Simplified analytical methodology for glucosinolate hydrolysis products: a miniaturized extraction technique and multivariate optimization†

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Miniaturized extraction techniques are one of the most significant advances in analytical chemistry today. Nowadays there is a growing tendency among food researchers to develop simpler and robust methodologies that allow the determination of multiple analytes in different samples. Based on this concept, the aim of this work was to develop an optimized and validated methodology for the determination of four isothiocyanates (ITCs) and one indole: allyl ITC, erucin, sulfophane, phenyl ITC and indole-3-carbinol present in Brassicaceae vegetables. Experimental design and multivariate analysis were the statistical tools used during the process. The dispersive liquid–liquid microextraction (DLLME) technique developed in this work was successfully applied to the analysis of nine Brassicaceae species.

1. Introduction

The Brassicaceae family (also known as Cruciferous or Brassica) includes 350 genera and about 3500 species.1 These plants are distributed all over the world due to their ability to adapt to a wide range of climatic conditions.2 This family includes genera of great economic value, such as Brassica, Nasturtium, Raphanus, Arabidopsis and Eruca.3 They represent a significant part of the human diet, being highly consumed by different ethnic groups around the world.4 In the last three decades, the world production of these vegetables has grown steadily, becoming a major source of nutrients and proteins for both, humans and animals.5 In addition to the nutritional and economic importance of Brassicaceae vegetables, these species are considered highly valuable for researchers given their health-promoting properties, which are related to their phytochemical composition.

In the Brassicaceae family, two different types of sulphur-containing compounds are found in the whole plant, glucosinolates (GLSs) and S-methyl sulfoxide.6,7 The presence of GLSs becomes evident after tissue disruption, which triggers the hydrolysis (by myrosinase enzymes (MS)) and the release of a small number of unstable products, which are then transformed into more stable compounds, such as isothiocyanates (ITCs), nitriles, epithionitriles and thiocyanates.8 The beneficial health properties associated with these vegetables are attributed to their quali-quantitative ITC levels.9 Among the functional properties, it is important to highlight that there is substantial evidence of the relationship between Brassica consumption and chronic degenerative disease prevention, such as cancer and cardiovascular disease.9 Other functional activities can also be mentioned such as antibacterial activity,10,11 antifungal activity,12 antiviral activity13 and antioxidant activity.14

At present, the analytical investigations of these vegetables have focused on achieving the individual determination of ITCs in complex matrices, such as plant extracts or biological fluids.15 Although there are numerous studies that have aimed their efforts at studying and improving the beneficial effects of these compounds, considering diverse species, the kind of crop, post-harvest treatments, cooking, and processing, there is scarce information related to modern analytical extraction techniques applied for multiple ITC determination.

Nowadays when developing a new analytical methodology, miniaturized extraction processes are being chosen over conventional extraction procedures. They follow the principles of green chemistry, in which lower solvent volumes are used and fewer polluting wastes are produced, and the analysis is faster, reducing electrical equipment consumption – all this, without sacrificing neither the efficiency nor effectiveness at the time of determining the analytes of interest. Considering food analysis, dispersive liquid–liquid microextraction (DLLME) is a highly employed extraction technique given its practicality and efficiency,
especially when analytes are found at low concentrations in the food matrix. However, there are no reports of liquid micro-extraction techniques applied to the determination of GLSs or ITCs in any matrices, despite their advantages over other sample preparation techniques traditionally used.

Therefore, considering the importance of obtaining a robust analytical method to contribute to the development of a proper dietary isothiocyanate database, the need to develop the present work arises. It allows the individual determination of five highly biologically active compounds derived from GLS hydrolysis (sulforaphane, indole-3-carbinol, allyl ITC, phenyl ITC and erucin) present in nine different vegetable matrices.

Here, we propose a novel methodology using DLLME for the extraction and pre-concentration of ITCs and indole-3-carbinol prior to their determination by HPLC-DAD. The optimum extraction conditions were obtained by employing a multivariate response surface design, and then analytical figures of merit were calculated under optimum conditions. Also, the matrix effect on DAD signals was investigated for the nine species under study. All the parameters obtained were satisfactory for the verification of the method suitability. The proposed methodology represents an improvement and modernization of other available methods, given the use of a miniaturized extraction technique. Hence, a superior methodology is achieved since it increases the sensitivity and decreases the analysis time, altogether minimizing solvent consumption. Furthermore, the optimized analytical methodology proved to be a helpful analytical tool that would allow a more in-depth study of bioactive compounds from the Brassicaceae family after their consumption.

