USDA and ODS-NIH Database for the Glucosinolate Content of Foods

Release 1.0

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List of Abbreviations:

Abbreviation	Definition
EU	European Union
FW	Fresh weight
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometry
GSL	Glucosinolate
HPLC	High-performance liquid chromatography
ISO	International Organization for Standardization
ITC	Isothiocyanate
UHPLC-HRAM/MS	Ultra-high-performance liquid chromatography coupled with high-resolution accurate-mass mass spectrometry
UHPLC-MS/MS	Ultra-high-performance liquid chromatography coupled with mass spectrometry

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A. Introduction

What are Glucosinolates?

Glucosinolates (GSLs) are sulfur-rich, anionic secondary metabolites predominantly found in plants belonging to the order Brassicales, with a notable concentration in the genus *Brassica* of the Brassicaceae (formerly Cruciferae) family (1). *Brassica* vegetables such as broccoli, cabbage, and cauliflower rank among the most widely consumed vegetables worldwide (2). Non-*Brassica* plant foods including horseradish, papaya, wintercress, and radish also contain GSLs. The vegetables belonging to the family Brassicaceae are also called cruciferous vegetables, for their cross-shaped flowers. Structurally, GSLs are β-thioglucoside N-hydroxysulfates (**Figure 1A**). The structures of GSLs are generally classified into three major groups based on their precursor amino acid and the types of modification to the side chain R group: *aliphatic*, side chain without sulfur (**Figure 1B)**; *aliphatic*, side chain with sulfur (**Figure 1C**); *aromatic* (phenyl) (**Figure 1D**) and *indole* (indolyl, indolic and heterocyclic are also used throughout literature) (**Figure 1E**). Aliphatic GSLs are biosynthesized from the essential amino acids alanine, leucine, methionine, isoleucine and valine, whereas aromatic GSLs are derived from phenylalanine and tyrosine, and the remaining indole GSLs are derived from tryptophan (3).

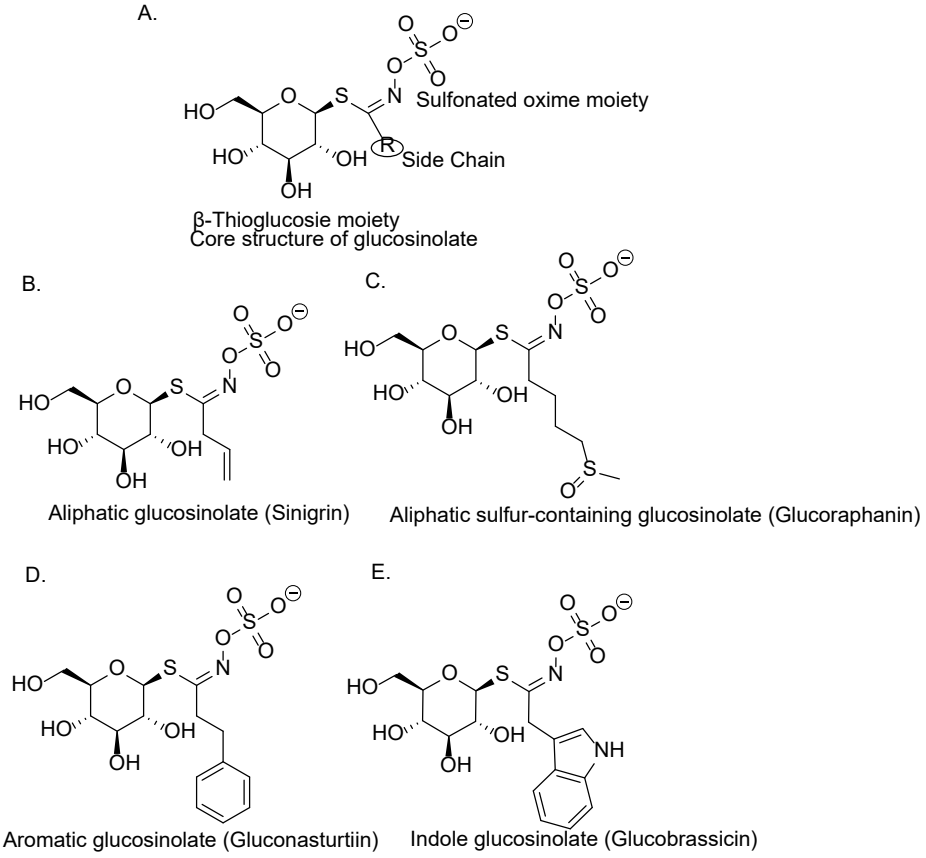

Figure 1. Core chemical structure and representative dietary glucosinolates

The side chain R of most GSLs can be extensively modified from their precursor amino acids. R groups are elongated by one or more methylene moieties. Both elongated and nonelongated R groups are subject to chemical transformations including hydroxylation, O-methylation, desaturation, glycosylation, and

acylation (4). In addition to side chain modification, substitutions with acyl groups at the thioglucose moiety also occur.

The Distribution of Glucosinolates in Foods

The most consumed GSL-containing vegetables, including their scientific names and common names, are presented in **Table 1** based on recently published literature (5). Most studies conducted to-date on dietary GSLs have focused on *Brassica* vegetables.

GSLs occur in all parts of plants, but in different profiles and concentrations. A single plant species typically contains 3 – 4 predominant GSLs, but as many as 15 different GSLs can be found in an individual plant (6). Also, GSL profiles and concentrations may vary significantly among different tissues in the same plant. The highest concentrations of GSLs are usually found in plant reproductive organs such as seeds and siliques (seed pods), and the lowest concentrations usually found in leaves (5). The GSL profile and content are influenced by the growth stage of the plant; in most cases, sprouts contain higher levels of GSLs than adult plants (7). Generally, GSLs constitute about 1% of the plants' dry weight, although the content is highly variable (1, 3).

