Review

Advances of Progress and Prospects on Glucosinolate Detection in Cruciferous Plants

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Abstract: This review provides a comprehensive summary of the latest international research on detection methods for glucosinolates in cruciferous plants. The article examines various analytical techniques, including high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and enzyme-linked immunosorbent assay (ELISA), while highlighting their respective advantages and limitations. Additionally, the review delves into recent advancements in sample preparation, extraction, and quantification methods, offering valuable insights into the accurate and efficient determination of glucosinolate content across diverse plant materials. Furthermore, it underscores the critical importance of standardization and validation of these methodologies to ensure reliable glucosinolate analysis in both scientific research and industrial applications.

Keywords: glucosinolate; cruciferous crops; HPLC; LC-MS/MS; determination

1. Introduction

Glucosinolates represent a category of organic compounds that are present in cruciferous vegetables, including broccoli, cabbage, kale, and Brussels sprouts [1–3]. They are of significant interest due to their potential health benefits and their role as bioactive compounds in plant defense mechanisms. The detection and quantification of glucosinolates are crucial for both nutritional research and the advancement of plant breeding initiatives [4–8]. Glucosinolates are extensively distributed throughout various plant parts, including roots, stems, leaves, and seeds. This widespread presence underscores the importance of their analysis. The significance of glucosinolates is not only attributed to their direct consumption by humans and animals but also to the potential health benefits they may confer [9,10].

Although some glucosinolates and their degradation products have been identified as having anti-nutritional effects on certain animals, such as cattle, which can lead to negative consequences, other glucosinolates demonstrate specific anti-cancer activities [11–14]. Research suggests that these compounds may enhance the activity of phase II detoxification enzymes, which are linked to cancer prevention [13,15–17]. Furthermore, research has underscored the essential role of glucosinolates in sulfur metabolism, indicating that their biological functions merit further investigation [16].

The quantification of glucosinolates can be performed using two principal methodologies: one entails the indirect measurement of enzyme degradation products, while the other emphasizes the direct quantification of intact glucosinolates [18]. In indirect analysis, it is generally necessary to measure the products released during enzymatic or chemical reactions, such as isothiocyanates, oxazolidinethiones, thiocyanate ions, or glucose. The structures of these degradation products can reflect the characteristics of various parent glucosinolates. However, due to the lack of specificity in indirect methods for individual glucosinolates, these approaches are typically employed to quantify the total glucosinolate content in natural products [19–21].

Currently, a range of analytical techniques has been utilized to quantify glucosinolates in various plant tissues, including leaves, roots, buds, and seeds. The most frequently employed methods encompass HPLC, Gas Chromatography (GC), and Capillary Electrophoresis (CE) [22–24]. As previously noted, the content of glucosinolates can be analyzed either by directly measuring intact glucosinolates or by indirectly assessing their degradation products. However, certain methods, such as GC, may not be suitable for direct analysis due to the non-volatile or thermally unstable nature of some glucosinolates. Consequently, it is often necessary to convert these analytes into volatile derivatives to facilitate effective detection.

HPLC has been extensively utilized for the analysis of glucosinolates; however, the sample preparation process often necessitates desulfation. This procedure can result in incomplete desulfation, self-dimerization, and self-degradation, potentially compromising the reliability of the final data. Furthermore, the absence of standards for certain desulfated compounds poses a significant challenge to this methodology [25].

Mass Spectrometry (MS), owing to its high sensitivity, has been employed for the identification and quantification of glucosinolate compounds. A method has also been developed that integrates liquid chromatography with MS without necessitating desulfation. However, the accuracy and precision of this method are constrained by insufficient mass resolution. For instance, the mass-to-charge ratios (m/z) of glycosides and glycoproteins (m/z 422.0255 and m/z 422.0585, respectively) are remarkably similar. Although they share the same nominal mass of 422, their isotopic masses differ by only 0.033 Da, making it challenging to distinguish them through mass analysis alone. While these compounds can be separated chromatographically, the application of LC-MS remains limited if certain glucosinolate peaks significantly overlap or if there is substantial background interference in the monitoring channel [12,26].

Similar challenges arise when utilizing High-Resolution Mass Spectrometry (HRMS) to detect isomeric glucosinolates. This limitation can be mitigated by employing LC-MS/MS, which introduces an additional dimension of mass spectrometric detection, allowing for the simultaneous scanning of precursor and fragment ions, thereby enhancing selectivity and sensitivity. Furthermore, compared to LC-MS, LC-MS/MS typically requires shorter analysis times, facilitating the confirmation of known glucosinolates and the identification of novel compounds based on their fragmentation patterns [27,28].

Recently, a novel computerized system, GLS Finder, has been introduced, utilizing data generated from high-resolution HPLC-MS/MS to analyze glucosinolates in 49 common cruciferous vegetables. However, some reviewers have noted that the reported results may be ambiguous, as the number of chromatographic peaks for glucosinolates or their isomers exceeds the known quantity of glucosinolates present in each extract. This phenomenon may stem from the initial methods' inability to comprehensively identify all existing glucosinolates. Furthermore, comparing results with reported compositions serves as an effective validation method [29].

