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Isolation of 4-Methylthio-3-butenyl Glucosinolate from *Raphanus sativus* Sprouts (Kaiware Daikon) and Its Redox Properties

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The most promising among glucosinolates (GLs) are those bearing in their aglycon an extra sulfur function, such as glucoraphasatin (4-methylthio-3-butenyl GL; GRH) and glucoraphenin (4-methylsulfinyl-3-butenyl GL; GRE). The GRE/GRH redox couple is typically met among secondary metabolites of *Raphanus sativus* L. and, whereas GRE prevails in seeds, GRH is the major GL in full-grown roots. During the 10 days of sprouting of *R. sativus* seeds, the GRE and GRH contents were determined according to the Eurpean Union official method (ISO 9167-1). In comparison to the seeds, the GRE content in sprouts decreased from about 90 to about 12 μmol g^{-1} of dry weight (dw), whereas a 25-fold increase—from about 3 to 76 μmol g^{-1} of dw—of the GRH content was measured. An efficient pure GRH gram-scale production process from *R. sativus* (kaiware daikon) sprouts resulted in significant yield improvement of up to 2.2% (dw basis). The reaction of GRH with both H_2O_2 and ABTS$^{\bullet+}$ radical cation was investigated. Whereas H_2O_2 oxidation of GRH readily resulted in complete transformation into GRE, ABTS$^{\bullet+}$ caused complete decay of the GL. Even though not directly related to its radical scavenging activity, the assessed reducing capacity of GRH suggests that *R. sativus* sprouts might possess potential for health benefits.

KEYWORDS: Glucosinolates; kaiware daikon; redox process; *Brassicaceae*; sprouts

INTRODUCTION

The largest and most commonly consumed group of edible plants within the *Brassicaceae* family (*Cruciferae*) are the vegetables of the Brassica genus. The most important Brassica vegetables include broccoli, cabbage, Brussels sprouts, cauliflower, and Chinese cabbage; other important edible plants of the *Brassicaceae* family include Japanese white radish, salad rocket, garden cress, watercress, horseradish, and wasabi.

Brassica vegetables contain carotenoids, vitamin C, fiber, flavonoids, and, in addition, a particular group of health-promoting compounds known as glucosinolates (GLs). These secondary plant metabolites have aroused great interest for their potential role in helping to maintain human health. There is strong epidemiological evidence for association of Brassica vegetable consumption with a highly significant cancer risk reduction, and it has been speculated that the isothiocyanates (ITCs) resulting from myrosinase (EC 3.2.1.147)-catalyzed hydrolysis of GLs (**Figure 1**) may contribute to the protective effects (*1*, *2*).

At least 120 different GLs have been identified, and their molecular structures share a common core of a β-D-glucopyrano moiety linked via a sulfur atom to a (*Z*)-*N*-hydroximinosulfate ester group and a variable aglycon side chain derived from α-amino acid biosynthetic precursors (*3*).

The two most relevant pairs of methionine-derived GLs that bear in the side chain an extra sulfur atom at different oxidation degrees (methylthio or methylsulfinyl group) are reported in **Figure 1**. The redox couple glucoraphasatin (4-methylthio-3-butenyl GL; GRH) and glucoraphenin (4-methylsulfinyl-3-butenyl GL; GRE) differs only by the presence of a double bond with respect to the other couple glucoerucin (4-methylthiobutyl GL; GER) and glucoraphanin (4-methylsulfinylbutyl GL; GRA). The side-chain structures of the above GLs are preserved in the related ITCs produced via myrosinase-catalyzed hydrolysis. Even though the biological activity of ITCs seems to be primarily due to the presence of the −N═C═S group, it is also known that the constitution of the ITC side chain strongly influences the properties and may play a relevant role in

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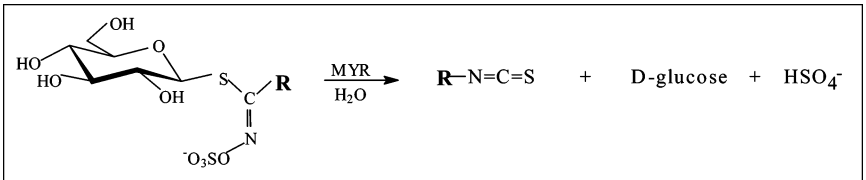


R side-chain	Sulfide (X=S)	Sulfinyl (X=SO)
$CH_3(X)CH_2CH_2CH_2CH_2$-	Glucoerucin (GER)	Glucoraphanin (GRA)
$CH_3(X)CH=CHCH_2CH_2$- (Z / E)	Glucoraphasatin (GRH)	Glucoraphenin (GRE)

Figure 1. Two pairs of alkylthio glucosinolates and the myrosinase-catalyzed reaction.

modifying the lipophilicity of the compound and also the electrophilicity of the ITC functional carbon atom (*3*).

