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Raphanus sativus functional potential: Impact of the analytical extraction technique on the bioaccessibility interpretation

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Abstract

Radishes (*Raphanus sativus* L.) are cruciferous vegetables with remarkable nutraceutical properties given their distinctive isothiocyanates (ITCs) profile. These compounds are formed after glucosinolates-Myrosinase enzymolysis. Although it is important to characterize radishes' ITCs levels, it is also necessary to evaluate the bioactive compounds' physiological fate after radishes ingestion. To do so, the extraction techniques should adapt to such conditions of high aqueous environment. In this work, we studied the bioaccessibility of ITCs and indole-3-carbinol (I3C) in radishes taproots considering the analytical implications of this biological process. Results showed that ITCs and indole profiles in the radish's taproots after aqueous-myrosinase hydrolysis followed by Dispersive Liquid-Liquid Microextraction were distinctively different from other reports. After in vitro digestion, raphasatin showed the highest bioaccessibility despite its low quantitative yields. Notably, I3C and S-Sulforaphene become promising phytochemicals, due to their bioaccessibility and their considerable remaining amounts after digestion.

KEYWORDS

analytical methodology, bioaccessibility, isothiocyanates, radishes

1 | INTRODUCTION

Vegetable-based diets, especially those rich in species belonging to the *Brassicaceae* family, have been attracting a large number of consumers given their health-promoting effects. Radishes (*Raphanus sativus*) are well-known cruciferous plants, specifically root crop vegetables, which are available in numerous varieties that differ in terms of size, shape, taste, and color (Hanlon & Barnes, 2011). They are extensively consumed, either in their raw version as salads ingredient or as pickles or sauces, especially in Asian countries where they represent one of the most popular consumed vegetables (Gutiérrez & Perez, 2004; Manivannan et al., 2019).

Besides the culinary importance of radish due to its characteristic pungent flavor, it is also a remarkable nutraceutical plant. Different radish extracts have been used to treat gastric and hepatic illnesses, as well as cardiac disorders throughout history (Manivannan et al., 2019; Takaya et al., 2003). Radish taproots, sprouts, and seeds have also been related to antimicrobial, anticancer, and antioxidant effects (Banihani, 2017; Gutiérrez & Perez, 2004; Manivannan et al., 2019; Takaya et al., 2003; Verkerk et al., 2009). These

beneficial properties have been attributed to the presence of different phytochemicals in their matrix, including glucosinolates (GLS), isothiocyanates (ITCs), and polyphenols (PF) (Gutiérrez & Perez, 2004; Hanlon & Barnes, 2011; Li et al., 2015; Lim, 2015; Manivannan et al., 2019). However, to fully characterize a functional food, not only the food product should be considered, but researchers also emphasize the importance of carrying out comprehensive studies analyzing the phytochemicals availability once they are consumed. The first biological barrier consists of the compounds' gastrointestinal (GI) digestion (Torres-Palazzolo et al., 2018). In this sense, bioaccessibility refers to the fraction of a chemical compound that can potentially be absorbed by the enterocytes or through tight junctions (Oliviero et al., 2018). Commonly, simulated GI digestion protocols are used to evaluate the bioaccessible fraction of phytochemicals. In this regard, even though several reports have studied phenolic compounds' bioaccessibility in cruciferous vegetable matrices, including broccoli, cabbage, and radish sprouts (Abellán et al., 2021; Hanschen & Schreiner, 2017; Oliviero et al., 2018; Sarvan et al., 2017; Tomas et al., 2021), there are no reports regarding radishes' sulfur compounds, like ITCs, after in vitro digestion, representing this last group the most distinctive *Brassicaceae* phytochemicals with highly recognized bioactivities.

ITCs, particularly, are formed from GLS upon tissue disruption during processing, chewing, or digestion. GLS are naturally occurring compounds (thioglycosides), present in *Brassicaceae* species, which differ in the structure of the aglycone side chain. They are considered biologically inactive per se, but upon tissue disruption, GLS are hydrolyzed by myrosinase (MYR; EC 3.2.3.1), an endogenous ß-glucosidase enzyme. In the presence of water, myrosinase cleaves off the glucose from GLSs (Lee et al., 2017). The type of product formed depends on the type of GLS, the presence of epithionitrile protein, and the reaction conditions (e.g., temperature, pH, and grade of tissue disruption) (Oliviero et al., 2018).