The accurate and effective detection of glucosinolates in plant materials is critically important for both scientific research and industrial applications. This review aims to summarize the latest advancements in detection methods for glucosinolates in cruciferous plants, providing valuable insights for future research.

2. Analytical Techniques for the Detection of Glucosinolates

Various analytical techniques have been employed for the detection of glucosinolates, including HPLC, LC-MS, GC, ultraviolet-visible spectrophotometry (UV-Vis), near-infrared spectroscopy (NIR), ELISA, and CE. Each technique presents distinct advantages and limitations regarding sensitivity, specificity, sample throughput, and cost. The selection of an appropriate method is contingent upon the specific requirements of the analysis, such as the number of glucosinolate species to be detected, the desired level of quantification, and available resources (Figure 1).
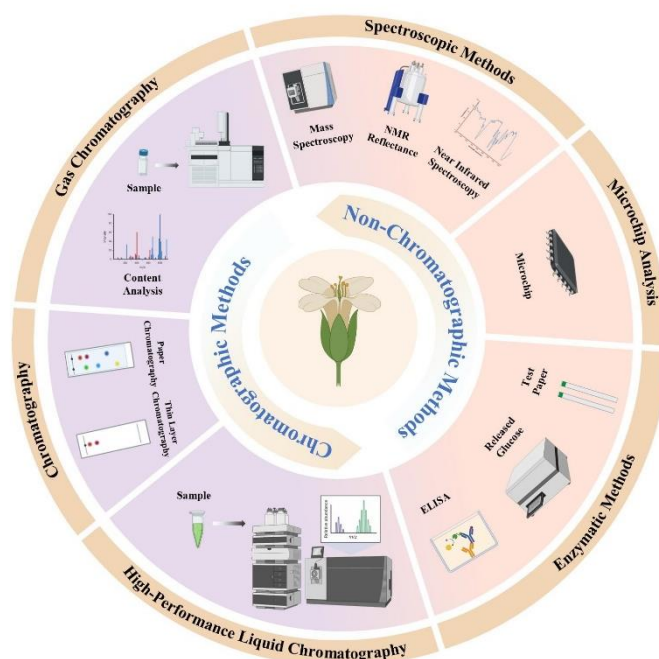
Figure 1. The characterization methods of gluconsinolates.

2.1. HPLC

HPLC is one of the most widely employed techniques for the analysis of glucosinolates, characterized by its high sensitivity, selectivity, and reproducibility. However, HPLC does have certain limitations, including extensive sample preparation requirements, costly equipment, and the necessity for skilled operators. The principle underlying this technique involves the separation and detection of thioglucoside components in vegetables, with quantification based on their retention time and peak area.

Initially, sample treatment is performed by chopping fresh cruciferous vegetables and extracting thioglucosides using suitable solvents (e.g., methanol or water). Following extraction, centrifugation and filtration are conducted to remove suspended matter; specifically, the extract is centrifuged to obtain a supernatant that is subsequently filtered. After obtaining the extract, chromatographic conditions are established by selecting appropriate columns and mobile phases while setting optimal flow rates and temperatures. Detection of thioglucosides can be achieved using either ultraviolet (UV) detectors or MS, allowing for accurate quantification of separated components through peak data recording [30,31].

2.2. LC-MS

LC-MS is a powerful technique that integrates the separation capabilities of liquid chromatography with mass spectrometric detection. It exhibits high sensitivity and specificity, enabling the simultaneous identification and quantification of multiple analytes. However, LC-MS is more complex and costly than HPLC, which limits its widespread application. The sample processing procedure closely resembles that of HPLC, involving the extraction of thioglucosides using appropriate solvents followed by separation via liquid chromatography upon completion. The separated components are then introduced into the mass spectrometer, which analyzes ions based on their mass-to-charge ratio (m/z) to provide molecular mass and structural information. Key advantages include enhanced sensitivity and selectivity, suitability for complex samples, and the capacity to yield insights into molecular structure [32–35].

2.3. ELISA

ELISA is a rapid, cost-effective, and straightforward method for the detection of glucosinolates. This technique relies on the specific binding of an antibody to a glucosinolate molecule. ELISA is well-suited for high-throughput analysis; however, it may be affected by cross-reactivity and limited specificity. The assay employs specific antibodies that bind to target molecules, generating a measurable signal through an enzymatic reaction, making it suitable for the quantitative analysis of particular thioglucosides [36,37]. Specific antibodies for the target thioglucoside are initially prepared, followed by sample processing once the antibodies are ready. The extracted sample is diluted and added to the ELISA plate, allowing the antigen to bind to the antibody. Subsequently, a substrate is introduced, and the enzyme reacts with this substrate to produce a measurable color change; absorbance is then quantified using a spectrophotometer. While this method offers advantages such as suitability for large-scale sample screening along with high sensitivity and specificity, it also presents limitations including the necessity of preparing specific antibodies in advance, a more complex procedure, and restricted capability for analyzing unknown compounds.