Alkylthio ITCs differing in the oxidation state of the side-chain sulfur were found to differently affect quinone reductase and glutathione *S*-transferase in mouse tissue. Since those first results on structure−effect relationship were published by Zhang et al. (*4*), several hundreds of papers have focused on the ITC resulting from myrosinase-catalyzed hydrolysis of GRA, sulforaphane (4-methylsulfinylbutyl ITC), which was identified as the most active health-promoting compound among analogues. Recently the reduced form of sulforaphane, that is, erucin (4-methylthiobutyl ITC), has been observed to be potentially more attractive in anticancer strategy, showing selectivity toward leukemia cells (*5*). Moreover, Barillari et al. (*6*) showed that erucin and its precursor GER exhibit a direct antioxidant activity, whereas other ITCs exhibit only an indirect antioxidant activity, by virtue of their capacity to induce phase II enzymes, for example, glutathione *S*-transferase (*7*).

Our interest was also recently attracted by the second redox couple GRE/GRH, which similarly differs in the oxidation degree of the side-chain sulfur atom (**Figure 1**). The above GLs are typical secondary metabolites of the radish (*Raphanus sativus* L.), and, whereas GRE prevails in seeds, GRH is the major GL in the root (*8*). The in vitro cytotoxic activity of GRE could be tested against several tumor cells, thanks to easy purification of a substantial amount of this GL from *R. sativus* cv. Pegletta seeds (*9*). In contrast, GRH has not been tested so far because isolation from radish roots according to the method of Visentin et al. (*8*) delivers only too small amounts of pure compound.

An ex vivo study showed that consumption of a mixture of sprouts including radish resulted in significant reduction in human lymphocyte DNA damage after a H_2O_2 stress (*10*), but no information about GLs content in sprouts was provided. Fahey et al. (*11*) reported that 3-day-old broccoli sprouts maintain the same level of GRA present in seeds, suggesting that no significant biological reduction process affects the sulfoxide function; however, partial oxidation of GER into GRA during rocket seed sprouting was recently described (*6*). The oxidation of an alkylthio GL into an alkylsulfinyl GL seems to occur more easily than the reverse reduction of an alkylsulfinyl GL, likewise considering that chemoselective oxidation of GER—isolated from *Eruca sativa* seeds—is an original way to produce GRA on the gram scale in mild conditions (*12*). We were interested in determining the GL content differences between seeds and sprouts, and thus we herein describe for the first time the change in the oxidation degree of the side-chain sulfur atom of a GL during the sprouting process of *R. sativus* seeds. Reductive conversion of GRE into GRH was clearly observed and, consequently, a suitable large-scale procedure was set up for the isolation and purification of GRH, starting from *R. sativus* sprouts.

Recently, Takaya et al. (*13*) isolated and identified 12 compounds with antioxidant activity from *R. sativus* sprouts, but GRH was not even mentioned. The latter GL was therefore examined with regard to its reactivity with H_2O_2 in water at two temperature values. The relative antioxidant activity of GRH was also tested as Trolox equivalent antioxidant capability using $ABTS^{\bullet+}$ radical cation.

MATERIALS AND METHODS

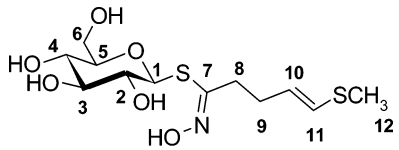
Chemicals. Potassium persulfate (reagent grade, ≥99%), hydrogen peroxide (35% w/w in water), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, reagent grade, ≥98%), were purchased from Merck, and ABTS−$(NH_4)_2$ [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, reagent grade, ≥99%] was from Fluka. GRE and 3-butenyl GL (gluconapin, GNA) were previously purified from seeds of *R. sativus* and *Brassica rapa*, respectively (*14*). All other reagents used were purchased from Sigma-Aldrich. HPLC solvents (analytical grade) were purchased from Carlo Erba (Milano). All solutions were prepared from doubly distilled deionized water.