2. Experimental

2.1. Reagents and analytical standards

Sulforaphane (SF, 90%), allyl ITC (AITC, 95% v/v), indole-3-carbinol (I3C, > 96% v/v) and phenyl ITC (PITC, 98% v/v) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Erucin was extracted from rocket (Eruca sativa) seeds, following the work of Vaughn and Berhow (2005). Defatted seeds were obtained following the optimized ultrasound process described previously by Da Porto et al. (2013). The identity and concentration of erucin were established according to Barillari et al. (2005).

All the standard solutions were diluted with methanol (MeOH) and stored at −20 °C. Working solutions were prepared monthly under the same conditions. Formic acid (FA, > 88% w/v) was obtained from Cicarelli (San Lorenzo, Santa Fé, Argentina). Sodium chloride (NaCl) and sodium sulphate (Na₂SO₄) were acquired from Biopack (Buenos Aires, Argentina). Hexane, dichloromethane (DCM), chloroform (CF), acetonitrile (ACN) and MeOH were of chromatographic grade and acquired from Sintorgan (Villa Martelli, Bs. As., Argentina). Ultrapure water (18 MΩ cm) was obtained from a Milli-Q purification system (Millipore, Paris, France).

2.2. Sampling

A total of 45 samples of nine commonly consumed Brassicaceae vegetables, including broccoli, cabbage, Brussels sprouts, radishes, green mustard, cauliflower, rocket seeds, and watercress, were purchased from local grocery stores located in the Cooperative Market of Mendoza. It represents the place where the primary producers, exporters and vegetable traders of Midwestern Argentina converge. One kg of each species was purchased in five different stores; after that, a single batch was formed and sent to the laboratory.

A subsample of each homogeneous batch was analyzed in triplicate. The edible part of these vegetables was conditioned by adequate cleaning. Phytochemical extraction and moisture content determination were performed on the same day of purchase. For moisture assays, the samples were processed, weighed (3 g of each vegetable, except rocket seeds) and dried in a convection oven (DALVO, Santa Fé, Argentina) at 100 ± 10 °C to constant weight.

2.3. Sample preparation

Phytochemical extraction was carried out following an optimized technique, previously reported by our research group. Ten grams of fresh vegetables were placed in a blender with 50 mL of water and homogenized (blender, 600 W, 60 Hz; model HR2030/10; PHILIPS, Buenos Aires, Argentina). Separately, two grams of defatted rocket seeds were placed in a conical tube and 10 mL of 0.1 M bicarbonate buffer (pH 8.1) were added. Then, the samples were sonicated in an ultrasound bath inside a 100 mL glass beaker for 5 min (US-bath, 40 kHz and 600 W; model tb 04; TESTLAB, Buenos Aires, Argentina). ITC formation was carried out by stirring an aliquot of 5 mL homogenate in a glass vial inside a water bath at 37 °C for 2 h.

2.4. Chromatographic equipment and operating conditions

The chromatographic analysis was performed using a liquid chromatograph (Shimadzu LC 20A), with a diode array detector (ODS Waters RP-C18 column, 150 mm × 4.6 mm × 5 μm) and a guard-column with the same characteristics. The elution of the analytes was performed with a mobile phase resulting from different ratios of MeOH (A) and water (B) at a flow rate of 0.6 mL min⁻¹ for 30 min. Both solvents contained 0.1% of FA. The system was equilibrated under the starting conditions for 10 min before the injection of the next sample. Before use, mobile phases were filtered through a 0.45 μm filter. The linear gradient program used is described below: 0 min 50% A, 20 min 80% A, and 30 min 80% A. The detector wavelength was set to 241 nm (Wilson, Ennahar, Marchioni, Bergaentzlé, & Bindler, 2012). The volume of injection was 10 μL, and the oven temperature was set to 25 °C. Peak identification in the samples was carried out by comparing retention times with reference standards.

2.5. DLLME procedure

One milliliter of ACN (dispersive solvent) mixed with 700 μL of CF (extraction solvent) was rapidly injected into each sample solution using a syringe. Subsequently, the mixture was centrifuged at 2000 rpm for 2 min (Gelec, G142, Buenos Aires, Argentina). The extractant phase was removed using a syringe and dried under a nitrogen stream, and then it was dissolved in...
500 µL of MeOH; finally, it was filtered before injection into the HPLC-DAD for analysis.

