (0.6)
C7	52.7 (7.9)	1.1 (0.6)	10.7 (3.2)	28.5 (20.1)	61.6 (13.7)	1.8 (2.1)	4.2 (3.5)
C8	22.4 (14.9)	0.8 (0.5)	5.7 (6.7)	9.8 (11.4)	83.4 (74.6)	5.3 (0.8)	3.0 (1.5)
C9	10.0 (5.9)	1.1 (0.4)	9.9 (11.6)	0.6 (0.7)	27.0 (9.8)	1.3 (1.5)	2.4 (1.8)
C10	32.7 (10.7)	3.9 (3.7)	3.0 (1.2)	58.0 (48.2)	60.0 (34.8)	3.2 (3.0)	3.1 (2.0)
C11	47.3 (13.1)	13.3 (12.1)	16.8 (19.1)	61.5 (35.2)	37.7 (16.7)	4.1 (2.9)	3.7 (2.9)
C12	67.0 (14.1)	4.4 (4.3)	16.5 (3.6)	45.6 (43.5)	65.4 (15.0)	4.6 (1.6)	16.0 (18.7)
C13	36.8 (12.2)	0.3 (0.2)	1.7 (1.0)	12.2 (5.5)	55.6 (8.6)	1.8 (2.1)	11.1 (5.7)
C14	47.8 (21.5)	1.4 (1.5)	11.4 (9.2)	14.7 (15.4)	50.3 (9.4)	4.0 (1.6)	15.3 (9.6)
C15	61.6 (13.5)	0.1 (0.1)	12.2 (6.4)	4.8 (1.5)	71.4 (10.8)	39.7 (46.5)	16.3 (10.2)
C16	46.6 (11.6)	0.1 (0.1)	3.2 (3.4)	13.8 (12.7)	48.5 (7.1)	4.8 (1.9)	10.0 (5.2)
C17	36.0 (21.7)	1.1 (1.5)	7.2 (4.5)	28.8 (44.4)	33.6 (21.1)	2.8 (2.6)	9.2 (6.8)
C18	32.0 (23.9)	20.2 (13.6)	8.6 (6.1)	106.3 (56.5)	15.4 (4.6)	4.8 (1.9)	ND
C19	28.5 (9.5)	15.3 (8.8)	9.0 (2.8)	92.8 (34.8)	24.2 (14.5)	2.4 (2.6)	9.3 (3.2)
C20	49.7 (30.0)	12.8 (15.2)	20.1 (13.3)	58.3 (54.6)	18.8 (2.2)	ND	6.5 (0.8)
C21	53.7 (16.3)	8.0 (8.5)	17.7 (14.4)	35.4 (25.1)	23.2 (11.2)	1.4 (1.9)	8.4 (5.6)
C22	27.6 (5.7)	2.4 (3.0)	5.7 (1.5)	22.3 (7.5)	15.3 (6.0)	6.0 (8.0)	1.1 (0.9)
C23	70.1 (30.5)	16.1 (17.8)	36.2 (20.8)	38.9 (18.4)	20.9 (10.5)	6.1 (5.9)	2.8 (0.9)
C24	23.9 (9.7)	26.2 (21.7)	17.5 (20.5)	54.8 (25.2)	28.2 (13.5)	1.0 (2.2)	4.6 (1.4)
C25	22.0 (7.7)	17.8 (18.1)	17.0 (18.7)	47.4 (27.8)	28.8 (15.8)	2.4 (2.7)	2.2 (2.0)
C26	39.0 (14.3)	19.3 (11.7)	14.5 (14.4)	106.2 (54.5)	43.2 (14.2)	0.9 (2.0)	7.6 (11.8)
C27	36.2 (23.2)	0.3 (0.3)	12.1 (2.2)	0.9 (1.5)	22.4 (16.3)	18.2 (21.4)	1.3 (0.4)
C28	98.0 (54.3)	0.6 (1.4)	76.9 (27.7)	1.9 (2.6)	13.9 (5.3)	2.9 (1.9)	1.8 (1.3)
C29	116.0 (45.9)	86.3 (44.1)	80.3 (17.3)	153.9 (92.2)	13.0 (7.2)	1.7 (3.4)	1.2 (0.8)
C30	38.9 (24.1)	18.8 (11.6)	12.2 (7.5)	79.5 (23.1)	11.0 (6.3)	1.9 (1.9)	ND
C31	71.2 (11.5)	5.9 (3.2)	52.7 (18.1)	12.7 (7.1)	3.5 (3.4)	3.4 (2.1)	0.4 (1.0)
C32	76.0 (22.9)	29.3 (24.8)	79.2 (63.4)	32.7 (39.9)	12.7 (5.8)	35.3 (9.0)	ND

ND denotes "Not Detected".
[a] Average of five samples. Figures in parenthesis denote standard deviation (SD).