Table 1. Common glucosinolate containing foods and their corresponding scientific names

Common name	**Scientific name**
Horseradish	*Armoracia rusticana*
Bittercress, Wintercress, Barbarea	*Barbarea vulgaris*
Ethiopian kale	*Brassica carinata*
Indian mustard, Oriental mustard	*Brassica juncea*
Chinese mustard	*Brassica juncea* var. *crispifolia*
Red giant mustard	*Brassica juncea* var. *integlifolia*
Wrapped heart mustard cabbage, Mustard greens	*Brassica juncea* var. *rugosa*
Yellow mustard	*Brassica hirta*
Canola/rapeseeds	*Brassica napus*
Siberian kale	*Brassica napus* var. *pabularia*
Rutabaga (swede)	*Brassica napobrassica*
Black mustard	*Brassica nigra*
Kale	*Brassica oleracea* var. *acephala*
Kai-lan, Chinese broccoli, Chinese kale	*Brassica oleracea* var. *alboglabra*
Cauliflower	*Brassica oleracea* var. *botrytis*
Romanesco broccoli	*Brassica oleracea* var. *botrytis* f. *romanesco*
White cabbage (drum)	*Brassica oleracea* var. *capitata* f. *alba*
Pointed cabbage	*Brassica oleracea* var. *capitata* f. *conica*
Red cabbage	*Brassica oleracea* var. *capitata* f. *ruba*

Savoy cabbage	*Brassica oleracea* var. *capitata* f. *sabauda*
Brussels sprouts	*Brassica oleracea* var. *gemmifera*
Kohlrabi	*Brassica oleracea* var. *gongylodes*
Broccoli	*Brassica oleracea* var. *italica*
Broccoflower	*Brassica oleracea* var. *italica* × *botrytis*
Komatsuna	*Brassica oleracea* var. *komatsuna*
Collard greens	*Brassica oleracea* var. *viridis*
Mustard spinach	*Brassica rapa*
Pak Choi (Cantonese)	*Brassica rapa* var. *chinensis*
Broad beak mustard	*Brassica rapa* var. *narinosa*
Mibuna	*Brassica rapa* var. *japonica*
Choy Sum (false Pak Choi)	*Brassica rapa* var. *parachinensis*
Chinese cabbage	*Brassica rapa* var. *pekinensis*
Komatsuna	*Brassica rapa* var. *perviridis*
Senposai	*Brassica rapa* var. *perviridis* × *pekinensis*
Purple stem mustard	*Brassica rapa* var. *purpuraria*
Turnip	*Brassica rapa* var. *rapifera*
Tatsoi (rosette Pak Choi)	*Brassica rapa* var. *rosularis*
Rapini (broccoli raab)	*Brassica rapa* var. *ruvo*
Caper	*Capparis spinosa*
Papaya, Papaw, Pawpaw	*Carica papaya*
Arugula, Rocket, Garden rocket	*Eruca vesicaria* ssp. *sativa*
Maca, Peruvian ginseng	*Lepidium meyenii*
Cress, Garden cress	*Lepidium sativum*
Watercress	*Nasturtium officinale*
Radish	*Raphanus sativus*
Daikon, Chinese radish, Korean radish, White radish, Winter radish, Oriental radish	*Raphanus sativus* var. *longipinnatus*
White mustard, Yellow mustard	*Sinapis alba*
White mustard, Yellow mustard	*Sinapis hirta*
Mashua	*Tropaeolum tuberosum*
Wasabi, Japanese horseradish	*Wasabia japonica*

Enzymatic Hydrolysis of Bioavailable Dietary Glucosinolates

In plants, GSLs naturally co-exist with the endogenous GSL-hydrolyzing enzyme myrosinase

(thioglucoside glucohydrolase, EC 3.2.3.1), although the two are stored separately in intracellular compartments (8). When plant tissue is disrupted, myrosinase rapidly hydrolyses GSLs, cleaving the glucose moiety and producing an unstable aglycone intermediate thiohydroximate-O-sulfonate, from both aliphatic and aromatic GSLs (9). This intermediate undergoes spontaneous rearrangement to form isothiocyanates (ITCs) or other breakdown products including nitriles, epithionitriles, oxazolidine-2-thiones, depending on the side chain structure, pH, reaction conditions and presence of specific protein factors such as epithiospecifier protein, nitrile-specifier protein and thiocyanate-forming protein (10). ITCs formed from indole GSLs are unstable and further decompose spontaneously into indole-3-carbinol, indole-acetonitrile, thiocyanate and 3,3′-diindolylmethane (3). These hydrolytic products function in plants as defense compounds, exhibiting toxicity or feeding deterrence against a wide range of insect herbivores (7). Hydrolytic products of GSLs such as ITCs and indole-3-carbinol represent the major metabolites found in humans after consumption of GSL-rich foods. Extensive research suggests that the disease-preventive benefits associated with cruciferous vegetables are largely attributable to these GSL hydrolytic products (1).

Analysis of Dietary Glucosinolates

More than 100 GSLs have been discovered in nature to date, with approximately 50 of them regularly detected in commonly consumed cruciferous vegetables (**Table 2**). The structural diversity of GSLs, together with the formation of hydrolytic products during sample preparation and extraction makes their analysis challenging (11). Mechanical procedures such as grinding, cutting, or even freeze-drying disrupt plant cells, which releases myrosinase and leads to enzymatic hydrolysis of GSLs (11). Conversely, tissue damage may also stimulate *de novo* synthesis, particularly for indole GSLs, which can partially or fully offset losses due to enzymatic hydrolysis (10).