3. Sample Preparation, Extraction, and Quantification

3.1. Sample Preparation

The preparation of samples for glucosinolate analysis is a critical step that significantly impacts the integrity and quantification of these compounds. Various methods and conditions have been investigated to optimize glucosinolate extraction, each carrying distinct implications for the final results. A primary concern in glucosinolate sample preparation is the degradation of these compounds during processing. For instance, González-Hidalgo et al. emphasize that the time elapsed between harvesting and processing can lead to cellular damage, facilitating contact between glucosinolates and myrosinase, which results in rapid hydrolysis and a subsequent reduction in glucosinolate concentrations compared to fresh samples [38]. This degradation highlights the necessity of minimizing the interval between harvest and processing to preserve glucosinolate levels.

Furthermore, the selection of extraction solvent and method is crucial for optimizing glucosinolate recovery efficiency. Cold methanol extraction has demonstrated effectiveness, as it not only preserves glucosinolate content but also mitigates complications associated with elevated temperatures that may lead to degradation [39]. Doheny-Adams et al. demonstrate that cold methanol extraction surpasses other methods, particularly in preserving glucosinolate concentrations, thereby establishing it as a preferred technique for sample preparation [39]. Furthermore, the application of lyophilization (freeze-drying) may inadvertently diminish glucosinolate levels, as noted by Major et al., who observed that various drying methods did not significantly affect glucosinolate content in certain instances, indicating that careful consideration of drying techniques is crucial [40].

The extraction process can also be affected by the physical state of the plant material. For instance, mechanical processing of vegetables, such as shredding, may initially reduce glucosinolate levels but could subsequently result in an accumulation of these compounds over time due to the release of myrosinase [41]. This phenomenon suggests that while immediate processing may result in losses, subsequent reactions can potentially enhance glucosinolate levels, underscoring the necessity for a balanced approach in sample preparation.

Furthermore, the analytical methods utilized for glucosinolate quantification must be both robust and reliable. Techniques such as HPLC are frequently employed; however, the extraction and purification steps can be labor-intensive and time-consuming [42]. Grosser and Dam propose a streamlined HPLC method that simplifies the extraction process while ensuring accuracy [43]. This is critical as the complexity of glucosinolate profiles necessitates precise analytical techniques to ensure accurate quantification.

In conclusion, the preparation of samples for glucosinolate analysis necessitates meticulous attention to detail, encompassing the timing of processing, selection of extraction solvents, and employed methodologies. The interplay among these factors can significantly influence the final

glucosinolate content and its bioactive potential. Therefore, adopting optimized extraction protocols and analytical methods is imperative for accurate glucosinolate profiling.

3.2. Extraction

Several extraction methods have been utilized to isolate glucosinolates from plant materials, including solvent extraction, ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE). The selection of the optimal extraction method is critical for achieving high recovery rates and purity of glucosinolates. The effective extraction of glucosinolates—bioactive compounds present in cruciferous vegetables—can be accomplished through various techniques, each possessing unique advantages and challenges that are essential for optimizing extraction efficiency.

Solvent extraction remains a conventional method for isolating glucosinolates, where the choice of solvent significantly affects the yield and purity of the extracted compounds. For instance, studies have demonstrated that an 80% methanol solution can effectively inactivate myrosinase, an enzyme responsible for degrading glucosinolates, thereby preserving their concentrations during extraction [39,44]. Furthermore, the application of hydroalcoholic mixtures has been shown to enhance glucosinolate extraction yields, with varying efficiencies observed depending on solvent composition [45]. However, traditional solvent extraction often necessitates substantial time and solvent volumes, which may result in increased operational costs and environmental concerns [46].

In contrast, UAE has emerged as a more efficient alternative. This method employs ultrasonic waves to generate cavitation bubbles that disrupt plant cell walls and facilitate the release of glucosinolates into the solvent [47]. In contrast, UAE has emerged as a more efficient alternative. This method employs ultrasonic waves to generate cavitation bubbles that disrupt plant cell walls and facilitate the release of glucosinolates into the solvent [48,49]. For instance, studies have demonstrated that UAE achieves higher extraction efficiencies for glucosinolates from Camelina sativa by optimizing parameters such as solvent type and extraction duration [49]. Additionally, the application of UAE has been shown to improve the extraction of phenolic compounds, which are often co-extracted with glucosinolates, thereby further increasing the nutritional value of the extracts [50].

MAE is another innovative technique that has garnered attention for its capacity to enhance extraction efficiency. MAE utilizes microwave energy to heat both the solvent and sample, resulting in rapid extraction of glucosinolates due to the elevated temperature and pressure within the plant matrix [51,52]. Studies have demonstrated that MAE can yield results comparable to or superior than those obtained through UAE and traditional solvent extraction methods, particularly regarding extraction speed and solvent efficiency [46,51]. The integration of MAE with optimization techniques such as response surface methodology has been shown to maximize glucosinolate yields while minimizing solvent consumption [52].

In summary, while traditional solvent extraction methods are effective for glucosinolate extraction, UAE and MAE present significant advantages in terms of efficiency, yield, and environmental impact. The selection of the appropriate extraction method should be informed by the specific requirements of the target glucosinolates and the desired purity of the final extract.

3.3. Quantification

The quantification of glucosinolates can be accomplished by constructing calibration curves with standard compounds or by comparing the peak areas of samples against those of internal standards. The choice of a quantification method is dependent on the analytical technique utilized and the availability of suitable standards.