There are numerous studies focused on the GLS content determination on the vegetable matrix employing Gas Chromatography coupled with Mass Spectrometry Detection (GC-MS) and/or Liquid Chromatography with Ultraviolet Detection (HPLC-UV) (Hanlon et al., 2007; Kim et al., 2013; Yi et al., 2016). However, there are few reports focused on ITCs composition in radishes (Blažević & Mastelić, 2009; Hanlon & Barnes, 2011; Kim et al., 2015; Lee et al., 2017; Li et al., 2015), and most of them have determined raphasatin and sulforaphene, without focusing on other ITCs or indoles. Moreover, some authors have shown that the analytical process, including the hydrolysis/extraction techniques employed to determine GLS breakdown products, influences the final ITCs and indole profile obtained (Arora et al., 2014). Hence, these effects should also be addressed when considering the phytochemicals biological fate. Regarding radishes analytical methodologies, traditionally employed techniques are mostly based on non-polar organic extractants added simultaneously during the myrosinase hydrolysis process (Hanlon & Barnes, 2011; Kim et al., 2015). However, to assess the phytochemical levels once the vegetable is consumed, it is necessary to adapt the extraction techniques to the digestive conditions (with a highly aqueous medium). Therefore, an alternative analytical procedure from the traditionally employed should be considered for this type of biological matrices.

Based on the foregoing, the present work aimed to study the bioaccessibility effects on the ITCs and I3C profile of radish's taproots. Previously, a comparison was made between two hydrolysis/extraction procedures for the determination of GLS degradation products. One was based on previously reported techniques (using organic solvents during the myrosinase hydrolysis), and on the other side, a validated analytical methodology based on Dispersive Liquid-Liquid Microextraction (DLLME) (Fusari et al., 2019) was used to extract and determine the ITCs and I3C present in the vegetable matrix after the enzymolysis process. This last method was also employed to determine the phytochemical composition and content after a GI in vitro digestion protocol. The radishes varieties considered for this study were *Raphanus raphanistrum* subsp. *Sativus* L. (red radish), Japanese Miyashige daikon (white radish), and Sakurajima Giant (giant radish). Results showed that the ITCs profile in the radish's taproots after the myrosinase hydrolysis employing DLLME was distinctively different from the others reported. ITCs and I3C bioaccessible percentages were also calculated. Notably, raphasatin and sulforaphene showed the highest bioaccessible percentages, despite their low quantitative yields. Overall, these results showed that when considering radish taproots, ITCs intake is highly dependent on the GLS myrosinase enzymolysis conditions. To our knowledge, this is the first time that a discussion about radishes' organosulfur compounds bioaccessibility is addressed considering the influence of the analytical techniques used.

2 | MATERIALS AND METHODS

2.1 | Reagents and analytical standards

Allyl ITC (AITC) 95% v/v and Indole-3-carbinol (I3C) > 96% v/v were obtained from Sigma-Aldrich. Erucin (E) > 98% w/w, Phenethyl ITC (FITC) > 98% v/v, S-Sulforaphene (SE) > 98%, and Benzyl ITC (B) > 97% w/w were purchased from LKT Laboratories Inc. Raphasatin (R) was extracted from *Raphanus raphanistrum* subsp. *Sativus* taproot according to Kim et al. (2015). The extracted raphasatin was isolated by fractions collection after HPLC separation. Subsequently, fractions were concentrated under reduced pressure and characterized by Diode Array Detection (DAD) and GC–MS analysis. Raphasatin quantity was calculated using erucin as the reference standard, which is similar to raphasatin in structure and is commercially available (a short study to compare their DAD signal was carried out to assure the calculation results). For GC–MS analysis, a Perkin Elmer Clarus 500 was used.

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). Chloroform (CF)

and Dichloromethane (DCM) p.a. were acquired from Sintorgan. Acetonitrile (ACN) and MeOH were chromatographic grade and acquired from Baker. Ultrapure water (18 MΩ.cm) was obtained from a Milli-Q system purification.

2.2 | Sampling

The vegetable material was purchased from a local organic farm located in Los Corralitos, Mendoza, Argentina. The varieties considered for this study were *Raphanus raphanistrum* subsp. *Sativus* L. (round, red radish taproot), Japanese Miyashige daikon (cylinder, white radish taproot), and Sakurajima Giant (giant, green radish taproot).

A subsample of three independently prepared batches per variety was analyzed. The edible part of such vegetables was conditioned by adequate cleaning. Phytochemicals extraction and moisture content determination were made on the same day of purchase. Samples consisted of 100 g of fresh vegetables and were placed in a blender with 100 mL of water and homogenized (Blender, 600 W, 60 Hz, model HR2030/10, PHILLIPS, Buenos Aires, Argentina). Afterward, the homogenates were centrifuged at 10,600 g for 20 min. Each sample was analyzed in triplicate by HPLC.