Table 3
Glucosinolate profiles of kale varieties. Amount (μg/100 mg[a] fresh weight).

Cultivar	GI	PG	SG	GR	GB	4mGB	1mGB
K1	36.4 (7.0)	12.6 (8.8)	39.4 (20.6)	17.5 (7.5)	9.0 (1.8)	10.6 (3.7)	ND
K2	0.7 (1.2)	44.4 (45.0)	74.1 (64.2)	25.0 (40.9)	15.8 (0.8)	11.4 (8.5)	76.0 (33.3)
K3	ND	ND	6.4 (7.8)	ND	6.6 (2.9)	3.8 (1.0)	0.4 (0.2)
K4	8.4 (1.7)	0.9 (1.3)	ND	31.0 (11.0)	7.9 (1.3)	4.7 (0.7)	0.7 (0.9)
K5	ND	2.3 (3.2)	76.3 (26.5)	ND	17.7 (3.2)	2.5 (1.1)	0.2 (0.2)
K6	15.0 (6.3)	5.7 (5.0)	20.8 (14.6)	10.7 (5.7)	12.9 (7.3)	1.4 (2.4)	0.2 (0.3)
K7	78.5 (53.1)	0.8 (1.3)	7.0 (10.5)	7.4 (7.8)	7.0 (3.3)	1.9 (3.2)	ND
K8	5.4 (2.8)	ND	11.3 (15.1)	0.8 (1.4)	6.0 (2.0)	2.7 (4.7)	0.1 (0.1)
K9	29.6 (16.5)	4.2 (4.5)	27.4 (24.1)	22.3 (16.6)	16.5 (3.6)	7.3 (4.0)	1.0 (0.6)
K10	60.9 (19.2)	9.7 (14.0)	51.1 (27.8)	18.5 (7.2)	11.7 (1.7)	2.2 (3.7)	0.7 (1.3)
K11	72.2 (13.3)	6.4 (2.5)	26.5 (11.0)	14.5 (5.7)	27.0 (10.0)	0.9 (0.6)	2.0 (0.9)
K12	11.1 (15.6)	2.6 (3.3)	26.4 (14.2)	1.6 (1.8)	19.8 (13.7)	2.5 (1.1)	2.5 (0.7)
K13	2.1 (2.9)	23.5 (5.4)	112.0 (27.1)	ND	27.6 (4.4)	2.1 (1.9)	4.1 (2.7)
K14	ND	29.1 (13.8)	ND	129.8 (9.9)	30.2 (4.5)	8.0 (6.2)	0.7 (0.5)
K15	42.5 (15.0)	8.7 (2.1)	74.8 (22.3)	23.2 (14.4)	2.7 (2.0)	ND	ND
K16	0.0 (0.0)	2.6 (2.1)	245.8 (39.5)	ND	0.4 (0.6)	3.5 (2.0)	ND
K17	119.8 (44.6)	2.8 (4.9)	23.9 (12.1)	10.3 (10.4)	14.0 (6.6)	2.6 (2.0)	3.1 (2.0)
K18	1.1 (1.4)	127.2 (60.0)	119.1 (27.3)	1.7 (2.0)	18.0 (4.0)	6.3 (2.1)	29.3 (18.6)
K19	2.9 (1.3)	ND	ND	145.6 (51.2)	30.1 (7.9)	37.7 (21.7)	18.8 (10.9)
K21	0.7 (1.0)	ND	ND	108.2 (4.1)	145.4 (44.8)	11.7 (4.7)	46.8 (33.4)
K22	1.2 (1.3)	ND	ND	94.5 (24.2)	19.3 (3.8)	17.0 (6.9)	ND
K23	ND	ND	ND	159.7 (69.8)	13.2 (7.4)	8.7 (3.6)	ND
K24	5.2 (10.1)	ND	ND	113.4 (44.6)	95.5 (57.3)	11.2 (8.3)	ND

ND denotes "Not Detected".
[a] Average of five samples. Figures in parenthesis denote standard deviation (SD).

also reported to have beneficial bio-activities [2]. Special attention was paid to the amounts of PG (Fig. 1), because it is the precursor of goitrin which is known to cause goiter in the thyroid gland [22].