### 2.6. Method development

To determine the optimum working conditions, the effect of different factors, such as the type of dispersive solvent (MeOH or ACN), the type of extraction solvent (DCM or CF), the extraction solvent volume (0.4–0.6 mL), the dispersive solvent volume (1–3 mL), pH modification, the salting-out effect, use of an ultrasound (US) bath to subserve the formation of the dispersion cloud, and the sample volume (3–6 mL), was evaluated using a Plackett–Burman experimental factorial design (Table S1 †). Peak areas of each analyte were investigated as responses to optimize the independent variables (factors). The solution used for the analysis was an unhydrolyzed vegetable homogenate (to avoid the presence of ITCs), and 150 µg g⁻¹ of each analyte standard was added.

Once the variables that significantly influenced the extraction process were selected, they were submitted to a multivariable experimental design using a response surface model (one-factor), which allowed us to study one qualitative variable and at least one quantitative variable. The obtained design is shown in Table S2 (ESI †).

### 2.7. Statistical software

The computer software Design-Expert 7.0.0 was used to design the experiments and to model and analyze the results.

### 3. Results and discussion

#### 3.1. Method development

3.1.1. Experimental design and response surface modelling. Eight factors were used for DLLME optimization using an experimental design. The Plackett–Burman model was selected to minimize the generalized variance of the parameter estimates for a prespecified model. Peak areas of each analyte were investigated as responses to optimize the independent variables (factors), which were selected due to their positive effects on the microextraction process, following the previous experience of other authors.¹⁶,²²,²³–²⁵

From the obtained results, it was possible to construct a Pareto chart and then evaluate the factors that influence the extraction process (Fig. 1). Table S3 (ESI †) comprises the Pareto chart’s information, summarizing the obtained results according to the influence of the different factors on each analyte. To determine if a factor is influential over the extraction process, the absolute value of the effect must be higher than the mean value assigned by the model (r-value); in case it exceeds the Bonferroni limit, its influence on the process is indisputable. The model is selected based on the factors that explain the 80% of the variability for each response. In all cases, the significance of each model was verified, and the presence of atypical data (outliers) and whether the residues for each analyte were in the order of normality were checked.

The use of an US bath, salting out effect and sample volume were the factors which at any level did not influence significantly the extraction process. Therefore, these factors were not considered in the extraction process, or if its use was strictly essential, only the lower level was considered as a reference.

The factors that had a significant influence on one of its levels were the type of dispersive solvent, the volume of the dispersive solvent and pH modification, in which case the level that influences at least one of the responses was considered for the extraction process.

Finally, the factors that influenced the analytes in different ways (type and volume of the extractant solvent) were selected for their optimization using the experimental design of surface response (one-factor) that allows obtaining maximum values for each analyte.

Fig. 2 illustrates the graphical analysis of the global desirability function. These plots were generated and studied to obtain an optimum set of conditions (maximum area) for each response, from d = 0, for a completely undesirable response, to d = 1, for a completely desirable response. This graph shows the interaction between two factors while the remaining factor was kept unchanged.

It can be observed from the plot that the desirability function reaches its maximum value (0.87) when it approaches coordinate –1 of variable B and 700 of variable A. This means that CF is the optimum extractant solvent and 700 µL is the optimum volume needed.

#### 3.2. Analytical performance of DLLME-HPLC-UV

Method validation was carried out following reference parameters established in the ICH guidelines, which are commonly used for method validation to determine low concentration compounds present in food samples.²⁶,²⁷ The parameters that were evaluated under optimum conditions were selectivity, the limit of detection (LOD), the limit of quantitation (LOQ), the linear range, precision, accuracy, and the matrix effect (ME).

Table 1 and 2 summarize the retention times of the analytes and the analytical figures of merit.

The calibration curves were found to be linear in a concentration range of 5–100 µg mL⁻¹ (5, 10, 20, 30, 50, and 100), with correlation coefficients (r) > 0.91 for all analytes. The detection limits (LODs) of the compounds, calculated as three times the signal/noise ratio (S/N = 3), ranged between 0.1 and 2.2 µg g⁻¹ (d.w.) and LOQs, calculated as ten times the signal/noise ratio (S/N = 10), ranged between 0.3 and 7.4 µg g⁻¹ (d.w.).

