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Antioxidant and Choleretic Properties of *Raphanus sativus* L. Sprout (Kaiware Daikon) Extract

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Brassica vegetables and glucosinolates contained therein are supposed to reduce the risk of cancer and to possess health-promoting properties. The benefits of a Brassica-based diet may be particularly expressed by eating sprouts, in which the glucosinolate content is higher than in mature vegetables. With this in mind, a first objective of this study was to evaluate the antioxidant properties of radish (*Raphanus sativus* L.) sprouts (Kaiware Daikon) extract (KDE), in which the glucosinolate glucoraphasatin (GRH), showing some antioxidant activity, is present at 10.5% w/w. The contribution of GRH to KDE's antioxidant activity was considered in two chemical assays (Trolox equivalent antioxidant capacity and Briggs−Rauscher methods). The total phenol assay by Folin−Ciocalteu reagent was performed to quantify the reducing capacity of KDE. Finally, on the basis of the putative choleretic properties of antioxidant plant extracts, the effect on the bile flow of KDE administration was investigated in an animal experimental model. The findings showed that KDE has antioxidant properties and significantly induced bile flow in rats administered 1.5 g/kg of body weight for 4 consecutive days.

KEYWORDS: Glucosinolates; Kaiware Daikon; antioxidant; Brassicaceae; choleresis

INTRODUCTION

A remarkable consensus has been building around the health benefit deriving from the dietary intake of Brassica vegetables (*1*). Several reports attribute the beneficial effect of a Brassica-based diet to the presence of a characteristic class of compounds named glucosinolates (GLs), which are hydrolyzed into isothiocyanates (ITCs) by vegetal myrosinase (thioglucohydrolase, E.C. 3.2.1.147) or by thioglucosidase activity of the intestinal microflora (*1*). Those compounds are supposed to prevent cancer and degenerative diseases by increasing cellular intrinsic mechanisms that deactivate potential carcinogens/toxics and reactive oxygen species (ROS), in the so-called "electrophile counterattack" (*2*).

Brassica vegetables are important dietary constituents in many parts of the world and appear to account for 10−15% of total vegetable intake, reaching almost 25% in countries with a high consumption (*3*, *4*). Among the more than 350 genera and 3000 species belonging to this plant family, the Japanese white radish (*Raphanus sativus* L.), also named daikon, is the vegetable for which the literature reports the highest per capita consumption, quoted at 55 g/day in Japan (*5*). In addition to this type of radish, the Japanese are heavy consumers of radish sprouts, under the name "Kaiware Daikon", with approximately 20000 t/year consumed (*6*).

Several studies sustain the beneficial role of sprouts in the human diet because these vegetables contain a wide variety of antioxidant compounds (*7*) and provide protection against oxidative damage (*8*). We recently found that glucoraphasatin (GRH), the major GL of Kaiware Daikon, shows a reducing capacity against both H_2O_2 and the $ABTS^{\bullet+}$ radical cation (*9*). Consumption of Kaiware Daikon sprouts could provide significant amounts of GRH, the content of which was found to be 10 times higher than in mature field-grown plants (*9*, *10*). GRH is, however, just one compound among the multitude of molecules existing in the vegetal matrix, some of which have exhibited an antioxidant activity evaluated by the bleomicin−Fe method (*7*). Botanical derivatives obtained from medicinal plants usually contain several classes of compounds endowed with a polyhedric mechanism of action, which often act synergistically on the same target. Recent research has shown that the complex mixture of phytochemicals in fruits and vegetables provides a better protective effect on health than single phytochemicals (*11*). For this reason it is worth carrying out an experimental evaluation of the antioxidant potential using whole food extracts.

With this in mind, the first objective of this study was to evaluate the antioxidant activity of the Kaiware Daikon extract

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(KDE) using two chemical in vitro methods based on the free radical scavenging properties of antioxidant: the Briggs−Rauscher method (BR) under acidic conditions and the Trolox equivalent antioxidant capacity (TEAC) assay under physiological conditions (pH 7.4). The GL content in KDE was quantified, and the contribution of GRH to the relative antioxidant activities was also considered. The total phenolic content was measured by using the Folin−Ciocalteu (FC) reagent, even though more selective and precise methods such as ^{1}H NMR or micro methods are now available. Indeed, the FC method was very recently recommended to quantify the reducing capacity (*12*, *13*). Because choleretic properties of different plant extracts have been related to their content in phenolics (*14*, *15*), the second part of our investigation was to evaluate the effect of KDE on bile flow after single or multiple administrations in rats.