Plant Source and Sprouting Process. *R sativus major* seeds, cultivar OP 38 (*Brassicaceae*), were supplied by Suba & Unico Longiano, Forlì, Italy. Seeds were surface sterilized by soaking for some minutes in 1% sodium hypochlorite and rinsed exhaustively with sterile distilled water. Twenty plastic trays (20 × 13 cm) each containing ∼5 g of seeds laid on three sheets of paper were incubated in a plant growth chamber under an 8 h day/16 h night photoperiod and a constant temperature of 22 °C. The resulting sprouts (known in Japan as kaiware daikon) were grown without added nutrients and watered twice a day. Sprouts of two independent trays were harvested every day by gently collecting the entire sprouts (including roots). They were then freeze-dried, ground to a fine powder, and stored at −20 °C until GL analysis was carried out.

Determination of the GL Content. GL content in *R. sativus* seeds and freeze-dried sprouts samples was assessed according to the EU official method (ISO 9167-1) (*15*), based on the HPLC analysis of desulfo-GLs resulting from removal of the sulfate group of GLs via sulfatase-catalyzed hydrolysis as previously described (*6*). The amount of GRE and GRH was determined using sinigrin as the internal standard and taking the relative response factors into account (*16*).

Purification of GRH. A sample of ∼35 g [dry weight (dw)] of 10-day-old freeze-dried *R. sativus* sprouts was extracted with 500 mL of boiling ethanol 70% (v/v), homogenized by U-Turrax at medium speed for 15 min at 75 °C, and then centrifuged (15300*g* × 30 min at 4 °C). The solid residue was extracted again with another 500 mL of boiling 70% ethanol and centrifuged. Subsequently, the pooled extracts were filtered by Hyflo Supercel Filter Aid (BDH Chemicals Ltd ., Poole, U.K.) and subjected to purification procedures to obtain GRH. The extract was loaded onto an open preparatory column (25 × 200 mm i.d., Pharmacia) containing DEAE-Sephadex A-25 conditioned with 25 mM acetate buffer (pH 5.6). After loading, the column was washed in sequence with starting buffer, formic acid/2-propanol/water (3:2:5)

Table 1. NMR Spectral Data (250 MHz in D_2O) for Desulfo-glucoraphasatin (DS-GRH)[a]



position	^{1}H (ppm)/*J* (Hz)	^{13}C (ppm)
1	d 4.99/J_{1-2} = 9.7	81.9
2	m 3.42–3.55	72.5
3	m 3.50–3.62	77.5
4	m 3.42–3.55	69.5
5	m 3.50–3.62	80.4
6a	bd 3.91/J_{6a-6b} = 12.4	61.0
6b	bdd 3.72/J_{6a-6b} = 12.4, J_{5-6b} = 4.9	61.0
7		155.9
8	bt 2.70/J_{8-9} = 7.1	32.0
9	m 2.47/J_{8-9} = J_{9-10} = 7.1	30.8
10	dt 5.53/J_{10-11} = 15.1, J_{9-10} = 7.1	125.3
11	bd 6.18/J_{10-11} = 15.1	125.5
12	bs 2.26/J_{11-12} = 1–2	14.2

[a] In the ^{1}H spectrum of desulfoglucoraphasatin, minor peaks corresponding to the *Z* stereoisomer could be detected.

solution, and the buffer again. The column was then eluted stepwise with 5 × 100 mL of 25 mM aqueous K_2SO_4 and finally with 2 × 135 mL of 50 mM aqueous K_2SO_4. Each fraction collected was then checked for GL content by HPLC, and those containing GRH (> 95%) were pooled and concentrated to $^1/_{10}$ of the initial volume. Inorganic salts were removed from the concentrate by adding absolute ethanol in 1:1 (v/v) ratio. After removal of precipitated salts by centrifugation, the ethanol was evaporated, and the aqueous solution containing GRH was freeze-dried. The GRH-containing sample of ~1.6 g was dissolved in water (400 mg/mL), and 2 mL was loaded onto an XK 26/100 column containing Sephadex G-10 (Amersham Biosciences, Milano, Italy) connected to an $AKTA_{FPLC}$ equipped with a Frac-900 fraction collector and UV monitor UPC-900 (Amersham Biosciences). Elution was performed using water as mobile phase at a flow rate of 2.5 mL min^{-1}, and the eluate was monitored at 254 nm. After the void volume was discarded, 10-mL fractions were collected. Individual fractions were then analyzed by HPLC, resulting in GRE and GRH present in fractions 10−15 and 18−23, respectively. Those containing pure GRH were pooled and freeze-dried. The purity of the white solid GRH was assessed by HPLC, and the compound was characterized by ^{1}H and ^{13}C NMR spectroscopy.