One major consideration in developing food composition databases for GSLs is minimizing both the loss or the formation of artificial compounds during sample preparation and extraction (12). Rapid inactivation of myrosinase is key to minimizing enzymatic hydrolysis. Current approaches include the immersion of samples in liquid nitrogen, microwave induced inactivation, and freeze-drying samples prior to homogenization (1). Microwaving and steaming have been shown to effectively preserve intact GSLs (12). The extraction of GSLs from plant material is best achieved using protic solvents, with a methanol-water mixture being the most frequently used. The purification of GSLs can be achieved by using anion exchange (1).

Table 2. Major GSLs in commonly consumed plant foods and their trivial and semisystematic names

Trivial Name	Semisystematic name	Molecular Wt.
Aliphatic GSLs		
Glucocapparin^{+}	Methyl GSL	333
Glucolepidiin	Ethyl GSL	347
Sinigrin^{+}	Allyl GSL; 2-Propenyl GSL	359
Glucoputranjivin	1-Methylethyl GSL, 2-Methylethyl GSL, Isopropyl GSL	361
N/A*	Propyl GSL	361
Gluconapin^{+}	3-Butenyl GSL	373
Glucocapparisflexuosain	Butyl GSL	375
Glucococonringianin	Isobutyl GSL	375
Glucocochlearin^{+}	1-Methylpropyl GSL	375
Glucosisymbrin	1-Methyl-2-hydroxyetyl GSL	377
Glucoarabidopsithalin	2-Hydroxypropyl GSL	377
Glucobrassicanapin^{+}	4-Pentenyl GSL	387
Progoitrin (2R) $^{+}$	2(R)-Hydroxy-3-butenyl GSL	389
Epiprogoitrin (2S) $^{+}$	2(S)-Hydroxy-3-butenyl GSL	389
Glucojiaputin (2S)	(2S)-2-Methylbutyl GSL	389
Glucokohlrabiin^{+}	Pentyl GSL	389
N/A*	3-Methylbutyl GSL	389
Glucocappariflexin	3-Hydroxybutyl GSL	391
4-hydroxybutyl^{+}	4-Hydroxybutyl GSL	391
Glucoviorylin	2-(Methylthio)ethyl GSL	393
Napoleiferin	2(R)-Hydroxy-4-pentenyl GSL	403
Gluconapoleiferin^{+}	2-Hydroxy-4-pentenyl GSL	403
Glucoraphasativusain^{+}	Hexyl GSL	403
Glucocleomin	2-Hydroxy-2-methylbutyl GSL	405
Glucomoracialapathin	2-Hydroxypentyl GSL	405
Glucoiberverin^{+}	3-(Methylthio)propyl-GSL	407
Glucosativin^{+}	4-Mercaptobutyl GSL	407
Glucoraphasatin^{+}	4-Methylthio-3-butenyl GSL	420
Glucoerucin^{+}	4-Methylthiobutyl GSL	421
Glucoiberin^{+}	3-(Methylsulfinyl)propyl GSL	423
N/A*	Heptyl GSL	427
Glucoraphenin^{+}	4-Methylsulfinyl-3-butenyl GSL	435
Glucoberteroin^{+}	5-Methylthiopentyl GSL	435
3-Methylsulfinylbutyl GSL^{+}	3-Methylsulfinylbutyl GSL	437
Glucoraphanin^{+}	4-Methylsulfinylbutyl GSL	437
Glucocheirolin	3-Methylsulfonylpropyl GSL	439
Glucolesquerellin	6-Methylthiohexyl GSL	450
Glucoalyssin^{+}	5-Methylsulfinylpentyl GSL	451

Glucoerysolin[+]	4-Methylsulfonylbutyl GSL	453
Glucosiberin	7-Methylsulfinylheptyl GSL	478
Glucohirsutin	8-Methylsulfinyloctyl GSL	494
Diglucothiobeinin	4-(β-D-glucopyranosyldisulfany)butyl GSL	521
Dimeric glucosativin	Dimeric 4-mercaptobutyl (DMB) GSL	812
Aromatic GSLs		
Glucotropaeolin (Glucotropeolin) [+]	Benzyl GSL	409
Gluconasturtiin (Gluconasturcin) [+]	2-Phenethyl GSL	423
Sinalbin (Glucosinalbin) [+]	p-Hydroxybenzyl GSL	425
Glucoarmoracialapicin	3-Phenylpropyl GSL	437
Glucoaubrietin	p-Methoxybenzyl GSL	439
Glucobarbarin (2S) [+ φ]	(2S)-2-Hydroxy-2-phenylethyl GSL	439
Glucosibarin (Epiglucobarbarin) (2R)[φ]	(2R)-2-Hydroxy-2-phenylethyl GSL	439
Glucoarmoracialafolin	4-Phenylbutyl GSL	451
Indolyl GSLs		
Glucobrassicin[+]	3-Indolylmethyl GSL	448
1-hydroxyglucobrassicin[+]	1-Hydroxy-3-indolylmethyl GSL	464
4-hydroxyglucobrassicin[+]	4-Hydroxy-3-indolylmethyl GSL	464
4-methoxyglucobrassicin[+]	4-Methoxy-3-indolylmethyl GSL	478
Neoglucobrassicin[+]	1-Methoxy-3-indolylmethyl GSL	478

* N/A: not available

[+] Glucosinolate identified in the USDA and ODS-NIH GSL Database

[φ] Zrybko et al. (1996) reported two isomers: glucobarbarin (2S) and glucosibarin (epiglucobarbarin) (2R); USDA combined values

Methods for quantifying GSLs generally fall into two categories: 1) chromatography-based methods (primarily HPLC), which measure individual GSLs; and 2) chromatographic methods or spectroscopic/colorimetric assays, which are used to determine total GSL contents by measuring the enzymatic breakdown products of GSLs (1, 11). In recent years, high-performance liquid chromatography (HPLC) or ultra-high-performance liquid chromatography coupled with mass spectrometry (UHPLC-MS) have become the primary methods to analyze GSLs. Currently, the most widely used chromatography-based method involves quantification of desulfo-GSLs. This method was harmonized into the European Union's (EU) official International Organization for Standardization (ISO) method ISO 9167-1 (1992) (13). ISO 9167 was amended in 2013 and 2019; the current version includes the modification of extraction solvent and relative response factors (https://www.iso.org/standard/72207.html). Despite its popularity, ISO 9167 is a multi-step, labor-intensive, and time-consuming method. To improve the method, commercially available dimethylaminopropyl (DEA)-based weak anion-exchange solid-phase extraction (SPE) can be used as a substitute for the self-prepared ion-exchange columns. This modification can reduce analysis time and improve repeatability and accuracy (14).