MATERIALS AND METHODS

Chemicals. Malonic acid (reagent grade, >99%), manganese(II) sulfate monohydrate (reagent grade, >99%), $NaIO_3$ (reagent grade, >99.5%), Na_2CO_3 anhydrous (reagent grade, >99.9%), and resorcinol (1,3-benzenediol, reagent grade, ≥99%) were purchased from Merck. Gallic acid (3,4,5-trihydroxybenzoic acid, Riedel-de Haën; reagent grade, ≥98%), 2,6-dihydroxybenzoic acid (2,6-DHBA) (Aldrich; reagent grade, ≥98%), $K_2S_2O_8$ (reagent grade, ≥99%), 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), FC reagent, $HClO_4$, and H_2O_2 were purchased from Fluka, and Trolox (6-hydroxy-2,5,8-tetrametylchroman-2-carboxylic acid) was from Aldrich. They were used without further purification. $HClO_4$ was analyzed by titration versus a standard 0.1 M NaOH solution (from Merck). H_2O_2 was standardized daily by manganometric analysis. HPLC solvents (analytical grade) were purchased from Carlo Erba (Milan, Italy). All other reagents used were purchased from Sigma-Aldrich. All stock solutions were prepared with doubly distilled, deionized water.

Plant Source and Extract Preparation. *R. sativus* major seeds, cultivar OP 38 (Brassicaceae), were supplied by Suba & Unico (Longiano, FC, Italy). Six-day-old sprouts were grown at room temperature by using a Freshlife model 2000 germinator (Tribest Corp.). The crude extract was prepared as follows. First, freeze-dried sprouts were treated by boiling 70% aqueous ethanol (1:20 w/v) to produce a quick deactivation of endogenous myrosinase. The solid residue was removed by centrifugation and re-extracted using the same weight/volume ratio. The two solutions were collected, and the ethanol was then removed by concentration using a rotary evaporator at a temperature of 45 °C. The concentrated extract was maintained in an ice bath overnight. Finally, the precipitated proteins were removed by centrifugation and the extract was freeze-dried.

Determination of the GL Content. The GL content in KDE was assessed by HPLC analysis of desulfo-GL, resulting from removal of the sulfate group via sulfatase-catalyzed hydrolysis, as previously described (*9*). The method is based on the EU official method (ISO 9167-1) (*16*), and the amount of GL was quantified by using sinigrin as an internal standard and the relative response factors (*17*).

Antioxidant Activity Assay Based on the BR Reaction. The chemical in vitro method reported by Cervellati et al. (*18*) is based on the inhibitory effects by free radical scavengers on the oscillations of the BR reaction. The BR system (*19*) consists of H_2O_2, acidic iodate, malonic acid, and Mn(II) as a catalyst, and it works at pH ≈2. The BR reaction method is based on the generation of free radicals in the reaction mixture. The generated hydroperoxyl radicals ($HOO^{\bullet}$) are among the main intermediates of the BR system. The mechanism of action of the antioxidants against $HOO^{\bullet}$ radicals in the BR system has been described in detail elsewhere (*18*, *20*). In brief, when antioxidant scavengers of free radicals are added to an active oscillating BR mixture, there is an immediate quenching of the oscillations, an inhibition time (t_{inhib}) that linearly depends on the concentration of the antioxidant added, and a subsequent regeneration of the oscillations. Relative antioxidant activity (rac) with respect to a substance chosen as a standard (resorcinol) is determined on the basis of concentrations of sample and resorcinol that give the same t_{inhib}; rac is expressed as micrograms per milliter of resorcinol equivalents (*21*). One milliliter of suitably diluted samples of KDE was added to 30 mL of an active BR mixture (maintained at 25.0 ± 0.1 °C) after the third oscillation. The oscillatory behavior was followed potentiometrically by recording the potential of the mixture using a coupled bright Pt electrode−reference electrode. Electrodes were connected to a multimeter (WTW, model pH 540 GLP) controlled by an IBM-compatible PC. More experimental details are reported in ref *21*.