Preparation of Desulfo-GRH (DS-GRH) Reference Standard. Starting from native GRH purified as described above, DS-GRH was obtained by using free purified sulfatase (*17*). The main steps of the preparation were (i) GL desulfation by adding free sulfatase to a 10-mL GRH solution (20 mg/mL in 50 mM acetate buffer pH 5.6) and (ii) purification of DS-GRH by HR 16/10 preparative column (Amersham Biosciences) packed with LiChrospher RP-18 (VWR International Milano) and connected to an $AKTA_{FPLC}$ equipped with a Frac-900 fraction collector and UV monitor UPC-900 (Amersham Bioscences). The DS-GRH solution was concentrated, and 2.0 mL was loaded onto the column. After a washing with 20.0 mL of acetonitrile 5% at a 1.0 mL min^{-1} flow rate, elution was carried out with a gradient up to 30% acetonitrile over 30 min and then over 30 min more with 30% acetonitrile; 4.0-mL fractions were collected and analyzed by HPLC, and those containing DS-GRH (peak purity > 99%) were collected and freeze-dried. The DS-GRH was characterized and unambiguously identified using ^{1}H and ^{13}C NMR (**Table 1**) and mass spectrometry techniques. The standard curve of DS-GRH was constructed by measuring the HPLC peak area obtained in the concentration range of 0.1−1.4 mM using the same instrument and chromatographic conditions described for GLs analysis.

Oxidative Reactions of GRH. A stock solution of 80 mM GRH was prepared by dissolving the appropriate amount of purified compound in water. The stock solution was diluted to a final concentration of 2 mM by a solution of H_2O_2 (100 mM). The reaction mixture was kept at either 25 or 37 °C by a thermostatic system to follow the kinetics of oxidation of GRH at the two established temperatures. Every time unit (5 min for the experiment at 37 °C and 10 min for that at 25 °C), 1 mL of solution was loaded as quickly as possible into an anion-exchange minicolumn to remove H_2O_2 from the reaction mixture and analyzed after desulfation as previously described. The residual amount of GRH in the mixture after reaction was determined through quantification of the DS-GRH derivative by the HPLC calibration curve. Measurements were repeated twice, and HPLC analyses were done in triplicate.

Trolox Equivalent Antioxidant Capacity (TEAC) Assay. To measure the relative antioxidant capacity, we used the well-known TEAC method (*18*), in which the $ABTS^{\bullet+}$ radical is produced in stable form, before the addition of GRH, via reaction between ABTS and potassium persulfate. On reaction with an antioxidant, the blue/green color of $ABTS^{\bullet+}$ disappears, and the decolorization resulting from GRH addition was measured spectrophotometrically (*E*6) at 734 nm after 6 min. All solutions and the cuvette compartment were thermostated at 30 °C and buffered to pH 7.4 with PBS. The reduction in absorbance was related to that of the standard Trolox. Measurements were performed in triplicate at four different Trolox concentrations, and a calibration curve Δ*E*6 [Abs (blank) − Abs (Trolox)] versus Trolox concentration (mM) was constructed. Similarly, a graph of ΔE6 versus concentration was obtained for GRH using a concentration range from 0.5 to 2 mM.