Total GSL content in plant material can also be estimated by the 1,2-benzenedithiol-based cyclocondensation assay (15). This method measures ITCs, the primary enzymatic hydrolytic products of GSLs. However, after enzyme hydrolysis, additional breakdown products other than ITCs may form depending upon the side chain structures, reaction conditions, and presence of non-enzymatic protein

factors. ITCs formed from indole GSLs are unstable and readily decompose into indole-3-carbinol and several other products (3). Therefore, this method is mostly suitable for aliphatic and aromatic GSLs and may measure only a portion of the total number of GSLs present in food.

In addition, data acquired through UHPLC coupled with high-resolution accurate-mass mass spectrometry (UHPLC-HRAM/MS) can be analyzed in a streamlined fashion using "GLS-Finder," a recently developed MATLAB-based expert system (16). This innovative tool offers a new way to quickly process data and interpret qualitative and semi-quantitative analyses of GSLs (16).

B. Glucosinolates and Human Health

Bioavailability of Glucosinolates in Human Body - Myrosinase-independent Hydrolysis

Many GSL-containing vegetables are consumed after domestic cooking using methods such as boiling, steaming, microwaving or stir-frying. These cooking methods, especially with longer cooking times and higher temperatures, are known to inactivate myrosinase. Nevertheless, studies in humans and animals have demonstrated that ITCs are still detectable in urine following consumption of cooked *Brassica* vegetables with no myrosinase activity (17, 18). This evidence suggested that GSLs could be hydrolyzed by "myrosinase-like" enzymes produced by the mammalian gut microbiota (19, 20). Although these microbial enzymes can hydrolyze GSLs, they are produced by diverse strains of bacteria in the gut, and their structure, function and activity may vary extensively. In general, the activities of these myrosinase-like enzymes are lower compared to myrosinase, which may limit the conversion of GSLs into bioactive ITCs. Consequently, the spectrum of degradation products formed by gut microbial metabolism may be more diverse and considerably varied among individuals compared to those generated by myrosinase.

Proposed Health Benefits of Dietary Glucosinolates

In humans, epidemiological evidence has linked consumption of cruciferous vegetables to reduced risk of several cancers, including lung (21), gastric (22), colorectal (23), breast (22), bladder (24) and prostate (25). A recent systematic review and dose–response meta-analysis (26) reported that these associations vary by cancer type, region, intake level, and follow-up duration. In the American population, cruciferous vegetables were predominantly associated with colorectal, renal, gynecological, and prostate cancer. In contrast, in Asian populations, significant associations were observed for lung cancer, head and neck squamous cell carcinoma, and esophageal cancer.

The biological activities of GSL rich vegetables have been largely attributed to their hydrolytic products, particularly ITCs and indole-3-carbinol (27). Beyond cancer prevention, GSLs and their hydrolytic products have also been shown to be protective against cardiovascular disease (28), neurodegenerative diseases (29, 30), Type 2 diabetes (31), several inflammatory disorders (32-34) and may exert beneficial effects on the risks of neurological and psychiatric conditions, such as depression, schizophrenia, autism, Alzheimer's disease, and multiple sclerosis (35).

A substantial body of evidence from both animal studies and human clinical trials has revealed multiple molecular mechanisms through which ITCs may exert cancer-preventive effects. These include the enhancement of carcinogen detoxification (36, 37), activation of antioxidant defenses via induction of phase II detoxification enzymes through the Nrf2-mediated pathway (38, 39), modulation of inflammatory responses (32), and induction of apoptosis in cancer cells (40).

Potentially Adverse Effects of Dietary Glucosinolates

GSLs in cruciferous vegetables have historically been considered anti-nutrients because of their potential to inhibit iodine absorption, which may impair thyroid function and contribute to the development of goiter (41). However, a recent systematic review questioned these previous assumptions (42). The data indicated that when consumed in reasonable and accessible amounts as part of a daily diet, *Brassica* vegetables are safe for thyroid function.

Due to their electrophilic reactivity, some ITCs can form adducts with DNA and induce gene mutations and chromosomal aberrations. Thus, the potential genotoxicity of ITCs should be carefully considered. However, the levels of ITCs from normal dietary consumption appear to be several orders of magnitude lower than the doses used in genotoxicity studies (43), making such toxic effects highly unlikely under normal dietary conditions. A related concern arises with dietary supplements and functional foods that contain highly concentrated ITCs. The potential health risks associated with these elevated exposures cannot be excluded with certainty (43).

In an analysis of three prospective cohorts of U.S. men and women (31), the consumption of cruciferous vegetables, and in particular cabbage and Brussels sprouts, were significantly associated with an increased risk of Type 2 diabetes. However, this study was criticized as having major flaws including a poor estimation of the intake of GSLs and exclusion of the bioactive forms of GSLs (44). In another study published by the same authors on the same cohorts, dietary GSL intake was also associated with a slightly higher risk of coronary heart disease (45). Similarly, methodological concerns apply to this study. Replication studies are needed to clarify these findings and to understand the underlying mechanism.