Antioxidant Activity Based on the TEAC Assay. We used the protocol suggested by Re et al. (*22*). The green $ABTS^{\bullet+}$ radical cation was prepared by mixing ABTS stock solution (7 mM in water) with 2.45 mM $K_2S_2O_8$. The mixture was kept in the dark for 12−24 h until the reaction was complete and absorbance stable. For the measurements, the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.800 ± 0.020 at 734 nm. A sample of KDE was dissolved in ethanol. This solution was suitably diluted. For the photometric assay 3.0 mL of diluted $ABTS^{\bullet+}$ solution and 30 μL of the sample solution were mixed in a photometric cuvette (1.00 cm optical path length) for 45 s, and the absorbance was measured after exactly 6 min at 734 nm (T = 24.0 ± 0.1 °C). A blank with distilled water was measured in the same way. The difference between the results of the blank and the sample gave ΔE6 ($E6_{blank} - E6_{sample} = \Delta E6$), the value used for further calculations of the Trolox equivalents in milligrams per liter. A stock solution of Trolox, 0.25 mg/mL, was prepared and diluted to an amount ranging from 0.05 to 0.1875 mg/mL. Absorbance was measured by using a Shimadzu UV-1601 spectrophotometer controlled by an IBM-compatible PC. The relative antioxidant activity is expressed as millimolar Trolox equivalents.

Determination of Total Phenolics (Total Reducing Capacity). This test is based on the oxidation of phenolic groups by phosphomolybdic and phosphotungstic acids (FC reagent). After oxidation, the absorbance of a green-blue complex can be measured at 765 nm. We used the procedure for the total volume (20 mL) of the reacting mixture (*23*). Two milliliters of a suitably diluted sample solution was mixed with 10 mL of FC reagent (diluted 1:10 with distilled water) in a 20 mL volumetric flask and allowed to stand at 24 °C for exactly 5 min. Eight milliliters of a sodium carbonate solution containing 0.6 g of anhydrous Na_2CO_3 was then added to the mixture. After exactly 2 h at 24 °C, absorbance was measured at 765 nm. The blank (2 mL of distilled water) was treated in the same way. Gallic acid was used as a standard, and the total phenolic content is expressed as gallic acid equivalents (GAE) in milligrams per liter.

Animals. Male Sprague−Dawley rats (150−180 g) were purchased from Harlan Italy (Correzzana, Milan, Italy). They were housed in standard conditions (22 ± 1 °C, 60 ± 5% humidity, 12 h light/dark cycle) and fed with standard diet and tap water ad libitum for 1 week prior to treatment. Procedures and animal comfort were controlled by the University Veterinary Service.

Bile Flow Assay. Rats were starved for 18 h before the experiment with free access to water. KDE was suspended in 0.5% carboxymethylcellulose (CMC) in distilled water prior to oral administration. Groups of six rats each were treated by gastric gavage with KDE at a dose of 1.5 g/kg of body weight (bw), in single or repeated administration (4 consecutive days). A group of six rats received chlorogenic acid at a dose of 40 mg/kg of bw. This compound is one of the most abundant and widespread phenolic constituents of plants, and it is known for its choleretic activity (*24*). Control animals received a similar volume of CMC solution (0.2 mL/100 g). Twenty minutes after the oral treatment, animals were anesthetized with urethane (1.2 g/kg of bw, ip). The abdomen was opened with a midline incision and the common bile duct exposed and cannulated just before the hepatic hilus to avoid contamination with pancreatic juice. Rectal temperature was monitored and maintained at 37.0 ± 0.5 °C throughout the experiment using a warming lamp. Bile was collected by gravity in pretared vials at 20 min intervals for 120 min. Bile flow was determined by weight assuming that the specific gravity of rat bile is 1.0.

Statistical Analysis. The data are expressed as mean ± standard deviation. Statistical evaluation was performed using the t test. Probability levels of <0.05 were considered to be significant.