RESULTS AND DISCUSSION

It is well-known that *R. sativus* mature vegetable contains GRH (*19*), whereas seeds of the same species constitute a good source for the oxidized counterpart GRE (*9*). The two representative HPLC chromatograms of GL content in seeds and 10-day-old sprouts are reported in **Figure 2**. Comparison of the two GL profiles shows that whereas GRE prevails in seeds, GRH is the major GL in sprouts. In comparison with the seeds, the GRE content in 10-day-old sprouts decreased from about 90 to about 12 μmol g^{-1} of dw, whereas a 25-fold increase—from about 3 to 76 μmol g^{-1} of dw—of the GRH content was found. The evolution of the two GLs over the 10 days of the sprouting process is reported in **Figure 3**. More than the physiological aspects of the sprouting process, we were interested in the general trend of the GL redox couple, which clearly showed for the first time the phenomenon of a hypothetical direct biological reduction of GRE to GRH during sprouting of *R. sativus* seeds. The large amount of GRH in 10-day-old sprouts when compared to the mature *R. sativus* (*19*) agrees with previously reported data regarding broccoli sprouts, in which the GL content was found to be 10−100 times higher than that in mature field-grown plants (*11*). Moreover, because the decrease of GRE in *R. sativus* sprouts reaches about the same value of the increase of GRH, our data are in line with anterior results indicating that no significant net synthesis of GLs occurs during 5−10 days of germination of broccoli (*11*), *Tropaeolum majus* L. (*20*), white mustard, and oilseed rape (*21*) seeds. However, although the high level of GLs in sprouts with respect to mature vegetables is expected, the most likely biological reduction of an alkylthio GL is an original observation. Even though it was previously reported (*22*) that erucin was found in the urine of rats after they had been injected with sulforaphane, the only available information related to changes in the oxidation state of the side-chain sulfur atom of such GLs concerns rocket (*Eruca sativa* Mill.) sprouts (*6*). In the latter, the oxidation process takes place, but not a reductive transformation of the methylsulfinyl group into the methylthio group as observed by us. Temperature and developmental stage can influence the GL content of sprouting seeds, as reported by

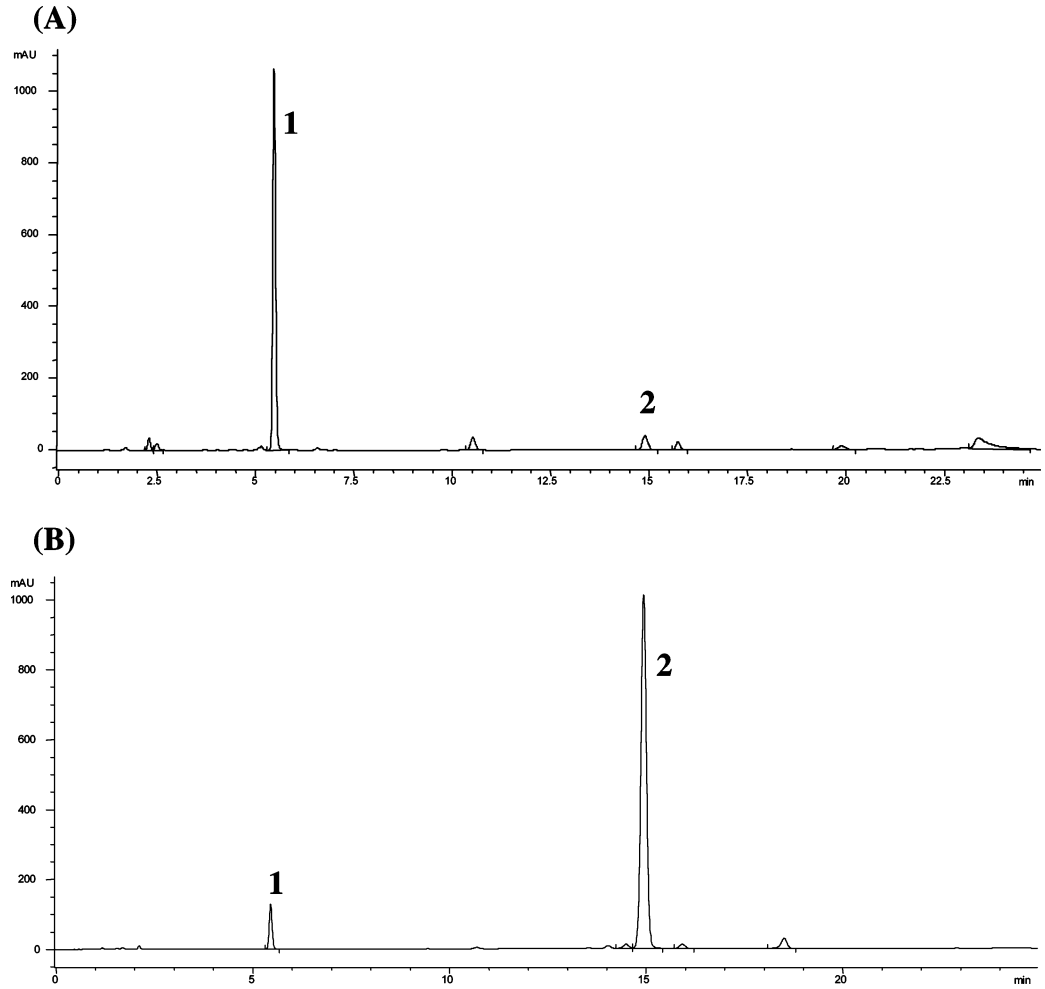


Figure 2. HPLC chromatograms (according to ISO 9167-1) of GLs present in *R. sativus* seeds (**A**) and 10-day-old sprouts (**B**): 1, glucoraphenin; 2, glucoraphasatin.