C. Variation of Glucosinolates in Brassica Vegetables and the Influential Factors

GSLs in plant foods are influenced by several factors. A plant's genetic background is a major factor in determining GSL composition. In one study (46), 21 different cultivars of broccoli were grown on the same field in the same year. The concentrations of the GSLs varied substantially across different cultivars. GSLs belong to a remarkably diverse group of plant secondary metabolites which are generally nonessential for the basic metabolic processes of the plant but help plants cope with continuous environmental challenges (47). Various environmental factors (e.g., climate, soil conditions and insects) and agricultural practices (e.g., fertilizer, growing seasons, organic vs. conventional farming) profoundly influence plant GSL profiles and contents. It is noteworthy that the effects of these factors are not always consistent. For example, organic farming was found to increase (48), decrease (49), or maintain GSL levels (50) compared to conventional farming.

D. Challenges for Dietary Guidance and the Need for Glucosinolate Data

Estimating Total Intake of Dietary Glucosinolates

The types of GSLs and their concentrations vary enormously among different cruciferous vegetables, both qualitatively and quantitatively (5, 10), making the estimation of total dietary GSL intake challenging. Reported intake estimates differ across regions and populations. In a German population, GSL intakes were estimated as 14.2 ± 1.1 mg/day for men and 14.8 ± 1.3 mg/day for women (51). For an adult Spanish population, mean intake was estimated as 6.5 mg/day, among which 35% were of indole type (52). In the United Kingdom, the national mean daily intake of GSLs was calculated as 46.1 mg in fresh materials, and as 29.4 mg from cooked foods (53). Nevertheless, these crude estimates were made based on very limited food composition data or restricted to exposure from GSL-containing vegetables. There is currently no

nationally representative estimate of GSL intake in the U.S. due to a lack of adequate dietary GSL composition data.

Need for a Glucosinolate Database

Food composition data are considered the foundation of dietetic practice and nutritional research, and are essential for advancing the multidisciplinary field of precision nutrition (54). In the United States, the USDA-ARS Methods and Application of Food Composition Laboratory (MAFCL) has developed several special interest databases for the dietary bioactive compounds of public health interest such as flavonoids (55), iodine (56) and purines (57), which have been widely used as a valuable tools to assess dietary intakes and for surveillance efforts (55, 58). Growing scientific interests in dietary GSLs have centered on their chemoprotective effects against cancer (59-61). Establishing a reliable and more comprehensive database of GSL composition in foods is, therefore, fundamental for accurate estimation to advance nutritional research.

An extensive literature search found only one published study which attempted to develop a dietary GSL database (62). This paper was published in 2003 and only included 18 references for developing the database. Of these, 10 used glucose-release methods, 5 used HPLC, and 3 used gas chromatography (GC) based methods. Due to the limitation of analytical methods, no data on individual GSLs were provided. Developing a comprehensive GSL database presents unique challenges compared to other food components, primarily due to the coexistence of GSLs with myrosinase in plant tissues, which can lead to enzymatic hydrolysis during sample handling (12). Ensuring data accuracy and reliability therefore requires careful consideration and evaluation of sample collection, preparation and extraction protocols, analytical methodologies, and quality control measures (63).

E. Creation of the USDA and ODS-NIH Glucosinolate Database

Purpose

The development of the USDA and ODS-NIH Database for the Glucosinolate Content of Foods (Release 1.0) serves an important scientific research purpose. First, it provides a tool for researchers and health professionals to estimate the dietary intake of GSLs for the U.S. population. Secondly, it supports epidemiological research for studying links between GSL intake and chronic diseases such as cancer and cardiovascular diseases. The development of food composition databases is a multi-step process which includes data acquisition, data evaluation, data compilation, data aggregation, and data dissemination (64, 65). Data collection and data quality evaluation are the core tasks of developing a valid food composition database (55, 66). For this database, the primary source of compositional data was published literature. Additional analytical data were generated in-house at USDA's MAFCL.

Database Format and Reporting Protocols

The USDA and ODS-NIH GSL database is presented as an Excel data file containing 6 tabs. Tabs contain North American food data (United States and Canada) which were divided into categories (broccoli, other *Brassica*, root vegetables) based on the volume of data sourced from literature. USDA's analytical data, which were collected between 2015–2016, are presented in a separate tab for accessibility. Sample size (N), sources of data, and detectable GSLs' (31 different types; **Table 2**) trivial names and molecular weights are reported. Plants' scientific names and their respective cultivars or genetic accessions were included when available. Data are sorted alphabetically by cultivar/accession to enable comparisons among species

varieties. In some cases, multiple plant cultivars were combined into a single composite sample by the original authors.

USDA data is in *Tab 4*. Except for microgreens, precise scientific names were unavailable for all the plants. Thus, plant scientific names were inferred using information sourced from literature. Data were combined across years for the same foods when the analytical methodology and processing conditions (i.e., cooking method and time interval) were identical.

Occasionally during the analysis, GSL compound peaks were observed, but their respective isomers could not be identified. Consequently, data columns denoted as "isomer(s)" represent unidentified chemical isomers (e.g., epiprogoitrin and progoitrin) which were included in the database for clarity and transparency. In addition, for one food (raw, chopped broccoli florets), 4-methoxyglucobrassicin data below the limit of detection (ND) was replaced with a 0.

GSL means, standard deviation (SD), minimum (Min), and maximum (Max) values were reported as milligrams per 100 grams fresh weight for foods. Descriptive statistics were re-calculated (e.g., standard error of the mean to standard deviation) when necessary. The database does not consider differences in seasonality – e.g., plant data collected from multiple seasons (e.g., Spring, Fall) were averaged to provide baseline GSL values, when applicable. Data cells with a dash ("- ") indicate data that were not calculable or measured, whereas values listed as "ND" (not detected) or "trace" in literature were reported when GSL measurements were below the studies' limit of detection. For all the data, footnotes are given where further descriptions or explanations of specific foods are needed.