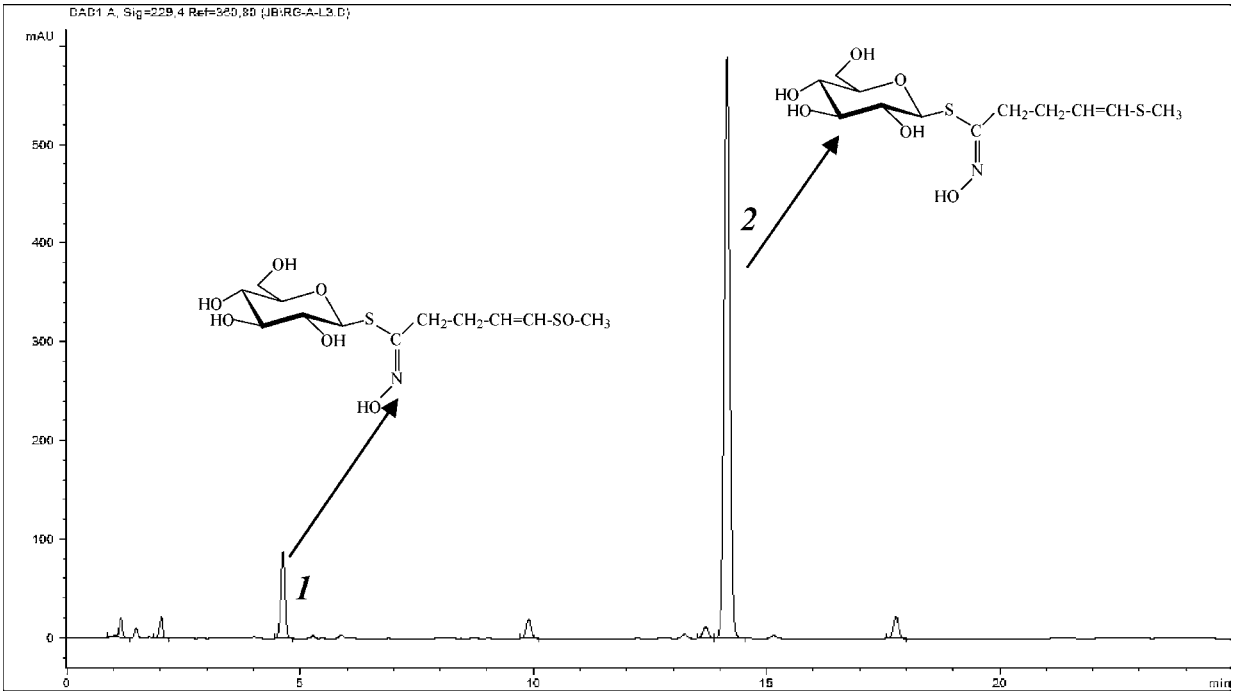

Figure 1. HPLC chromatogram (according to ISO 9167-1) of GLs present in KDE. Peaks 1 and 2 correspond to desulfo-GRE and desulfo-GRH, respectively. Chemical structures of desulfo-GRE and desulfo-GRH are reported next to the corresponding peak.

Table 1. Relative Antioxidant Activity (rac) Values for KDE

KDE (μg/mL)	t_{inhib} (s)	rac	$(rac)_m \pm \sigma$
25	1212	0.0227	
29	1457	0.0215	
34	1810	0.0204	0.021 ± 0.001[a]
38	2047	0.0201	
42	2304	0.0196	

[a] 1.00 μg/mL of KDE solution has the same activity as a resorcinol solution containing 0.021 μg/mL. In terms of μmol equivalents, 1.0 mg/L of KDE shows the same activity as a 0.19 μM resorcinol solution.

Table 2. TEAC Values for KDE

[KDE] (mg/mL)	ΔE6	$[Tr]_{equiv} = \Delta E6/0.3319$	TEAC = [Tr]/[KDE]	$(TEAC)_m \pm \sigma$
2.56	0.190	0.572	0.223	
3.20	0.217	0.654	0.204	
6.41	0.3667	1.105	0.172	0.18 ± 0.03[a]
9.615	0.498	1.500	0.156	
12.82	0.6483	1.953	0.152	

[a] A solution containing 1.0 mg/mL of KDE sample has the same antioxidant activity as a 0.18 mM Trolox solution.