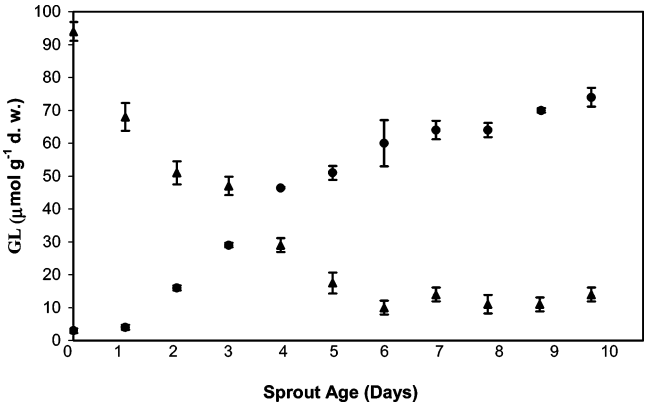


Figure 3. Quantitative determination of glucoraphenin (GRE, ▲) and glucoraphasatin (GRH, ●) contents during sprouting of Japanese radish seeds. Values and error bars represent the mean and standard error of two independent trays containing each 5 g of seeds, analyzed in duplicate.

Pereira et al. (*23*), but our interest in GRH was first justified by selecting a rich vegetable source of this GL so as to facilitate further investigations on alkylthio GLs and the derived ITCs (*6*). Sprouting at room temperature (ranging from 28 to 30 °C) gave a less suitable starting material, whereas lower temperature gave better results (data not shown), making available a good source for GRH isolation. The choice of *R. sativus* sprouts as a vegetable source was a crucial step in obtaining GRH on the gram scale. Indeed, the method described by Visentin et al. (*8*) allowed only a very low yield, whereas sprouts, as alternative starting material to *R. sativus* roots, resulted in significant yield improvement (2.2% dw basis). Besides the choice of vegetable source, some other factors such as the stepwise elution method contributed to the efficiency of the purification process. In fact, elution with 25 mM K_2SO_4 caused a partial release of GRE from DEAE-Sephadex A-25. The general method for GL purification usually requires extraction of compounds from the dried collected fractions by boiling methanol after ion-exchange chromatography, but GRH was shown to undergo partial oxidation during the process and, therefore, the problem had to be solved by adding an equal volume of ethanol to the concentrated fractions. Salts were eliminated by centrifugation and, finally, possible residues were completely removed by size-exclusion chromatography using Sephadex G-10. The above purification step allowed a good separation of GRH from GRE, probably due to a different interaction of compounds with the resin related to their individual molecular weights, which differ by one oxygen atom. Starting with 35 g of dried sprouts, ~770 mg of pure GRH—assessed by ^{1}H NMR spectroscopy (*8*)—was obtained using not more than two chromatographic steps. The purification procedure described above enabled us to isolate GRH on a large scale and to investigate its reactivity toward both H_2O_2 and $ABTS^{\bullet+}$.

Reactive oxygen species have been implicated in several cellular processes, including growth signal transduction, gene expression, and apoptosis (*24*). Hydrogen peroxide is one among six major reactive oxygen species causing oxidative damage in

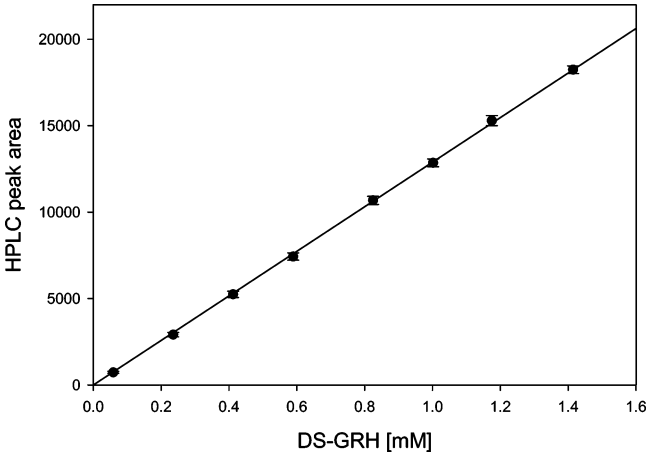


Figure 4. HPLC calibration curve for DS-GRH reference compound: $y = 12.880x$ ($R^2 = 0.999$); y represents the HPLC response (peak area) and x represents the concentration of the DS-GRH solution injected on column (mM). Error bars represent the standard error of the mean for triplicate analyses.