Glucosinolate Data from Scientific Literature

1. Data sources

A systemic literature search was conducted spanning 1980–2024 and PubMed, SciFinder and Google Scholar were selected as the primary search databases. The following keywords were used in different combinations: glucosinolate, *Brassica*, cruciferous, food composition, analysis, isothiocyanate, vegetable. Reference lists of the retrieved research and review articles were reviewed to identify references not found using electronic search engines. Only data in original research articles were included; secondary data in review articles or books were excluded. A total of 31 articles were found to meet all inclusion and exclusion criteria. Detailed criteria are described as follows:

a) Because the database is intended for U.S. foods, only plant materials obtained from retailers or fields in the U.S. were included. According to USDA's Economic Research Service, Mexico and Canada are the major exporters of GSL-containing vegetables to the U.S. (67). Therefore, data from the studies conducted in these two countries, if any, were also included.

b) Information on the food source and the identification of samples, preferably with full scientific names and cultivars, were provided. Only data on fresh, raw (not processed) samples were included.

c) Only the data of commonly consumed plants and the edible parts of the samples were included. Wild plants, medicinal plants, animal feeds, non-edible plant parts, or by-products of plants, such as seeds of certain vegetables (68), were excluded.

d) The analytical methods were carefully described. Only the methods that quantify individual GSLs were included. HPLC analysis of desulfated GSLs (13) has been the primary quantification method in recent years. Data generated by gas chromatography (GC) or gas chromatography mass spectrometry (GC-MS) used in several early studies, and HPLC-MS, were also accepted.

e) For publications examining the effects of certain treatments or experimental manipulations on GSL concentrations, only the data from untreated or control groups were extracted.

2. Data Evaluation Methods

Five data evaluation qualities (sampling plan, number of samples, sample handling, analytical method and analytical quality control) were carefully assessed by a team of USDA scientists to ensure data quality using an established procedure (64). It is noteworthy that none of the accepted 31 papers were designed to develop a composition database. These studies were conducted for different purposes – for example, to examine the effects of plant fertilization (69), or to evaluate seasonal variation (70). Hence, the experimental designs in these studies generally did not take the factors discussed above into full consideration.

3. Data Extraction

Individual data points were extracted from the accepted studies. If both individual and total GSL values were reported, only individual data were extracted. Reporting units from literature were converted to the unit of milligrams per 100 g fresh weight (FW) for consistency in observing data across studies. Reported dry weight values were converted to FWs using the water content provided in the specific study, or the moisture level reported in USDA National Nutrient Database for Standard Reference, Legacy Release (71) if moisture information was not available in the literature.

4. Literature Data Discussion

In general, the sampling plans from studies meeting USDA inclusion criteria were not comprehensive. Samples in some studies were collected directly from the field, hence information about the cultivars, location and growing conditions were provided. In others, samples were obtained from the local supermarket without further information. Another commonly neglected issue was the insufficient or incorrect description of the plant materials. For example, only the common names (e.g., “broccoli”) of plant samples in five studies were provided. Plants may have multiple common names across different cultures, and in some cases, they can refer to different cultivars or even species (1, 7, 72). For studies where scientific names were not explicitly provided, they were inferred based on the descriptions of the plant materials and assigned using the most plausible taxonomic identification available.

The number of samples (*n*) and cultivars varied considerably across studies based on their experimental designs. For example, fifty cultivars of broccoli were included in one study to investigate the variation of GSLs in *Brassica* vegetables (73). However, in this publication, only one biological sample was analyzed for GSL content due to preparation methods (e.g., homogenized using multiple plants) and sample limitations. Sample handling was also different between different studies. Plant materials were analyzed either as raw or dry forms, and with or without cold storage. HPLC analysis of desulfated GSLs was used in 21 of the 30 studies. GC or GC-MS were mainly used in the early published papers (before 2005), and HPLC-MS was only used in two recent studies. The sample extraction and detailed analytical procedure also varied across different studies. Lastly, analytical quality controls, which ensure that the results of analysis are consistent, accurate and comparable, were not mentioned in any of the 31 studies.

Overall, a total of 357 individual food descriptions representing approximately 20 types of vegetables were sourced from literature. Among all the studies, broccoli was predominantly analyzed (n = 222 food descriptions; > 60% of the database entries), followed by horseradish roots (n = 38 food descriptions) and Brussels sprouts (n = 18 food descriptions). Cultivar and accession data for each vegetable were included for research purposes, when applicable. However, this information may not represent cultivars sold in the U.S. commercial market. A few of the studies composited multiple cultivars for the same food types (radish, turnip, and rutabaga) to increase the sample n and to provide average GSL values. Cooking data was only available for 3 vegetables from 4 of the 31 studies, highlighting gaps for future research to address.

The number of detectable GSLs and their contents varied per food type, cultivar, cooking, and even across studies for the same vegetables. Some of the common GSLs like glucoraphanin, glucobrassicin, and gluconasturtiin were broadly detected across the studies. However, in several cases detectable GSLs were limited to a few studies which only analyzed one biological sample. For example, glucobarbarin was only present in one sample of raw winter cress (74), and glucosinalbin was observed in one sample of broccoli and mustard leaf from two different studies (75). Likewise, the GSLs glucokohlrabiin and glucoraphasativusain were only detected in 3 broccoli samples (76). Furthermore, a few of the studies reported the GSLs as not detected (ND) or as trace but did not specifically state their detection limits. The lack of consistency and detectability of GSLs in vegetables, particularly for the same foods, presents a major research challenge – without this data, it is difficult to elucidate total GSL contents, to predict which GSLs are most likely to occur in certain food types, and to suggest which foods may offer increased beneficial health outcomes.