RESULTS

Determination of the GL Content. Figure 1 reports a typical HPLC chromatogram of GLs present in KDE. As shown, KDE contains both the GLs of redox couple GRH and glucoraphenin (GRE), which differs from the former in the oxidation degree of the side-chain sulfur atom (sulfinyl instead of thio). The two GRH and GRE are both metabolites of Kaiware Daikon, and their amounts in KDE are 230 ± 11 and 59 ± 3 μmol/g of dry weight (dw), respectively.

Antioxidant Activity at Acidic pH. Data points (concentration, t_{inhib}) for KDE are well fitted by a straight line ($R^2 = 0.9958$). **Table 1** reports the experimental data and the rac values, calculated as the ratio

$$rac = [std]/[smp]$$

where [smp] is the concentration of the KDE added to the BR mixture giving a certain inhibition time and [std] is the concentration of the standard (resorcinol, Re) that should give the same inhibition time. The latter concentration is obtained from the straight-line equation of resorcinol, which is

$$t_{inhib} = 4224\ (\mu g^{-1}\ mL\ s) \times [Re]\ (\mu g\ mL^{-1}) - 1181(s); \quad R^2 = 0.9944$$

When possible, it is convenient to calculate a mean value of rac in the linear concentration range of the sample and the standard. This mean value, $(rac)_m$, is more significant than the rac value calculated at only one inhibition time.

Antioxidant Activity at pH 7.4. TEAC measurements were also performed at different concentrations of the sample added to the reacting mixture. Data points (concentration, ΔE6) for KDE are well fitted by a straight line ($R^2 = 0.9921$). The straight line ΔE versus concentration for the standard Trolox (Tr) is

$$\Delta E6(734\ nm) = 0.3319 \times [Tr]\ (mM);\ R^2 = 0.9962$$

Experimental data and calculated TEAC values (expressed as millimolar Trolox equivalents) are given in **Table 2**.

Total Phenolic Content (Total Reducing Activity). TPC measurements were carried out at different concentrations of the sample added to the reacting mixture. All measurements were performed in triplicate. Data points [concentration, Abs-(765 nm)] for KDE are well fitted by a straight line ($R^2 = 0.9995$). The straight line for gallic acid (GA) is

$$Abs(765\ nm) = 0.0101 \times [GA]\ (mg/L);\ R^2 = 0.9991$$

Results, expressed in milligrams per liter of gallic acid equivalents, are reported in **Table 3**.

Bile Flow Assay. The effect of orally administered KDE on bile flow is shown in **Figure 2**. Control rats presented a slight regular decrease in bile flow level throughout the experiment.

Table 3. Total Phenolic Content (Total Reducing Capacity) Values for KDE

KDE (mg/L)	Abs (765 nm)	corresponding [GA] (mg/L)	GAE (mg/L)	GAE_m (mg/L) $\pm \sigma$
13.09	0.085	8.416	0.64	
26.18	0.169	16.73	0.64	
39.27	0.246	24.36	0.62	0.62 ± 0.02[a]
52.36	0.324	32.08	0.61	
65.45	0.395	39.11	0.60	

[a] A solution containing 1.0 mg/L of KDE sample has the same total phenolic content (total reducing capacity) as a solution containing 0.62 mg/L of gallic acid.

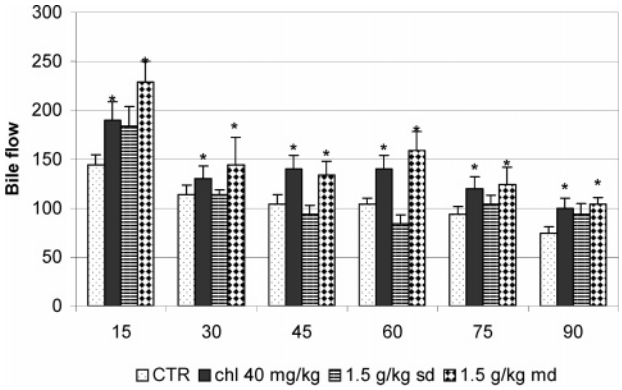

Figure 2. Effect of chlorogenic acid (chl; 40 mg/kg of bw) and KDE (1.5 g/kg of bw) in single (sd) and multiple doses (md) on bile flow in rat. Results are expressed as mean ($n = 6$) ± SD. $P < 0.05$ versus control (CTR), *t* test.