The quantification of glucosinolates, a class of sulfur-containing compounds predominantly present in the Brassicaceae family, is essential for elucidating their biological activities and nutritional significance. Various methodologies have been developed to accurately assess glucosinolate content in plant tissues, each possessing distinct advantages and limitations.

HPLC is one of the most widely employed techniques for quantifying glucosinolates. This method facilitates the separation and identification of individual glucosinolates based on their distinct chemical properties (**Table 1**). For instance, research has demonstrated that HPLC can effectively differentiate among various glucosinolates present in different cruciferous crops and Brassica species, thereby providing comprehensive profiles of their content [53–55]. The quantification process generally entails the extraction of glucosinolates from plant tissues, followed by hydrolysis to liberate glucose, which is subsequently measured spectrophotometrically [56,57]. This methodology has been validated in numerous studies, affirming its reliability for both total and individual glucosinolate measurements (Table 2) [52,58–60].

Table 1. The individual compounds of glucosinolates found in cruciferous plants.

Family	Species	Tissues and organs	GSLs compounds
Brassicaceae	*Brassica napus*	Seed, leaves, stems, roots	6-15
	Brassica juncea		7-17
	Capsella bursa - pastoris (L.) Medic.		3-7
	Brassica oleracea L. var. botrytis L.		4-9
	Brassica rapa		2-11
	Brassica carinata A Braun		3-8
	Camelina sativa	Seed, leaves, stems	3-12
	Camelina rumelica subsp. rumelica		
	Camelina macrocarpa		
	Brassica oleracea L. var capitata	Seeds, leaves	3-12
	Brassica oleracea L. convar capitata var alba	Florets, seedlings	3-14
	Brassica oleracea L. var italica	Seed, leaves, stems, roots, seedlings	7-16
	Raphannus sativus L.	Roots, seeds	6-14
	Arabidopsis thaliana	Leaf, florets, flowers, seedlings	3-23

Note: This table referenced some previous reports [2,61–63].

Table 2. The profiles of glucosinolates detected in cruciferous plants.

Glucosinolate types	Chemical Names	Common Names	Characterization Methods
AliphaticGSLs	Methyl GSL	Glucocapparin	MS, NMR
	1-Methylethyl GSL	Glucputranjivin	UV, IR, MS, NMR
	(1R)-Methyl-2-hydroxyethyl GSL	Glucosisymbrin	MS, NMR
	3-Methoxycarbonyl-propyl GSL	Glucoerypestrin	NMR
	Ethyl GSL	Glucolepidiin	Thiourea-type
	4-Oxoheptyl GSL	Glucocapanglin	Deducted from IR and 5-oxooctanoic acid
	5-Oxoheptyl GSL	Gluconorcappasalin	Thiourea-type, IR compared to GSL
	5-Oxooctyl GSL	Glucocappasalin	UV, IR of GSL and desGSL
	2-Hydroxy-2-methylpropyl GSL	Glucoconringiin	MS, NMR
	(1S)-1-Methylpropyl GSL	Glucocochlearin	MS, NMR of GSL and desGSL

(1R)-1-(Hydroxymethyl)-propyl GSL	Glucosisaustricin	MS, NMR of desGSL
(2S)-2-Methylbutyl GSL	Glucojiaputin	UV, IR, MS, NMR of GSL and des GSL
(2S)-2-Hydroxy-2-methylbutyl GSL	Glucocleomin	NMR of desGSL
3-(Methylsulfanyl)propyl GSL	Glucoibervirin	MS, NMR of GSL
4-Oxoheptyl GSL	Glucocapangulin	Deduction from IR,5-oxooctanoic acid
4-(Methylsulfanyl)butyl GSL	Glucoerucin	UV, IR, MS NMR of GSL
5-(Methylsulfanyl)pentyl GSL	Glucoberteroin	UV, IR, MS, NMR of GSL; UV, MS, NMR of desGSL
6-(Methylsulfanyl)hexyl GSL	Glucolesquerellin	UV, IR, MS, NMR of GSL
(R)-11-(Methylsulfinyl)-propyl glucosinolate	Glucoiberin	MS, NMR, X-Ray of GSL; UV
(R/S)-4-(Methylsulfinyl)-butyl glucosinolate	Glucoraphanin	MS, NMR of GSL, UV
(R/S)-5-(Methylsulfinyl)pentyl GSL	Glucoalyssin	MS, NMR of GSL
(R/S)-6-(Methylsulfinyl)-hexyl GSL	Glucohesperin	UV, IR, MS, NMR of GSL
(R/S)-8-(Methylsulfinyl)-octyl GSL	Glucohirsutin	UV, IR, MS, NMR of GSL;
(R/S)-9-(Methylsulfinyl)-nonyl GSL	Glucoarabin	UV, IR, MS, NMR of GSL;
(R/S)-10-(Methylsulfinyl)decyl GSL	Glucocamelinin	MS, NMR of GSL
3-(Methylsulfonyl)-propyl GSL	Glucocheirolin	MS of GSL; NMR of desGSL
4-(Methylsulfonyl)butyl GSL	Glucoerysolin	MS of GSL; MS, NMR of desGSL
(R/S, 3E)-4-(Methylsulfiny1)-but-3-enyl GSL	Glucoraphenin	MS, NMR of GSL; UV,NMR of desGSL
(R)-4-(Cystein-S-yl) butyl GSL	Glucorucolamine	MS, NMR of desGSL
4-(β-D-Glucopyranosyl-disulfanyl)-butyl GSL	Diglucothiobeinin	MS of GSL; MS, NMR of desGSL
6-Benzoyl-4 (methylsulfanyl)butyl GSL	6'-Benzoyl-glucoerucin	UV, MS, NMR of desGSL
6'-Benzoyl-4(methylsulfinyl)butyl GSL	6'-Benzoyl-glucopharanin	UV, MS, NMR of desGSL
(R/S,3E)-6-Sinapoyl-4 (methylsulfinyl)but-3-enyl GSL	6'-Sinapoyl-glucoraphenin	UV, IR, MS, NMR of desGSL
Allyl glucosinolate	Sinigrin	MS, NMR, X-Ray of GSL; UV
But-3-enyl GSL	Gluconapin	MS, NMR of GSL; UV,
Pent-4-enyl GSL	Glucobrassicanapin	MS of GSL
(2S)-2-Hydroxypent 4-enylGSL	Gluconapoleiferin	MS of GSL