To evaluate the precision of the analytical method, the dispersion of five replicates on the same day and nine replicates on three consecutive days was performed and the RSD (relative standard deviation) was calculated. The critical parameters were found to be within the acceptance intraday (%RSD < 3.9) and interday criteria (%RSD < 15). To evaluate the extraction recovery of the technique, the samples were contaminated with three levels of each standard compound (20, 30 and 50 µg mL⁻¹) and they were extracted following the optimized DLLME in triplicate. Calculations were performed according to Rezaee et al., (2006).²⁸ The mean of the obtained results showed that extraction recoveries ranged between 80 and 110%, which
indicated that the DLLME technique for ITC and indole extraction from Brassicaceae samples was suitable (see Table S4 – ESI†).

According to the parameters previously established and accepted for the analysis of fruits and vegetables, values from −20 to +20% for the matrix effect are considered adequate indicating a low ME. When a positive value is obtained, it means that in the matrix there are components that can interfere by increasing the analytical signal; the signal is decreased if the value is negative. In both cases, the value of ±20% was considered as a reference value to justify the use of external calibration with analytes dissolved in solvents instead of dissolving them in a medium that is similar to the matrix, thereby considerably reducing the cost of the analyses.

The obtained results in the different matrices indicated that in most cases (95%), the ME was <20%; only in two cases this effect is the highest. One of them was SF in watercress, and the other was PITC in red cabbage. In these cases, a correction

Fig. 1 Pareto chart obtained after screening the design of the effect that influences the extraction process. Responses: (A) allyl ITC, (B) phenyl ITC, (C) sulforaphane, (D) indole-3-carbinol and (E) erucin.
factor should be used, for the quantification with an external standard on these matrices. However, there are no reports indicating the presence of any of these two analytes in these plant matrices.

3.3. Comparison of the presented DLLME with other sample preparation techniques

After analyzing all the results of the analytical performance of the optimized method, a comparison of the proposed methodology was made with other studies reported in the literature. The search for new methodologies that allow us to improve the analytical figures of merit, to shorten the times of analysis or to reduce the use of polluting compounds constitutes the driving force of modern research in analytical chemistry. Therefore, it is important to compare the performance of different experimental procedures or the optimization of a given methodology under various experimental conditions.

When comparing two or more analytical methodologies, the following parameters are usually considered: linearity, accuracy, and sensitivity. For Brassicaceae, although some literature exists on analytical methodologies to determine the compounds derived from GLSs, only a small number of them focus on the study of the analytes present in our research, and further only a few investigations have determined the method performance by calculating the analytical figures of merit. So, our methodology based on DLLME coupled to HPLC with a UV detector was compared with other analytical methods based on traditional liquid extraction and solid phase extraction (SPE), in all cases coupled to HPLC-UV.

Table 3 shows the linear range, limit of detection (LOD) and accuracy of four different methodologies for the extraction and determination of ITCs and indole in the samples of Brassicaceae vegetables. The obtained results are satisfactory in terms of linearity, sensitivity, and accuracy, without many differences between the different methods. In our case, given the use of DLLME as the extraction technique, it is also possible to observe a few advantages over the other studies; the time of analysis is clearly reduced compared to traditional liquid–liquid extraction techniques; plus analytical wastes and costs are reduced.

Also remarkable, when comparing our analytical methodology with other available methods, is the green aspects that have been taken into account in the proposed method. A modern analytical protocol should assess an environmental impact of the analytical procedures.

### Table 1: Retention time, precision, limits of detection and quantification and correlation coefficient of each compound using the optimized methodology

| Standard compound | Retention time (min) | RSD% | LOD (μg g⁻¹ d.w.) | LOQ (μg g⁻¹ d.w.) | r²  |
|-------------------|----------------------|------|-------------------|------------------|-----|
|                   |                      | Intra-day⁶ | Inter-day⁷       |                  |     |
| Sulforaphane      | 7.3                  | 2.3   | 6.7               | 0.1              | 0.3 | 0.9978 |
| Indole-3-carbinol | 10.0                 | 2.2   | 8.2               | 0.5              | 1.6 | 0.9669 |
| Allyl ITC         | 13.4                 | 3.6   | 7.1               | 0.8              | 2.7 | 0.9122 |
| Erucin            | 20.7                 | 1.9   | 5.9               | 2.2              | 7.4 | 0.9973 |
| Phenyl ITC        | 24.4                 | 2.1   | 5.6               | 1.3              | 4.2 | 0.9983 |