Because of these methodological and reporting inconsistencies, comparisons for each GSL and vegetables across the 31 studies are challenging. For demonstration purposes, **Table 4** shows the broad ranges for 5 GSLs representing 5 vegetables with the largest number of entries in the database, irrespective of cooking, storage, or other processing conditions. GSLs are not distributed equally and can vary in several magnitudes of difference depending on the vegetable. It is also worth noting that in some studies, certain plant accessions were prioritized for analysis due to their hypothesized high GSL content or were purposefully grown to evaluate significant differences in GSL contents at different growth stages. Overall, the compilation of these USDA reviewed North American publications into a unified, novel resource provides a framework for researchers seeking to understand the variability of GSL content in *Brassica* and related foods.

Table 4. Ranges of Five Selected Glucosinolates in Vegetables Sourced from North American Literature

Vegetable	Glucosinolate (mg/100g FW)				
	Sinigrin	**Glucoraphanin**	**Glucobrassicin**	**Gluconasturtiin**	**Neoglucobrassicin**
Broccoli	0.0 – 23.1	1.2 – 217.9	0.5 – 54.6	0.0 – 13.6	0.1 – 46.0
Brussels sprouts	1.4 – 55.7	0.2 – 35.6	0.0 – 267.2	0.0 – 3.6	0.0 – 4.9
Cabbage	3.2 – 59.9	0.3 – 19.6	1.8 – 105.6	ND – 2.3	0.0 – 8.4
Horseradish roots	8.5 – 1092.9	NA	0.0 – 14.8	NA	NA
Rutabaga roots	NA	2.4 – 4.8	0.1 – 41.2	1.7 – 22.0	0.05 – 0.3

ND = not detected; NA = not analyzed

USDA Analytical Glucosinolate Data

1. USDA Samples: Sources and Sampling

Selected cruciferous vegetables of the *Brassica oleracea* species (broccoli, kale, collard greens, and red cabbage) were purchased from local grocery stores in Beltsville, MD in 2015–2016. In addition, six microgreens in Brassicaceae, including red cabbage, broccoli, arugula, kale, China rose radish, and collard greens were also obtained from USDA's Food Quality Laboratory and were prepared using previously described methods (14).

The vegetables were rinsed with tap water for approximately 60 – 90 seconds to remove dirt and residue, followed by a second rinse with distilled water. Inedible parts of the plants (e.g., leaves/scales, hard stems) were cut off, and the vegetables were cut into 5 cm x 3 cm pieces and pieces mixed thoroughly prior to further processing to ensure homogeneity of the materials. Samples that did not require further preparation (e.g., cooking) were lyophilized and stored in a -60°C freezer immediately until analysis. Four common cooking methods — blanching, boiling, microwaving, and steaming — were used to cook the vegetables. The vegetables were cut just before cooking to minimize oxidation and hydrolysis of the GSLs. A brief description of each cooking method is provided below:

- *Blanching*: Tap water was added into a stainless-steel pot and was heated to boiling. Vegetable pieces were immersed in the pot for 30 seconds, then quickly removed from the boiling water and spread on a plate to cool.
- *Boiling*: Tap water was added into a stainless-steel pot with a lid. The water in the pot was brought to boil over high heat. The lid was removed, and vegetable pieces were submerged into the boiling water at various time intervals (30 – 85 seconds) based on the type of vegetable. Samples were removed from the water with a plastic colander, patted dry with a paper towel, and were spread on a plate to cool.
- *Microwaving*: Vegetable pieces were put in a microwave safe bowl with a teaspoon of water. The bowl was covered with microwave-safe plastic wrap and microwaved at full power (1200W, 2450 MHz) in a microwave oven (Panasonic Model NE-12521, Newark, NJ, USA) at various time intervals (30 – 90 seconds). The samples were removed from the microwave and were spread on a plate to cool.
- *Steaming*: A steamer basket was inserted into a stainless-steel pot containing approximately 2.5 cm (1 inch) of water, with the bottom of a steamer basket approximately 5 cm (2 inches) above the water surface. The water in the pot was brought to boil over high heat. Vegetable pieces were scattered over the steamer basket and steamed in medium heat at various time intervals (2 – 15 minutes). When the vegetables were done, they were quickly removed from the steamer basket and were spread on a plate to cool.

After cooking, all vegetables were lyophilized and stored in a freezer at -60 °C until analysis.

2. Analytical Laboratory Methodology and Quality Control

The extraction of GSLs was performed following the ISO 9167-1 method (13), as previously described in a USDA publication (12). Freeze-dried vegetable samples were ground into powders using a mini rotary processor or coffee mill. An aliquot of 100 mg of each sample was transferred into a pre-heated test tube at 75 °C for 1 minute. 5 mL of methanol was added and vortexed for 20 seconds. Subsequently, 200 µL of 5 mM sinigrin solution, which served as an internal standard, was added to the tube. The extraction solution

was kept in the heating block for another 20 min. After cooling the solution, additional methanol was added back to the liquid level marked by an indicator. The extracts were filtered with a 0.45 μm syringe filter and filtrates were transferred to HPLC vials for analysis. Each sample was extracted a total of three times.

For GSLs requiring desulfation, anion exchange resin in mini-columns were used to bind the GSLs from the vegetable extracts. The bound GSL molecules were desulfated overnight by incubating on the resin. For this procedure, 100 mg vegetable sample was transferred to a test tube and was processed following previously described procedures without the addition of sinigrin (internal standard). Each sample was extracted a total of three times using procedures briefly described below (13): The certified reference material ERM-BC367 (rapeseed) was used as a quality control throughout the experiment following the same sample preparation procedure.