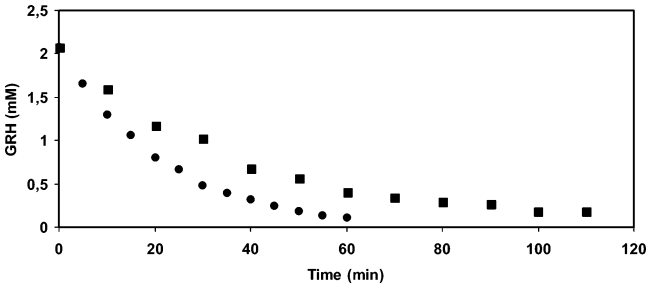


Figure 5. Pseudo-first-order kinetics of GRH incubated with H_2O_2 at 37 °C (●) and 25 °C (■).

the human body (*25*). In this context, GRH capability to directly react with H_2O_2 is of particular interest.

The residual amount of GRH in the mixture after reaction with H_2O_2 was determined by the HPLC calibration curve (**Figure 4**), which in turn was constructed using a pure DS-GRH reference compound characterized by ^1H and ^{13}C NMR (**Table 1**). The calibration curve of DS-GRH standard was obtained within 0−1.4 mM concentration values (triplicate analyses), and the regression coefficient (R) was >0.999. **Figure 5** reports the decrease during the oxidation reaction of GRH concentration versus time. It was found that when dissolved in water, the methylthio group of GRH can react with H_2O_2 to undergo complete transformation into the oxidized methylsulfinyl group of GRE in <1.5 h, similar to the oxidation of GER into GRA (*6*).

The plots of the natural logarithm of GRH concentration versus time (minutes) resulted in pseudo-first-order kinetics. Data were well fitted by straight lines, related to the equations below:

$$\ln [\mathrm{GRH}]_{25\ ^\circ\mathrm{C}} = -0.0218 \times \text{time (min)} + 0.5955$$
$$R^2 = 0.9822, \text{ where } 0.0218 = k[\mathrm{H_2O_2}]^\alpha = k'$$
$$t_{1/2,25\ ^\circ\mathrm{C}} = \ln 2/k' = 31.8 \text{ min}$$

$$\ln [\mathrm{GRH}]_{37\ ^\circ\mathrm{C}} = -0.0489 \times \text{time (min)} + 0.7644$$
$$R^2 = 0.9987, \text{ where } 0.0489 = k[\mathrm{H_2O_2}]^\alpha = k'$$
$$t_{1/2,37\ ^\circ\mathrm{C}} = \ln 2/k' = 14.2 \text{ min}$$

Those results show that in the described experimental conditions, the rate of disappearance of GRH at 37 °C is more than twice that at standard temperature (25 °C). This is a rough indication that the reaction follows Arrhenius-type dependence on temperature.

Table 2. Measured $\Delta E6$ in the $ABTS^{\bullet+}$ Reaction and Calculated TEAC Mean Value

[GRH] (mM)	$\Delta E6$ GRH	[Trolox] (mM)	TEAC[a] (mM)	$(TEAC)_m$[b] $\pm \sigma$ (mM)
0.437	0.0198	0.0597	0.137	
0.546	0.0250	0.0753	0.138	
1.09	0.0450	0.136	0.125	0.130 ± 0.006
1.64	0.0680	0.205	0.125	
2.18	0.0923	0.278	0.127	

[a] TEAC values = [Trolox]/[GRH], where [GRH] is the concentration of GRH giving a certain $\Delta E6$ and [Trolox] is the concentration of the standard that should give the same ΔE6. [b] Because the slopes of the straight lines of GRH and the standard are different, it is convenient to calculate a mean value of TEAC in the concentration range of the sample and the standard. This mean value, $(TEAC)_m$, is more significant than the TEAC value calculated at only one concentration.