This decrease is a usual response to the experimental condition adopted. A similar pattern was observed for rats administered 300 mg/kg of bw (data not shown), meaning that no significant pharmacological effect is associated with very low dosages of KDE. When the dosage was increased to 1.5 g/kg of bw, different effects on bile flow rate were observed as shown in **Figure 2**. A significant increase of the volume of bile output in the time course considered was recorded only for the repeated administration.

DISCUSSION

KDE was prepared starting from 6-day-old sprouts, in which the hypothetical direct biological reduction of the methylsulfinyl group of GRE into the methylthio group of GRH gives amounts of this latter compound of about 1 order of magnitude higher than in mature plant (*9*, *10*). Although the scientific literature often reports remarkable differences in GRH content among different cultivars of Japanese white radish, the most common strain in Japan contains just 0.71 μmol/g of fresh weight (fw) (*10*). In addition to the variety of factors (i.e., agrotechnical processes, climatic condition, postharvest manipulation) that influence the ultimate content of GRH in the vegetable, a further cause of the discrepancy in the quantitative data is due to the fact that its response factor relative to sinigrin in HPLC analysis (ISO 9167-1) was only recently corrected from 1.00 to 0.44 (*17*).

The extraction process did not change the ratio existing in the sprouts between GRH and GRE, being, respectively, 10.5 and 2.8% (w/w) in KDE.

The presence, concentration, and composition of some phytochemicals are well-known to influence the healthy properties of foods. GRH-ITC, enzymatically produced from its corresponding GL, plays a great role in inducing the flavor and color of radish and is a parameter of the good quality of the vegetable (*10*). Moreover, plant foods such as sprouts are rich sources of both antioxidant vitamins and nutrients, including phenols. The literature reports that vitamin C, like phenolic compounds, shows a rising trend during the sprouting process of some Brassica seeds (*25*). We cannot overlook that many phytochemicals that are naturally present in plant extracts can exert their activity synergistically. Takaya et al. (*7*) isolated and identified 12 compounds with antioxidant activity from Kaiware Daikon. However, constituents obtained from the methanol extract by partition with solvents did not reproduce the activity of the whole methanol extract (*7*). In addition, we found that GRH purified from sprouts exhibited a reducing capacity against H_2O_2 and $ABTS^{\bullet+}$ radical cation (*9*). The objective in the first part of our study was therefore to evaluate the antioxidant potential of KDE, more than a 10th part of which consists of GRH.

To better appreciate the antioxidant parameters of KDE, we report here the data obtained for extracts of other edible vegetables. An MeOH extract of laurel (*Laurus nobilis*) leaves showed $(rac)_m = 0.05 \pm 0.01$, $(TEAC)_m = 0.65 \pm 0.03$, and $(GAE)_m = 0.50 \pm 0.06$ (*26*). A commercial hydroalcoholic (EtOH 55%) extract of artichoke (*Cynara scolymus*) leaves showed $(rac)_m = 0.013 \pm 0.002$, $(TEAC)_m = 0.160 \pm 0.006$, and $(GAE)_m = 0.292 \pm 0.004$ (*15*). Hence, it is possible to conclude that the antioxidant properties of KDE (**Tables 1**−**3**) are similar to or better than those of these other vegetables.

The BR method works at pH ≈2, similar to that of human gastric juice. Kanner and Lapidot (*27*) observed that some plant-derived antioxidants are able to prevent lipid peroxidation, amplified in the acidic pH of gastric fluid. The conception of the stomach as a bioreactor, where ROS and food nutrients interact, highlights the importance of determining the antioxidant activity of dietary source at acidic pH. However, GRH was found to react with H_2O_2, being oxidized to GRE (*9*). The oxidation reaction was investigated at 25 °C using a 50-fold excess of oxidant, and it was found that GRH was completely transformed into GRE in about 1 h (*9*). Because the H_2O_2 concentration is 1.2 M in the BR mixture, the ratio $[H_2O_2]/[GRH]$ in the mixture with the maximum KDE added (42 mg/L) is about 10^7 ($M_{GRH} = 457.6$ g/mol). It is thus conceivable that all of the GRH contained in KDE is completely oxidized by H_2O_2 before having the possibility to react with hydroperoxyl radicals. The relative antioxidant capacity of KDE at acidic pH is therefore underestimated.