⁶ n = 5 extractions made on the same day (30 μg mL⁻¹). ⁷ n = 9 extractions made on three consecutive days (30 μg mL⁻¹).
friendly approach considering all the different steps of the analytical process. For this reason, we focused on making the different stages of the analytical process as non-polluting and waste-free as possible. We took into account from the beginning that the homogenate preparation and enzymatic hydrolysis only involved aqueous media with no organic solvents involved. Otherwise the results would not be representative of what a consumer might eventually acquire when eating these vegetables. On the other hand, the combination of a short ultrasound time (5 min) and a miniaturized extraction technique contributed significantly to the increase the analyte recoveries because of a concentration effect, without the need for subsequent steps of sample preparation. In addition, the use of a chromatographic gradient and a column with a small particle size allowed the optimal separation of the analytes in a short time and with a low expenditure of solvents when using a chromatographic gradient and a column with a small particle size. We must emphasize the main advantage of this method: it can be applied to different types of samples, an alternative that until now was not available. On the other hand, sample pretreatment is quite simple despite the complexity of the matrices, as shown in the previous sections. So, the application of the analytical method developed in this work proved to be reliable on different types of samples.

### 3.4. Brassicaceae sample analysis

After validation of the analytical method (DLLME-HPLC-UV), different Brassicaceae vegetables were studied, and their ITC and indole contents were determined. The obtained results are shown in Table 4.

The quali-quantitative levels found by applying our method are consistent with those in previous reports which have determined each ITC in different vegetables. AITC, SF, and I3C were found in almost every sample. The concentration of these analytes varies widely from one plant to another; green mustard produced the highest level of AITC (95.7 ± 10.4 μg g⁻¹ d.w.) and SF was the highest ITC found in broccoli (260.4 ± 9.2 μg g⁻¹ d.w.) while I3C in Brussels sprouts was the highest (70.5 ± 8.8 μg g⁻¹ d.w.). It was also observed that the main glucosinolate hydrolysis product in rocket seeds was erucin (6.47 ± 1.5 μg g⁻¹ d.w.); this coincides with reports by Barillari et al., 2005 (ref. 20) and Pasini, Verardo, Caboni, & D’Antuono (2012). Notably, levels of AITC in mustard leaves and PTC in radishes found in other studies are lower than those found in this study. Here we demonstrate the efficiency of the extraction, purification, and pre-concentration of the samples developed in this work. It should also be noted that thanks to the application of our methodology, multiple ITCs could be detected in the same vegetable sample, facilitating rapid ITC profile characterization of each vegetable.

### Table 2 Matrix effect during the extraction and analysis of the analytes in the different Brassicaceae vegetables

| Brassicaceae vegetables | Sulforaphane | Indole-3-carbinol | Allyl ITC | Erucin | Phenyl ITC |
|-------------------------|--------------|------------------|----------|--------|------------|
|                         | %ME         | r²               | %ME      | r²     | %ME        | r²     | %ME        | r²     | %ME        | r²     |
| Broccoli                | 9.4         | 0.98             | -3.5     | 0.88   | -16.1      | 0.99   | 12.0       | 0.99   | -4.0       | 0.98   |
| White cabbage           | 0.6         | 0.97             | -14.4    | 0.98   | -13.9      | 0.99   | 15.6       | 0.99   | 13.5       | 0.99   |
| Red cabbage             | 5.6         | 0.99             | -0.5     | 0.87   | -3.6       | 0.99   | 15.9       | 0.98   | 28.0       | 0.95   |
| Brussels sprouts        | -14.2       | 0.99             | -15.0    | 0.99   | -15.8      | 0.99   | 14.7       | 0.99   | -8.6       | 0.99   |
| Radishes                | 12.0        | 0.98             | -19.8    | 0.90   | -8.5       | 0.94   | 15.0       | 0.95   | 6.2        | 0.93   |
| Watercress              | -27.4       | 0.99             | -14.6    | 0.99   | -1.1       | 0.96   | 5.9        | 0.97   | -18.4      | 0.99   |
| Rocket seeds            | 10.6        | 0.98             | -16.3    | 0.95   | 8.4        | 0.97   | 14.7       | 0.97   | 14.7       | 0.99   |
| Cauliflower             | -16.0       | 0.99             | -13.4    | 0.98   | -4.8       | 0.99   | 14.7       | 0.98   | 4.4        | 0.98   |
| Green mustard           | -0.8        | 0.89             | -11.9    | 0.97   | 16.4       | 0.97   | 15.8       | 0.96   | -19.1      | 0.99   |