To evaluate the reducing capacity of GRH, we have also tested the compound with $ABTS^{\bullet+}$ radical cation in the TEAC assay (*18*). $ABTS^{\bullet+}$ was generated by persulfate oxidation, and because the reaction was complete (*18*), we can assume that no free oxidants able to oxidize GRH into GRE were present in the mixture. Values of TEAC are reported in **Table 2**, and parameters of the straight-line equations for Trolox and GRH were

$$\Delta E6 = 0.3319[\text{Trolox}] \qquad R^2 = 0.9962$$
$$\Delta E6 = 0.04212[\text{GRH}] \qquad R^2 = 0.9995$$

Reaction with $ABTS^{\bullet+}$ caused the complete decay of GRH, because DS-GRH was not detected by HPLC analysis. On the other hand, we found that GRE showed no appreciable reaction with the $ABTS^{\bullet+}$. A similar reluctance was also observed with gluconapin (3-butenyl GL), a GRH analogue devoid of the methylthio group. These findings suggest the formation of an intermediate sulfide radical cation, $R_2S^{\bullet+}$, in the reaction between GRH and $ABTS^{\bullet+}$ as found in enzymatic and biomimetic oxidations of sulfides to sulfoxides (*26*). Even though we could not identify the products resulting from the reaction between GRH and $ABTS^{\bullet+}$, it can be supposed that the sulfur atom of the vinyl sulfide moiety is able to act as an electron donor, as illustrated in **Figure 6**. The equilibrium of the reaction should be shifted toward the reactants, but the radical cation on sulfur decays very rapidly, so that after 6 min, GRH is totally consumed (*27*). Although the reducing capacity of a compound is not directly related to its radical scavenging capacity, this stands as an important parameter of its antioxidant potential. However, it should be noted that the relative antioxidant capacity (TEAC) of GRH is much less than that of polyphenolic compounds (*18*, *28*).

In conclusion, we have set up an improved chromatographic methodology for the purification of GRH on the gram scale, bringing about a solution to the problem of the inadequate separation of GRH from structurally related GRE, allowing production of better suited amounts of pure GRH, which should facilitate our further studies of the GRH-derived ITC. The present paper therefore discloses work in progress for evaluating the role of the oxidation degree of the side-chain sulfur functions in dietary GLs. The implication of redox mechanisms in the pathogenesis of human diseases has led to the affirmation that plant-derived compounds, able to modulate these processes,

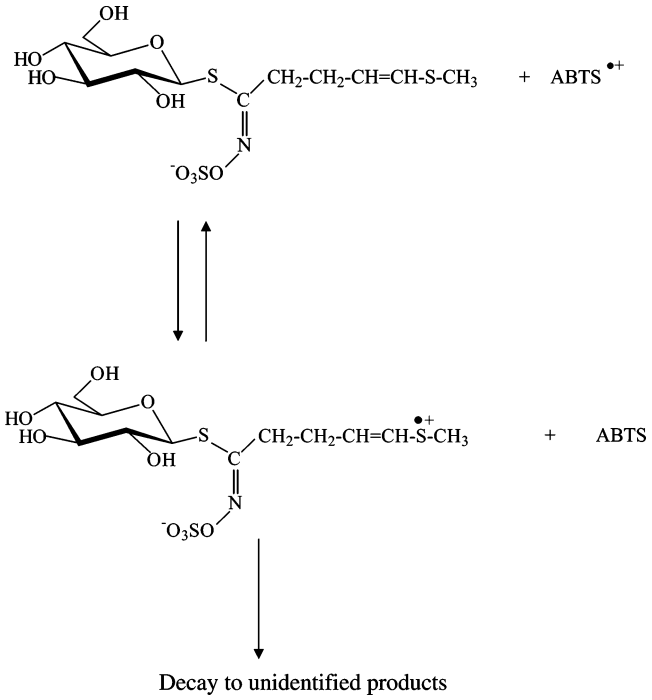


Figure 6. Hypothesis of reaction between GRH and $ABTS^{\bullet+}$.

might bring health benefits as prophylactic agents. Daikon is one of the most popular and important vegetables in Japan, where it is consumed either full-grown or as sprouts. Several papers sustain the beneficial role of sprouts in the human diet because these vegetables provide a wide variety of antioxidant compounds (*13*) and protection against H_2O_2 damage (*10*). Although the GRH content is considered to be a predominant parameter that can influence the quality of both processed and fresh Japanese radish (*18*), almost no information is available on the biological activity of this GL. The literature refers only to the antimicrobial activity of GRH-derived ITC (*29*) and indirectly to its antimutagenic effects (*30*). Our current investigation stimulates an interest in the role of *R. sativus* sprouts—insofar as source of GRH—in the human diet and, moreover, opens the way to many further studies.

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