Glucosinolate types	Chemical Names	Common Names	Characterization Methods
	(2R)-2-Hydroxybut 3-enylGSL	Progoitrin	MS, NMR of GSL; UV, MS, NMR of desGSL
	(2S)-2-Hydroxybut 3-enylGSL	Epiprogoitrin	MS, NMR of GSL; UV, MS, NMR of desGSL
Aromatic GSLs	(1R)-2-Bezoyloxt-1-methylethyl GSL	Glucobenzosisymbrin	UV, IR of ITC
	(1R)-1-(Benzoyloxymethyl) propyl GSL	Glucobenzsisaustricin	Thiourease-type, IR compared to GSL
	Benzyl GSL	Glucotropaeolin	MS, NMR of GSL; UV, MS, NMR of desGSL
	3-Hydroxybenzyl GSL	Glucolepigramin	MS of GSL; MS, NMR of desGSL
	3-Methoxybenzyl GSL	Glucolimnanthin	MS, NMR of GSL; UV, MS, NMR of desGSL
	4-Hydroxybenzyl GSL	Glucosinalbin	UV, MS, NMR of GSL and desGSL
	4-Methoxybenzyl GSL	Glucoaubrietin	MS, NMR of desGSL
	3,4-Dihydroxybenzyl GSL	Glucomatronalin	MS of GSL
	4-Hydorxy3-methoxybenzyl GSL	3-Methoxysinalbin	UV, MS, NMR of desGSL
	3-Hydroxy-4-methoxybenzyl GSL	Glucobretschneiderin	UV, IR, MS, NMR of GSL
	4-Hydorxy-3,5-dimethoxybenzyl GSL	3,5-Dimethoxy-sinalbin	UV, MS, NMR of desGSL
	2-Phenylethyl GSL	Gluconasturtiin	NMR of GSL; UV, MS, NMR of desGSL
	2-hydroxy-2-phenylethyl GSL	Glucobarbarin	MS, NMR of GSL and desGSL
	(2R)-2-Hydroxy-2-phenylethyl GSL	Epiglucobarbarin	MS, NMR of GSL and desGSL
	2-(4-Methoxy-phenyl) ethyl GSL	Glucoarmoracin	NMR of GSL, MS, NMR of desGSL
	(2R)-2-Hydroxy-2-(4-hydroxyphenyl) ethyl GSL	p-Hydroxy-epiglucobarbarin	MS, NMR of GSL; UV, MS, NMR of desGSL
	(2S)-2-Hydroxy-2(4-hydroxyphenyl) ethyl GSL	p-Hydroxy-glucobarbarin	UV, MS, NMR of desGSL
	4-(4'-O-Acetyl-α-c-4-rhamnopyranosyloxy)-benzyl GSL	4-Acetyl-glucomoringin	MS of GSL and ITC
	6'-Isoferuloyl-2 phenylethyl GSL	6'-Isoferuloyl gluconasturtiin	MS of GSL, UV
	6'-Isoferuloyl-(2R) 2-hydroxy-2phenylethyl GSL	6'-Isoferuloyl epiglucobarbarin	MS, NMR of GSL; UV
	6'-Isoferuloyl-(2S) 2-hydroxy-2phenylethyl GSL	6'-Isoferuloyl glucobarbarin	MS, NMR of GSL; UV, MS, NMR of desGSL
	4-(α-L-Rhamnopyranosyloxy) benzyl GSL	Glucomorinigin	MS, NMR of GSL and desGSL
Indolic GSLs	4-Methoxyindol-3-yl GSL	Glucorapassicin A	UV, IR, MS, NMR of synthesized GSL