1. 10 g of DEAE Sephadex A25 resin was mixed in excess of 2 mol/L acetic acid solution and was left to settle for approximately two minutes. An additional 2 mol/L of acetic acid was subsequently added until the volume of the liquid was equal to twice the volume of the sediment. **NOTE**: Alternatively, and according to ISO 9167 4.71 and 4.72, 10 g of DEAE Sepharose A25 resin may be used directly without the inclusion of acetic acid.
2. The mini-column was placed vertically on a stand, and 500 μL ion-exchange of resin was added to each column.
3. 1 mL of immidazole formate solution (6 mol/L) was added to each mini-column, which were then rinsed twice with 1 mL of water.
4. 1 mL of the vegetable extract was transferred to a prepared column without disturbing the resin surface. Two 1 mL portions of sodium acetate buffer (pH = 4) were added, and the buffer was drained after each addition.
5. 100 μL of diluted purified sulfatase solution was added to the column. The column was left to react overnight at ambient temperature. A tube was placed under the column to collect the eluate.
6. The desulfo-GSL was obtained with two 1 mL portions of water. Water was drained after each addition and while the eluate well was mixed.

Analyses were performed on an Agilent 1290 UHPLC (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector. Quantification was conducted using desulfated GSLs, with desulfo-sinigrin as internal standard and response factors were calculated against internal standard (14).

3. Data Discussion

USDA's analytical laboratory data (*Tab 4*) shows GSL values for a total of 32 foods representing 6 vegetables processed as raw or under different laboratory conditions (as whole material, via chopping, and/or cooking). Except for microgreens, all other vegetables were purchased in local grocery stores, and scientific names were not obtained. Like the literature data on samples from retailers, scientific names were assigned using the most plausible taxonomic identification available.

During USDA's analysis, several compound peaks were observed, but their respective isomers could not be identified. Thus, the database contains a total of 21 unique GSLs and 4 unidentified GSL isomers or stereoisomers (for progoitrin, glucoraphasativusain, hydroxyglucobrassicin, and methoxyglucobrassicin). All samples were analyzed in triplicate. Although data were combined when vegetable(s) and processing condition(s) were identical, many of the GSL values represent one biological sample. This lack of

variability is attributed to the experimental nature of the analyses which were not originally intended for compilation in a database.

The GSL profiles identified in USDA's material varied depending on vegetable type. Interestingly, the aliphatic GSL glucoraphanin and the indolyl GSLs glucobrassicin, neoglucobrassicin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin (including unidentified isomers) were detected in nearly all the samples, regardless of the vegetables and/or processing conditions. This may suggest a similar genetic background of these vegetables as *Brassica* vegetables. In other cases, aliphatic GSLs such as gluconapin, gluconapoleiferin, and glucoiberin were observed in leafy vegetable varieties. Arugula microgreens were the only samples which contained the aliphatic GSLs glucosativin and 3-methylsulfinylbutyl, because it is a non-*Brassica* vegetable. Furthermore, one of six samples of raw, chopped broccoli florets analyzed for 4-methoxyglucobrassicin, the measured content fell below USDA's limit of detection. To calculate descriptive statistics, these values were assumed as zero.

Because most *Brassica* vegetables are consumed after domestic cooking, the impact of domestic preparation methods on GSL content is highly relevant. Cooking in general (either via myrosinase-induced degradation or heating) can induce biological, physical, and chemical modifications to GSL profiles. For *Brassica* vegetables in particular, cooking is considered one of the most important factors affecting the enzymatic activity of myrosinase and thus the contents of GSLs measured in foods (5). The effects of four commonly used cooking (blanching, boiling, steaming and microwaving) on GSLs in selected vegetables were evaluated. Differences in GSL content were observed in the cooked samples compared to the raw samples. Generally, steaming and microwaving led to significantly higher GSL values compared to boiling and blanching because they effectively deactivated myrosinase. The effects of cooking varied by vegetable. For example, GSLs in untreated broccoli were similar to those of blanched broccoli, while GSLs were not detected in untreated kale. Importantly, these cooking methods could be considered as ways to inactivate myrosinase, which will thus result in more accurate measurement of GSLs.

F. Database Strengths and Limitations

The USDA and ODS-NIH GSL database provides the contents of individual GSLs in commonly consumed GLS-containing plant foods. The database integrates publicly available literature data and USDA-generated analytical data. For literature data, a thorough and systematic assessment of data quality was performed before the data was included in the database. Metadata for the food samples including scientific names, variety, growing location and harvest season were included when available. For USDA's analytical data, the analytical method was thoroughly reviewed and validated, and both sample preparation and extraction procedures were optimized to ensure accuracy and consistency. The database includes GSL profiles for both raw and cooked vegetables representing several of the most widely consumed cruciferous foods.

This database is the first publicly available resource to provide substantial GSL data and is intended for research use. Despite these strengths, there are notable limitations with the current database that researchers should consider. First, most available data were from a few select and commonly consumed vegetables (e.g., broccoli). Data on other GSL-containing vegetables are still limited. Secondly, none of the accepted literature sources were originally designed to generate data for the purpose of developing a composition database. As a result, no standard procedure was followed across different studies, and the experimental designs in different studies were also different. These factors made it difficult to directly compare the data from different studies. Thirdly, the GSL data on cooked vegetables are still limited. Future studies should

consider this critical factor to generate GSL content data that more accurately represents dietary intake patterns.

G. Conclusions

The USDA and ODS-NIH Glucosinolate Database Release 1.0 includes values for over 380 food descriptions representing approximately 20 vegetables. Values were sourced from published North American literature between 1980–2019 and USDA analytical data. The database provides new insights into the GSL content of commonly consumed vegetables using established review methods. It also highlights the challenges of analyzing GSLs in foods. Future research data and methodology gaps to address include 1) continuous identification of GSLs to establish commonalities among food types, 2) guidance for selecting additional, commonly consumed vegetables, 3) increased sampling size within food types to improve representability, and 4) the examination of how processing (cooking methods, storage conditions, temperature) affect GSL contents in foods over time. This database is the first publicly available resource to provide substantial GSL data and is intended for research use. As new U.S. data becomes available, subsequent database releases are anticipated.

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