For moisture assays, the samples were processed, weighed (3 g of each vegetable), and dried in a convection oven (DALVO, Santa Fé, Argentina) at 50 ± 10°C to constant weight.

2.3 | Comparative study of two methodologies employed for ITCs determinations

2.3.1 | Solvent assisted hydrolysis and liquid-liquid extraction (procedure #1)

First, an aliquot of 20 mL of supernatant from the radish samples was subjected to Myrosinase enzymolysis at 37°C for 10 min in agitation. Then, 25 mL of DCM were added to the mixture and kept shaking for 15 more minutes at room temperature (25°C). The resulting mixture was added to 10 mL of DCM in a separation funnel. After separation, the DCM layer was collected, and 2 g of anhydrous sodium sulfate was added. Finally, the organic layer was dried under a nitrogen stream and redissolved in the mobile phase (H_2O: MeOH—acidified with formic acid 0.1%; 50:50 v/v). Then, samples were filtered through a 22 µm membrane filter (J. Prolab) for their HPLC analysis.

2.3.2 | Aqueous hydrolysis and extraction using dispersive liquid-liquid microextraction (procedure #2)

Phytochemical hydrolysis/extraction was carried out following an optimized technique, previously reported by our research group (Fusari et al., 2019). Two milliliters of the homogenized and centrifuged radish samples were sonicated in an ultrasound bath for 5 min (US-bath, 40 kHz and 600 W, model tb 04, TESTLAB, Buenos Aires, Argentina). The GLS hydrolysis for ITC formation was carried out by stirring the samples in a glass vial inside a water bath at 37°C for 2 h. Once the samples were cooled, 1 mL 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 g for 2 min (80-2B centrifuge, Buenos Aires, Argentina). The extractant phase was removed using a syringe, dried under a nitrogen stream, and then it was dissolved in 500 µL of mobile phase; finally, it was filtered before injection into the HPLC-DAD for analysis.

2.4 | Bioaccessibilty study of phytochemicals in radish taproots

Simulated gastric and intestinal fluids, as well as the in vitro digestion protocol, were carried out according to Torres Palazzolo et al. (2018) and Hedrén et al. (2002).

Briefly, 1.5 g of the radish samples that had already been homogenized and centrifuged were added to three samples of 5 mL of gastric solution, and then pH was adjusted to 2. Next, samples were incubated at 37°C with orbital agitation at 5 g for 60 min. Once the gastric digestion had been completed, pH was adjusted to 6.8, and 3 mL of simulated intestinal fluid was added. Samples were incubated for 120 min at 37°C with orbital agitation at 5 g; at the endpoint, they were immediately centrifuged for 30 min at 10,600 g. The supernatant was subjected to the DLLME technique previously mentioned, and ITCs and I3C were quantified by HPLC-DAD.

Bioaccessibility (%) was determined as the ratio between ITCs and I3C concentration in the supernatant (or accessible fraction, $A_{fraction}$) and the initial ITCs amount in the radish taproot (Torres-Palazzolo et al., 2018)

$$\text{Bioaccessibility } (\%) = \frac{A_{fraction}}{\text{Initial content}} \cdot 100 \quad (1)$$

2.5 | HPLC determination of ITCs and I3C

ITCs and I3C separation and determination were performed according to Fusari et al. (2019), using a Shimadzu LC 20A chromatograph coupled to a diode array detector; ODS Waters RP-C18 column (150 mm × 4.6 mm × 5 µm) and a guard-column with the same characteristics. All samples were previously filtered through a 0.22 µm nylon membrane filter. The elution of the analytes was performed with a mobile phase resulting from different ratios of MeOH (A) and water (B) following a linear gradient program consisting of 50% A at 0 min and 80% A between 20 and 30 min. The flow rate was 0.6 mL min^{-1} for 30 min. Both solvents contained 0.1% of FA. The system was equilibrated using 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 detector

wavelength was set to 241 nm (Wilson et al., 2012). The injection volume was 10 µL, and the oven temperature was set at 25°C. Peak identification in samples was carried out by comparing retention times and UV spectra with reference standards, and the compounds were quantified using an external calibration curve (Table S4).

2.6 | Statistical analysis

For statistical treatment of data, INFOSTAT software was employed. All values are expressed as the mean ± standard deviation. In vitro digestion results were compared using the ANOVA test considering both radish variety and hydrolysis/extraction procedure ($p < 0.05$).