Indol-3-ymethyl GSL	Glucobrassicin	UV, IR, MS, NMR of GSL and desGSL
4-Hydroxyindol-3-ylmethyl GSL	4-Hydroxy-glucobrassicin	MS of GSL; UV, MS, NMR of desGSL
4-Methoxyindol-3-ylmethyl GSL	4-Methoxy-glucobrassicin	UV, MS, MS, NMR of GSL and desGSL
1-Methoxyindol-3-ylmethyl GSL	Neoglucobrassicin	UV, IR MS, NMR of GSL; MS, NMR of desGSL
1,4-Dimethoxyindol-3-ymethyl GSL	1,4-Dimethoxy-glucobrassicin	UV, MS, NMR of desGSL
1-Acetylindol-3-ymethyl GSL	N-Acetyl-glucobrassicin	MS of desGSL
1-Sulfoindol-3-ylmethyl GSL	N-Sulfo-glucobrassicin	UV, IR, MS, NMR of GSL
6'-Isoferuloylindol-3-ylmethyl GSL	6'-Isoferuloyl-glucobrassicin	MS of GSL; UV, MS, NMR of desGSL

Note: This table references some previous reports [2,64,65].   .

Concurrently, spectrophotometric methods have been developed for the rapid and cost-effective quantification of glucosinolates. For instance, a straightforward spectrophotometric approach was proposed to estimate total glucosinolates in mustard de-oiled cake, illustrating the potential for less resource-intensive techniques [66]. Furthermore, near-infrared spectroscopy (NIRS) has emerged as a promising alternative for non-destructive analysis of glucosinolates in intact seeds and plant tissues [67,68]. This method capitalizes on the unique spectral signatures of glucosinolates, enabling swift assessments without extensive sample preparation.

The quantification of glucosinolates is influenced by a variety of factors, including plant species, developmental stage, and environmental conditions. For instance, glucosinolate content can vary significantly among different cultivars of Brassica and in response to abiotic stresses such as salinity and temperature [52,69–71]. Moreover, genetic factors play a pivotal role in shaping the glucosinolate profiles of various plant varieties, which can be leveraged for breeding programs aimed at enhancing nutritional quality [72–74]. Additionally, the biological activity of glucosinolates is intricately linked to their hydrolysis products—such as isothiocyanates—which are recognized for their health-promoting properties [52,75,76]. Therefore, understanding quantification methods not only facilitates the assessment of the nutritional value of Brassica vegetables but also provides insights into their potential health benefits."

The quantification of glucosinolates is a complex process that can be accomplished using various analytical techniques, primarily HPLC and spectrophotometry. The choice of method may depend on specific research goals, available resources, and the nature of the samples being analyzed. Further research into the factors affecting glucosinolate content will enhance our understanding of these important phytochemicals.

4. Standardization and Validation of Glucosinolate Detection Methods

4.1. Standardization

Standardizing glucosinolate detection methods is crucial to ensure accurate and reliable results. This involves establishing standardized protocols for sample preparation, extraction, and quantification, as well as using certified reference materials and participating in inter-laboratory comparison studies. The standardization of these methods is essential for ensuring consistency and reliability in the analysis of bioactive compounds found in cruciferous vegetables, which offer significant health benefits. Various methodologies have been developed and validated, each with its own advantages and limitations.

HPLC is a widely utilized technique for glucosinolate analysis. Vastenhout et al. have demonstrated a method for evaluating the kinetics of glucosinolate hydrolysis using HPLC, confirming the linearity of absorbance with concentration through UV-Vis spectroscopy, which is essential for accurate quantification [77]. Similarly, Gallaher et al. developed and validated a spectrophotometric method for quantifying total glucosinolates in cruciferous vegetables, highlighting the reliability and speed of their approach, which has broad applicability [78]. The use of HPLC in combination with MS has also been emphasized by Frank et al., who employed LC-TOF-MS to screen mustard seeds for glucosinolates, demonstrating the method's effectiveness in identification and quantification of these compounds [79].

Additionally, Nuclear Magnetic Resonance (NMR) spectroscopy has been suggested as a potential approach for quantifying glucosinolates. Yuan et al. observed that although NMR is not commonly utilized for this purpose, it offers advantages such as eliminating the need for calibration standards, which can streamline the analysis process [53]. This is particularly pertinent when considering the complexity of plant matrices, where conventional methods may encounter difficulties due to interference from other compounds.

The extraction protocols employed prior to analysis are equally critical and can significantly influence the results. Neal et al. described a method for extracting glucosinolates from Arabidopsis thaliana leaves, where the identities of the peaks were confirmed using standards, thereby ensuring the reliability of the findings [80]. Furthermore, Major et al. discussed the impact of myrosinase activity during extraction, emphasizing that enzymatic activity can lead to a reduction in total glucosinolate concentration, thus necessitating meticulous control over extraction conditions [40].

Recent advancements have also concentrated on optimizing extraction methods to enhance glucosinolate yield. For instance, Meza et al. developed a UPLC-DAD method that refined sample preparation procedures to achieve high specificity and accuracy in glucosinolate quantification [81]. Furthermore, ultrasound-assisted extraction has been investigated as a more environmentally friendly alternative, demonstrating potential in improving extraction efficiency while minimizing solvent usage [82].

Thus, the standardization of glucosinolate detection methods necessitates a combination of reliable analytical techniques, optimized extraction protocols, and careful consideration of enzymatic activity. The integration of HPLC with MS, along with emerging methodologies such as NMR and ultrasound-assisted extraction, constitutes a comprehensive approach to glucosinolate analysis that can enhance the accuracy and reproducibility of results across various studies.

