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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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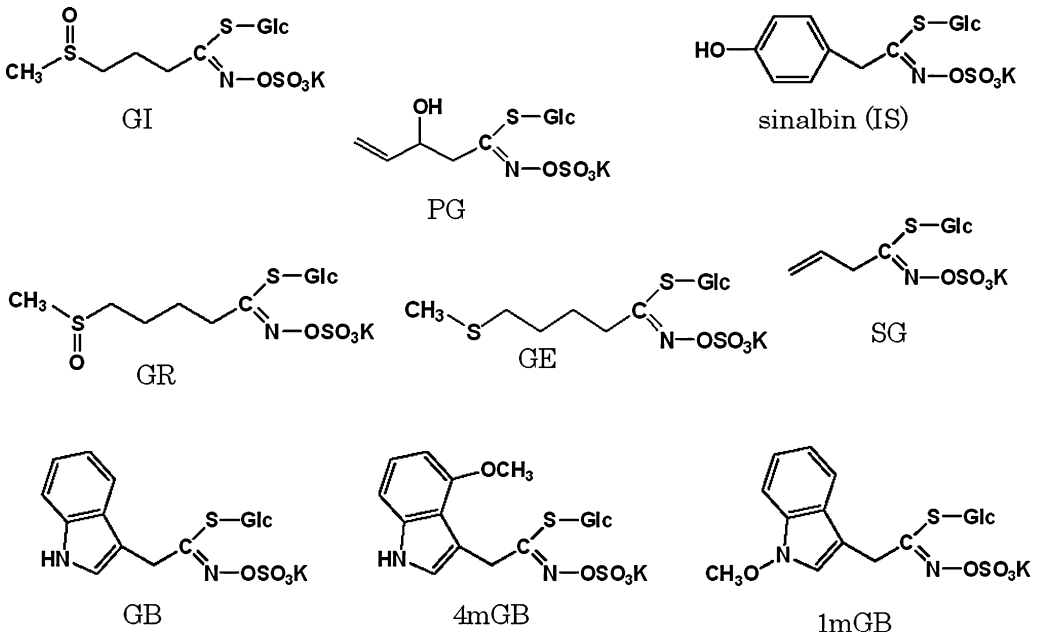

Fig. 1. Structures of GSLs detected in *B. oleracea* crops and sinalbin used as an internal standard (IS). GI: glucoiberin, PG: progoitrin, SG: sinigrin, GR: glucoraphanin, GE: glucoerucin, GB: glucobrassicin, 4mGB: 4-methoxyglucobrassicin, and 1mGB: 1-methoxyglucobrassicin.

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.

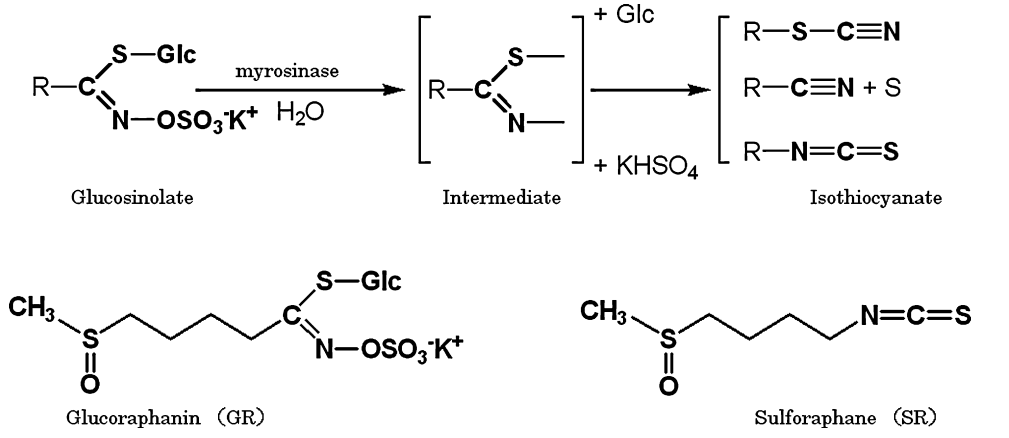

Fig. 2. Structure of sulforaphane derived from glucoraphanin, which is catalyzed by myrosinase.