3 | RESULTS AND DISCUSSION

3.1 | Comparison of two different hydrolysis/extraction conditions for ITCs and I3C determination

Several authors have studied the influence of myrosinase hydrolysis/extraction conditions on the GLS degradation products profile (Arora et al., 2014). Such implications must be considered when discussing the phytochemical characterization of any vegetable from the *Brassicaceae* family as well as their bioavailability. Radish taproots characterization and intake should be supported, therefore, on this basis. To do so, two different analytical conditions for ITCs extraction were assessed. One is based on the simultaneous GLS hydrolysis and liquid-extraction, which is extensively used for these vegetables, while the other is based on DLLME after the enzymolysis process. The resulting ITCs and indole levels from radish taproots analysis under the two different conditions previously discussed are shown in Table 1.

Overall, the amounts of most analytes (ITCs and I3C) evidenced significant differences for all the samples under study when comparing the two analytical extractive conditions. This fact was expected as both methodologies differ in terms of hydrolysis time, hydrolysis conditions (adding or not adding organic solvent), and extraction conditions (sonication and preconcentration vs. simple liquid-liquid extraction). Previous studies have shown that the addition of non-polar solvents used as extractants during the hydrolysis process, so that extraction occurs simultaneously the myrosinase hydrolysis, helps non-polar ITCs formation (Kim et al., 2015; Lv et al., 2021), so it is expected that compounds with such characteristics like raphasatin in radishes would be affected by changes in the hydrolysis conditions. Additionally, sonication employed during extraction leads to the vegetable cell structure rupture and mass transfer from the vegetable matrix; therefore, an optimized extraction process can be achieved (Tomšik et al., 2016).

In this study, the second methodology—the aqueous hydrolysis followed by DLLME—stands out because higher concentrations of analytes result to be recovered. The above-mentioned could occur given the sonication step, which may help the GLS extractability, a longer GLS hydrolysis time, and the use of DLLME, which not only helps to separate analytes from samples, but also a pre-concentration effect is observed (Rezaee et al., 2006; Shariati et al., 2011). To confirm this hypothesis and to deepen the causes of the observed differences, more studies would be necessary.

An exception to the latter occurs when considering Raphasatin. In this case, Table 1 shows that ***Procedure 1*** (solvent-assisted hydrolysis-LE), which represents the traditionally used hydrolysis/extraction technique, presented high yield values for Raphasatin, as expected according to other reports (Blažević & Mastelić, 2009; Kim et al., 2015; Yi et al., 2016), as well as for AITC. On the contrary, raphasatin low stability in an aqueous or polar medium could cause its degradation and/or transformation into other compounds (Hanlon

TABLE 1 Isothiocyanates and indole levels found in *Raphanus sativus* taproots employing DCM liquid extraction versus DLLME.

Radish Taproots varieties						
Phytochemicals	*R. sativus* L.[a]		*R. sativus* L. var. Japanese Miyashige Daikon		*R. sativus* L. var. Sakurajima Giant	
ITCs and indole	SH-LE	AH-DLLME	SH-LE	AH-DLLME	SH-LE	AH-DLLME
S-Sulforaphene	0.721 ± 0.034^{A*}	0.678 ± 0.025^{A}	Nd	0.361 ± 0.133	2.684 ± 0.062^{A}	0.48 ± 0.402^{B}
Indole-3-carbinol	0.584 ± 0.098^{A}	0.875 ± 0.107^{B}	Nd	1.092 ± 0.214	0.396 ± 0.098^{A}	1.563 ± 0.025^{B}
Allyl isothiocyanate	0.110 ± 0.005^{A}	0.203 ± 0.007^{B}	0.638 ± 0.011^{A}	0.353 ± 0.027^{B}	9.618 ± 0.378^{A}	1.556 ± 0.321^{B}
Raphasatin	2.055 ± 0.057^{A}	0.079 ± 0.001^{B}	0.221 ± 0.001^{A}	0.049 ± 0.003^{B}	0.203 ± 0.003^{A}	0.138 ± 0.050^{B}
Benzyl isothiocyanate	Nd[b]	Nd	Nd	Nd	Nd	Nd

Abbreviations: AH-DLLME, Aqueous Hydrolysis-Dispersive Liquid-Liquid Microextraction; DCM, dichloromethane; ITCs, isothiocyanates; SH-LE, Solvent Assisted Hydrolysis-Liquid Extraction.

[a]Mean values ($n = 3$) expressed as µg g-1 ± SD.

[b]Nd, not detected.

*Compound values followed by different superscript capital letters are significantly different between the analytical procedures, according to Tukey's Test ($p < 0.05$).