4.2. Validation

The validation of glucosinolate detection methods entails the assessment of parameters such as linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). This process ensures that the method is appropriate for its intended purpose and yields reliable results. The validation of these methods is essential to confirm their reliability and suitability for specific applications. Key parameters for validation include linearity, precision, accuracy, LOD, and LOQ; each parameter plays a crucial role in verifying that the analytical methods employed can consistently produce valid results.

Linearity refers to the method's capacity to yield results that are directly proportional to the analyte concentration within a specified range. For instance, studies have shown that HPLC methods can achieve exceptional linearity for glucosinolate quantification, with correlation coefficients (R^2) frequently exceeding 0.99, thereby indicating a robust linear relationship between concentration and response [43,83]. This is crucial for ensuring that the method can accurately quantify glucosinolates across a spectrum of concentrations typically encountered in plant samples.

Precision, which evaluates the reproducibility of the method, is another critical parameter for validation. It is typically assessed through repeatability and intermediate precision studies. For instance, the precision of HPLC methods for glucosinolate analysis has been reported with relative standard deviations (RSDs) generally below 5%, indicating high reproducibility in measurements

[39,43]. Similarly, methodologies such as UPLC-DAD have demonstrated consistent results across various laboratories, further reinforcing the reliability of these techniques [52,81].

Accuracy, which quantifies the proximity of measured values to true values, is a fundamental aspect of method validation. Various studies have utilized recovery experiments to evaluate accuracy, wherein known quantities of glucosinolates are added to samples and the recovery rates are subsequently calculated. Reports indicate that recovery rates for glucosinolates typically range from 90% to 110%, thereby confirming the reliability of the methods employed [39,83]. Such accuracy is essential for ensuring that reported glucosinolate levels accurately reflect true concentrations in the samples.

LOD and LOQ are critical for assessing the sensitivity of analytical methods. LOD denotes the lowest concentration of the analyte that can be reliably detected, while LOQ represents the lowest concentration that can be quantified with acceptable precision and accuracy. Studies have demonstrated that HPLC methods can achieve LODs in the low micromolar range for various glucosinolates, rendering them suitable for detecting trace levels of these compounds in complex matrices [43,83]. This sensitivity is particularly vital in food safety and nutritional studies, where low glucosinolate levels may carry significant biological implications. The validation of glucosinolate detection methods through an assessment of linearity, precision, accuracy, LOD, and LOQ is essential to ensure result reliability. A rigorous evaluation of these parameters confirms that the employed methods are appropriate for their intended purposes, thereby bolstering research and applications across fields such as nutrition, food science, and plant biology.

5. Future Prospects

5.1. Development of New Analytical Techniques

The development of novel analytical techniques, such as ion mobility spectrometry (IMS) and NMR, has the potential to significantly enhance the sensitivity, selectivity, and throughput of glucosinolate analysis. Glucosinolates, sulfur-containing compounds found in cruciferous vegetables, are of considerable interest due to their health benefits and roles in plant defense mechanisms. Traditional methods for glucosinolate analysis—primarily HPLC—while effective, may be limited in terms of sensitivity and specificity. The integration of advanced analytical techniques like IMS and NMR can effectively address these limitations.

NMR spectroscopy is particularly valuable due to its non-destructive and highly informative characteristics. Recent advancements in NMR technology, including the development of hyperpolarization techniques, have significantly enhanced the sensitivity of NMR measurements. For instance, hyperpolarized water has been employed to improve the detection of various compounds, including glucosinolates, by increasing the signal-to-noise ratio in NMR experiments [84,85]. Furthermore, the application of dynamic nuclear polarization (DNP) has demonstrated promise in further enhancing NMR sensitivity, facilitating the detection of low-abundance metabolites—crucial for analyzing glucosinolates within complex biological matrices [86].

Furthermore, the incorporation of magnetic nanoparticles in conjunction with NMR has emerged as a potent strategy for enhancing detection capabilities. Magnetic nanoparticles can alter the local magnetic environment, thereby influencing the relaxation rates of adjacent nuclei and improving the overall sensitivity of NMR measurements [87,88]. This approach is particularly advantageous for glucosinolate analysis, as it facilitates the detection of these compounds at lower concentrations and within more complex samples.

IMS is another promising technique that can complement NMR in the analysis of glucosinolates. IMS facilitates rapid separation and identification of ions based on their mobility in a gas phase, thereby providing high-throughput analytical capabilities. The integration of IMS with MS further enhances the specificity of glucosinolate detection by enabling the identification of specific molecular ions associated with glucosinolates and their degradation products [89,90]. This dual approach significantly improves the analytical workflow, allowing researchers to obtain comprehensive profiles of glucosinolates across various samples.

The integration of advanced analytical techniques, such as NMR and IMS, offers a significant opportunity to enhance the analysis of glucosinolates. These methods not only improve sensitivity and selectivity but also facilitate high-throughput analysis, rendering them invaluable tools in the investigation of these important phytochemicals.