### Table 3 Comparison of different methods for the extraction and determination of ITCs and indoles in vegetables of the Brassicaceae family

| Methodology          | Analytes | Linear range (μg mL⁻¹) | LOD (μg g⁻¹) | % Accuracy | Vegetable sample                          | References                                      |
|----------------------|----------|------------------------| ------------|------------|--------------------------------------------|------------------------------------------------|
| LLE-HPLC-UV          | SF       | 2–100                  | 0.2         | 109        | White cabbage and cauliflower              | (Sangthong and Weerapreeyakul, 2016)⁴²         |
|                      | AITC     | 0.2                    | 102         |            | Radishes                                   | 22                                             |
|                      | PTC      | 0.1                    | 108         |            | Broccoli                                   | (Pilipczuk, Kusznierekiewicz, Chmiel, Przychodziński, and Bartoszek, 2017)⁴⁹ This work |
| SPE-HPLC-UV          | SF       | 0.3–30                 | 0.8         | 92–102     | Broccoli, white cabbage, radishes and watercress | (Pilipczuk, Kusznierekiewicz, Chmiel, Przychodziński, and Bartoszek, 2017)⁴⁹ This work |
| SPE-HPLC-UV          | SF       | 9–18                   | 0.3         | 83–104     | Broccoli, white cabbage, radishes and watercress | (Pilipczuk, Kusznierekiewicz, Chmiel, Przychodziński, and Bartoszek, 2017)⁴⁹ This work |
| UAE-DLLME-HPLC-DAD    | SF       | 5–100                  | 0.1         | 87–107     | Broccoli, watercress, rocket seeds, white cabbage, radishes, red cabbage, Brussels sprouts, green mustard and cauliflower | This work                                      |
Table 4 Isothiocyanate and indole levels found in vegetables of the Brassicaceae family

| Brassicaceae sample | Sulfuraphane\(^a\) | Indole-3-carbinol\(^a\) | Allyl ITC\(^a\) | Erucin | Phenyl ITC |
|---------------------|---------------------|-----------------------|----------------|--------|------------|
| Broccoli            | 260.4 ± 9.2         | 54.0 ± 9.8            | 91.9 ± 12.4    | 13.0 ± 1.8 | ND         |
| White cabbage       | 10.4 ± 0.1          | 27.4 ± 1.0            | 9.1 ± 0.4      | ND     | ND         |
| Red cabbage         | 4.7 ± 0.3           | 17.9 ± 1.7            | 78.2 ± 2.0     | ND     | ND         |
| Brussels sprouts    | 2.6 ± 0.1           | 70.5 ± 8.8            | 12.2 ± 0.4     | ND     | ND         |
| Radishes            | 16.9 ± 0.3          | 18.6 ± 1.4            | 69.4 ± 5.4     | ND     | 11.5 ± 1.2 |
| Watercress          | ND                  | 42.9 ± 2.7            | 89.0 ± 10.8    | ND     | ND         |
| Rocket seeds        | 1.17 ± 0.5          | ND                    | 0.98 ± 0.08    | 6.47 ± 1.5 | ND         |
| Cauliflower         | ND                  | 24.1 ± 4.4            | 6.7 ± 0.1      | ND     | ND         |
| Green mustard       | ND                  | 11.9 ± 0.5            | 93.7 ± 10.4    | ND     | ND         |

\(^{a}\) Mean values (\(n = 3\)) expressed as \(\mu g\) g\(^{-1}\) of dry matter ± SD. ND means that the compound was not detected in that vegetable, considering the LOD showed in this table.

4. Conclusions

The present work proposes a novel methodology based on DLLME coupled to HPLC-DAD for the extraction, pre-concentration, and determination of the hydrolysis products of GLSs present in Brassicaceae samples. A multivariate experimental design was developed to optimize the analytical conditions.

The target analytes can be determined accurately and precisely, and the time of analysis is noticeably reduced. Additionally, the proposed methodology represents an environmentally friendly procedure, since it requires lower volumes of organic solvents compared to previously reported methods. The figures of merit results were robust and comparable with those previously reported, and the optimized analytical methodology has been efficiently applied for the extraction and determination of ITCs and indoles in different vegetable matrices, proving to be a helpful analytical tool to complement diverse studies.

It is important to highlight that this is the first time that a DLLME-HPLC-DAD methodology is reported for ITC and indole determination in vegetable matrices. Altogether, the proposed methodology constitutes an excellent analytical implementation for the screening and quantitation of GLS hydrolysis products, with prospects of being used as a protocol analysis to develop a phytochemical and nutritional database of the Brassicaceae family.

Conflicts of interest

There are no conflicts to declare.

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