This drawback does not occur with the TEAC measurements because the oxidant $K_2S_2O_8$ necessary to produce $ABTS^{\bullet+}$ following the reaction

$$2ABTS + S_2O_8^{2-} \rightarrow 2ABTS^{\bullet+} + 2SO_4^{2-}$$

is added in defect with respect to ABTS. In this way an oxidation of GRH by the persulfate is impossible. Because the $(TEAC)_m$ for pure GRH is known [0.13 mM Trolox (*9*)], we calculated a contribution of about 16% from GRH content to the antioxidant capacity of KDE.

Finally, a GRH content contribution to the TPC of KDE is also possible because the FC reagent is a strong oxidant, but this is difficult to quantify. The FC method suffers from a number of interfering substances present in plants, such as ascorbic acid, sulfur-containing compounds, and mono- and disaccharides (*12*, *28*). Despite its limitations for quantifying phenolic compounds, the FC is the recommended method for measurement of total reducing capacity (*12*, *13*). Several

publications have reported excellent linear correlations between the FC method and other chemical assays involving a single electron transfer, such as TEAC (*13*, *28*). The reducing capacity of a sample is an important parameter reflecting one aspect of its antioxidation ability, because reducing activity is involved in maintaining the cellular redox status, in signal transduction, and in metal cycling and possible prooxidant effects (*12*, *13*).

Antioxidant activity plays a basic role in many cases in the pharmacological effects of plant extracts (*28*), and growing evidence links antioxidant plant extracts and their phenolic content to choleretic effects (*14*, *15*). Our results showing both antioxidant and choleretic properties of KDE seem to support, at least in part, such a relationship. In agreement, black radish (*Raphanus sativus* L. var. *niger*) root has been used in folk medicine since ancient times as a natural drug for stimulation of bile function (*29*), and a juice from black radish root was recently found to exhibit antioxidant properties (*30*). Black radish and Japanese white radish, although belonging to the same species, can differ in their GL profiles (*31*) and consequently in their hydrolysis-derived ITCs. Some authors have reported that GLs were not detectable in the juice (*30*). Although details of the squeezed juice preparation were omitted, we can assume that the non-inactivation of the enzyme myrosinase allowed GL hydrolysis in the respective ITCs. Indeed, GL hydrolysis greatly depends on processing conditions. If myrosinase remains active after processing, this provides an optimal condition for enzymatic GL hydrolysis and a high yield of the corresponding GL hydrolysis products. A comparison of red, black, and white radishes showed that the proportion of aliphatic GLs in these cultivars exceeded 90% (*31*). The pattern for GRH is reported to be generally similar to that of GL content and, depending on the cultivar, GRH accounted for 75−95% of the total GLs in *R. sativus* root (*31*). GRH-ITC could therefore be expected to represent the main ITC in the juice.

Hydrolyzed plant extracts are expected to contain more active antioxidants because, for example, the glycosides of phenolic substances are weaker antioxidants than the corresponding aglycons (*28*). However, the antioxidant activity of a hot water extract of white radish was higher than that of room temperature water extract, using the thiocyanate method with linoleic acid as the substrate (*32*). Although the authors do not mention the GL-myrosinase system, the preparative conditions lead us to suppose that the thermal process inactivates the enzymatic fraction, including myrosinase.

Comments on GRH/GRH-ITC availability have more than a mere academic interest. Biliary secretion is modulated by the action of multidrug resistance-associated protein 2 (MRP2), the expression of which is stimulated by some derived ITCs, such as sulforaphane (*33*). Although glucoraphanin, a precursor of sulforaphane, was not found in KDE, the structurally related GRH and GRE account for >13% of the weight. Degradation of GLs takes place in the rat intestine, and the literature reports that about 10% of the parental GLs is converted into ITCs (*34*). This class of compounds is indicated as indirect antioxidants, by virtue of their capacity to induce phase II enzymes. Coexpression of MRP2 with relevant phase II metabolizing enzymes seems to occur in several studies (*35*). Although this has not been ascertained, we can speculate that the induction of bile flow may, at least in part, be due to derived ITC.

The potential healthy effects of a Brassica-based diet may be particularly expressed by eating sprouts of Brassicaceae, where GL content is higher than in mature plants, and the possibility of their consumption as raw vegetables can increase the bioavailability of GL-derived ITCs.

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