5.2. Miniaturization and Automation

The adoption of advanced analytical techniques, including NMR and IMS, provides a considerable opportunity to enhance the analysis of glucosinolates. These methodologies not only improve sensitivity and selectivity but also facilitate high-throughput analysis, thereby establishing them as essential tools in the investigation of these important phytochemicals.

HPLC and Ultra-Performance Liquid Chromatography (UPLC) represent the cutting edge of glucosinolate analysis. Notably, UPLC is recognized for its superior separation efficiency, reduced solvent consumption, and shorter run times compared to conventional HPLC methods. For example, Meza et al. demonstrated that UPLC provides a more environmentally sustainable quantification of glucosinolates in Camelina seeds, thereby minimizing ecological impact while enhancing throughput [81]. Similarly, Shi et al. underscored the efficacy of HPLC coupled with diode array detection for quantifying glucosinolates, highlighting its significance in high-throughput applications [91]. These methodologies enable the simultaneous detection of multiple glucosinolates, thus streamlining the analytical process.

Furthermore, the integration of MS with chromatographic techniques has significantly enhanced glucosinolate detection. Wu et al. employed LC-MS/MS in multiple reaction monitoring mode to analyze glucosinolate profiles in red cabbage, demonstrating the method's sensitivity and its capacity to rapidly provide detailed compositional data [35]. This combination improves detection limits and facilitates the identification of various glucosinolates within complex matrices, which is crucial for comprehensive profiling in high-throughput applications.

Automation plays a pivotal role in these advancements, facilitating the rapid processing of samples with minimal human intervention. Techniques such as automated sample preparation and robotic liquid handling systems can significantly reduce analysis time and variability in results. However, the reference by Li et al. does not specifically address glucosinolate analysis; rather, it focuses on genetically modified organisms [92]. Consequently, it is inappropriate to support claims regarding automation in glucosinolate detection based on this source. Furthermore, the proposed application of biosensors and microfluidic devices for real-time monitoring and analysis of glucosinolates lacks direct corroboration from the cited references [93,94].

In conclusion, the miniaturization and automation of glucosinolate detection methods are revolutionizing analytical practices by facilitating high-throughput analysis, minimizing resource consumption, and expediting analysis times. The integration of advanced chromatographic techniques with MS, alongside automation, is paving the way for more efficient and effective glucosinolate profiling.

5.3. Application of Machine Learning(ML) and Artificial Intelligence

ML and artificial intelligence algorithms can be employed to optimize detection methods, predict glucosinolate content, and classify plant materials based on their glucosinolate profiles. The application of ML and artificial intelligence (AI) in refining detection techniques, forecasting glucosinolate levels, and categorizing plant materials according to their glucosinolate profiles represents an emerging area of research that holds significant promise for agricultural and food sciences. Glucosinolates, a class of sulfur-containing compounds predominantly found in Brassica species, are recognized for their health benefits, including cancer prevention and antioxidant properties [52,85,95]. The integration of ML and AI has the potential to enhance the efficiency and accuracy of glucosinolate analysis, which is critical for both breeding programs and food quality assessment.

One of the primary applications of ML in this context is the optimization of detection methods. Traditional techniques for glucosinolate quantification, such as HPLC, while effective, can be time-

consuming and necessitate extensive sample preparation [81]. Recent advancements in ML algorithms have demonstrated their ability to analyze complex datasets generated from chromatographic techniques, enabling rapid identification and quantification of glucosinolates [96]. For instance, the GLS-Finder platform employs UPLC coupled with HRMS to conduct qualitative and semi-quantitative analyses of glucosinolates, significantly reducing analysis time [96]. Furthermore, ML can be trained to predict glucosinolate profiles based on various growth conditions and environmental factors, thereby facilitating more targeted breeding strategies [85,97].

In addition to optimizing detection methods, ML and artificial intelligence (AI) can be utilized to predict glucosinolate content in various plant materials. Factors such as soil composition, climatic conditions, and plant genetics significantly influence glucosinolate levels [97]. Statistical modeling approaches, including regression analysis and neural networks, have been effectively employed to forecast glucosinolate concentrations in crops such as Chinese cabbage and kale based on these variables [85,97]. For instance, studies have demonstrated that environmental factors like temperature and humidity are correlated with glucosinolate accumulation, facilitating predictive models that assist in selecting optimal growing conditions for desired glucosinolate profiles [89,97].

Furthermore, artificial intelligence (AI) can facilitate the classification of plant materials based on their glucosinolate content. By employing supervised learning techniques, researchers can categorize different cultivars of Brassica species according to their glucosinolate profiles, which is essential for both breeding and consumer preferences [95]. ML algorithms are capable of analyzing spectral data from methods such as hyperspectral imaging to classify plant materials rapidly and non-destructively, providing a valuable tool for quality control in agricultural practices [98]. This classification capability can also extend to identifying plant varieties with enhanced health-promoting properties, thereby guiding breeding programs aimed at improving nutritional quality [95]. Thus, the integration of ML and AI into glucosinolate research offers significant advancements in detection methods, predictive modeling, and the classification of plant materials. These technologies not only enhance the efficiency of glucosinolate analysis but also contribute to the development of crops with optimized health benefits, aligning with the increasing demand for functional foods in the market.

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18

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