3.4. Cultivars with high GR contents

3.4.1. Broccoli variety

Broccoli has been reported to be a good supply source of GR [23–25]. The six cultivars tested contained fairly high amounts of GR (Table 1), which is consistent with previous reports [24,26]. The contents ranged from 12.2 to 119.4 mg/100 g FW. The median was 57.7 and the average was 59.5. The highest value (119.4) recorded in the B1 cultivar (F_1 hybrid) was taken as a criterion to judge the GR levels in the other varieties tested (cabbage and kale). Worthy of note is the absence of PG in all cultivars. Because PG is the precursor of goitrin, its absence favors broccoli as a supply source of GR.

3.4.2. Cabbage variety

The GR contents (mg/100 g FW) in the cabbage cultivars are listed in Table 2. High GR contents, comparable with the highest of the broccoli, were observed in three cultivars: C18 (106.3), C26 (106.2) and C29 (153.9). The former two were preferred to the last one because of the low PG contents (20.2 and 19.3 mg, respectively). There was wide variation in the GR contents among the cultivars: range 0.1–153.9 mg, median 35.4 mg and average 44.4 mg. The PG contents also varied widely (0.1–86.3). Although the levels detected in this study are unlikely to pose any health problems, it will be better for consumers and food industries to select or create cultivars with high GR and low PG levels.

3.4.3. Kale variety

As presented in Table 3, six cultivars of kale contained high amounts of GR: K14, K19, K21, K22, K23 and K24. The highest content detected in K19, 159.7 mg, exceeded the highest of the broccoli (119.4 mg). Of the six cultivars with high GR contents, only one cultivar (K14) contained PG; the other five were devoid of PG (K19, K21, K22, K23 and K24). The five devoid of PG are domestic cultivars from Italy and are categorized as *Cavolo nero* (black kale); the high GR and null PG contents suggest they would make ideal sources of GR. The GR contents of the other kale varieties tested were lower than those of broccoli, with the exception of the K14 cultivar. In addition, a large amount of PG was detected in the K18 cultivar. Therefore, evaluation of the kale cultivars would have been very different if the black kales were not included in the analysis.

Although the buds of broccoli are a good source of GR, the total yield is not sufficiently high, because there are only a few florets per plant and they can be harvested only once during a cultivation period. The broccoli sprouts are also known to be a potential source of GR [27,28] but the harvest yields are low because of their tiny leaves. The mature leaves of broccoli are unpalatable due to their well-developed stiff midribs. For the same reasons, the leaves of cauliflower, Brussels sprouts and kohlrabi were excluded from this study. Cabbage and kale leaves are advantageous over broccoli florets: their leaves are large and can be harvested throughout the cultivation period. Cultivars used in this study were collected mainly from Europe where the crops were first domesticated and are supposed to have produced wide genetic variations. The unfavorable environment for Cole crops in Japan might have created a different type of genetic variation as they adapted to the high humidity and acidic soil, etc. The diversity of the collection enabled us to find excellent cultivars in both cabbage and kale. One of the valuable cabbage cultivars is of French (C18) origin while the other two are from Japan (C26 and C29). The black kales of Italian origin seem to be an excellent source of GR, they could supply GR through daily meals without the risk of PG intake. To avoid an excessive intake of SR, which was reported to be cytotoxic at higher doses [29], natural vegetables are preferable to artificial dietary supplements as a supply source. It may be an interesting challenge to achieve an even higher GR content by optimizing the culture conditions (fertilizer, soil, etc.). Aside from the health functions, GSLs are also important in endowing characteristic taste and odor to the vegetables. From a culinary point of view, the GSL profiles revealed in the present study provides useful information. The data will also be useful if new functions, either for or against, health is found in future for any of the GSLs.

4. Conclusion

The GR content of three cultivars of cabbage (106.2–153.9 mg/100 g FW) and six cultivars of kale (94.5–159.7)

were comparable to or even higher than the highest of broccoli, which is reputed to be rich in GR (12.2–119.4). The black kales were especially suitable for supplying GR through food. The analysis of a large number of samples was facilitated by a new clean-up method and improved LC–MS conditions. Qualitative profiles of seven GSLs were obtained in 62 cultivars using broccoli (6), cabbage (32) and kale (24) varieties.

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