& Barnes, 2011; Kim et al., 2015). The latter could explain an overall raphasatin decreased yield in such polar conditions during the GLS breakdown process, as presented during ***Procedure 2*** here proposed (AH-DLLME).

Notably, the I3C values were higher in samples subjected to aqueous hydrolysis-DLLME; this increase could be due to the compound stability under conditions with a predominantly aqueous environment (Anderton et al., 2003). This is highly interesting since I3C was one of the main compounds in White Radish (*R. sativus* L. var. Japanese Miyashige Daikon) and Red Radish (*R. sativus* L.) samples. I3C presence in different radish varieties has been previously reported, and this is related to the fact that glucobrassicin, the I3C precursor, is one of the three most found GLS in this species (Banihani, 2017; Blažević & Mastelić, 2009; Kim et al., 2013).

On another note, for S-Sulforaphene, there was no difference between the hydrolysis/extraction conditions. Regarding this, Kim et al. (2015) stated that the optimal hydrolysis conditions, and in turn, to improve sulforaphene extraction yield, the use of water as a reaction medium was implied. In our study, given the use of aqueous media in both hydrolysis/extraction methods, sulforaphene extractability might not be altered.

Considering the different varieties of radish taproots, Tables S1 and S2 showed a statistically significant difference in ITC and indole profiles among the varieties of radishes. This discrepancy in the phytochemicals profile, as well as their concentration among the different varieties, has already been reported, and our results matched this information (Blažević & Mastelić, 2009; Hanlon & Barnes, 2011). Numerous authors have argued that the reason behind these differences in the ITCs content is related to the fact that each Brassica species, and in particular each variety, has a different glucosinolate profile. As previously mentioned, GLS is the starting point for ITCs formation, and these profiles are influenced, in turn, by factors such as growth stage, environmental conditions, insect attack, and microorganism intrusion (Wu et al., 2017).

Based on all the aforementioned, as the objective of the present work was to assess radishes' bioaccessibility, the second procedure—aqueous-MYR hydrolysis coupled to DLLME—showed to be the most suitable enzymolysis/extraction conditions to address GLS-breakdown products levels of radish taproots. The fact that the extraction step is carried out post the hydrolysis process and considering that good extractability can be achieved using this method, representative information about health-promoting ITCs and indoles upon radish intake can be assessed.

3.2 | Bioaccessibility study of radish

3.2.1 | Evaluation of the bioaccessibility

Table 2 shows the ITCs and I3C bioaccessibility percentages, whereas Figure 1 represents the concentration values for each ITCs and I3C considering the initial samples and the accessible fraction after the in vitro digestion protocol. For more detail, the quali-quantitative ITCs and I3C levels in the samples, before and after the in vitro digestion, are shown in Table S3.

Table 2 shows that the bioaccessibility percentages varied significantly among radish varieties. Notably, all samples had in common high raphasatin bioaccessibility percentages (values between 58% and 92%). For this particular case, even though it was not possible to find data to compare these results because there are no other reports to compare raphasatin bioaccessibility levels, other lipophilic organosulfur phytochemicals present in vegetable matrices have also presented similar behavior (Bhatt & Patel, 2013; Torres-Palazzolo et al., 2018). Abellán et al. (2021), also stated that those ITCs formed from aliphatic GLS, like in this case, presented higher bioaccessibility considering Brassicaceae sprouts in vitro digestion, including radish sprouts.

High bioaccessibility percentages were also observed for S-Sulforaphene, representing the second most accessible ITCs in these samples (Table 2). The latter is similar to sulforaphane in structure, and it only differs in the presence of a double bond, which would explain the similarity between our results from those corresponding to sulforaphane in digested broccoli samples (Oliviero et al., 2018; Sarvan et al., 2017). Other bioavailability studies would be necessary to define their final bioavailability; however, their presence in the GI tract is undoubtedly promising, as several

TABLE 2 Isothiocyanates and indole bioaccessibility [%] in *Raphanus sativus* taproots.

Compounds	*R. sativus* L.[a]	*R. sativus* L. var. Japanese Miyashige Daikon[a]	*R. sativus* L. var. Sakurajima Giant[a]
S-Sulforaphene	44^{A*}	45^{A}	49^{A}
Indole-3-carbinol	35^{A}	47^{B}	46^{B}
Allyl isothiocyanate	3	---	4
Raphasatin	86^{B}	58^{A}	92^{C}
Benzyl isothiocyanate	---	---	---

[a]Mean values ($n = 3$) expressed as [%].

*Compound values followed by different superscript capital letters are significantly different (Tukey Test, $p \leq 0.05$).

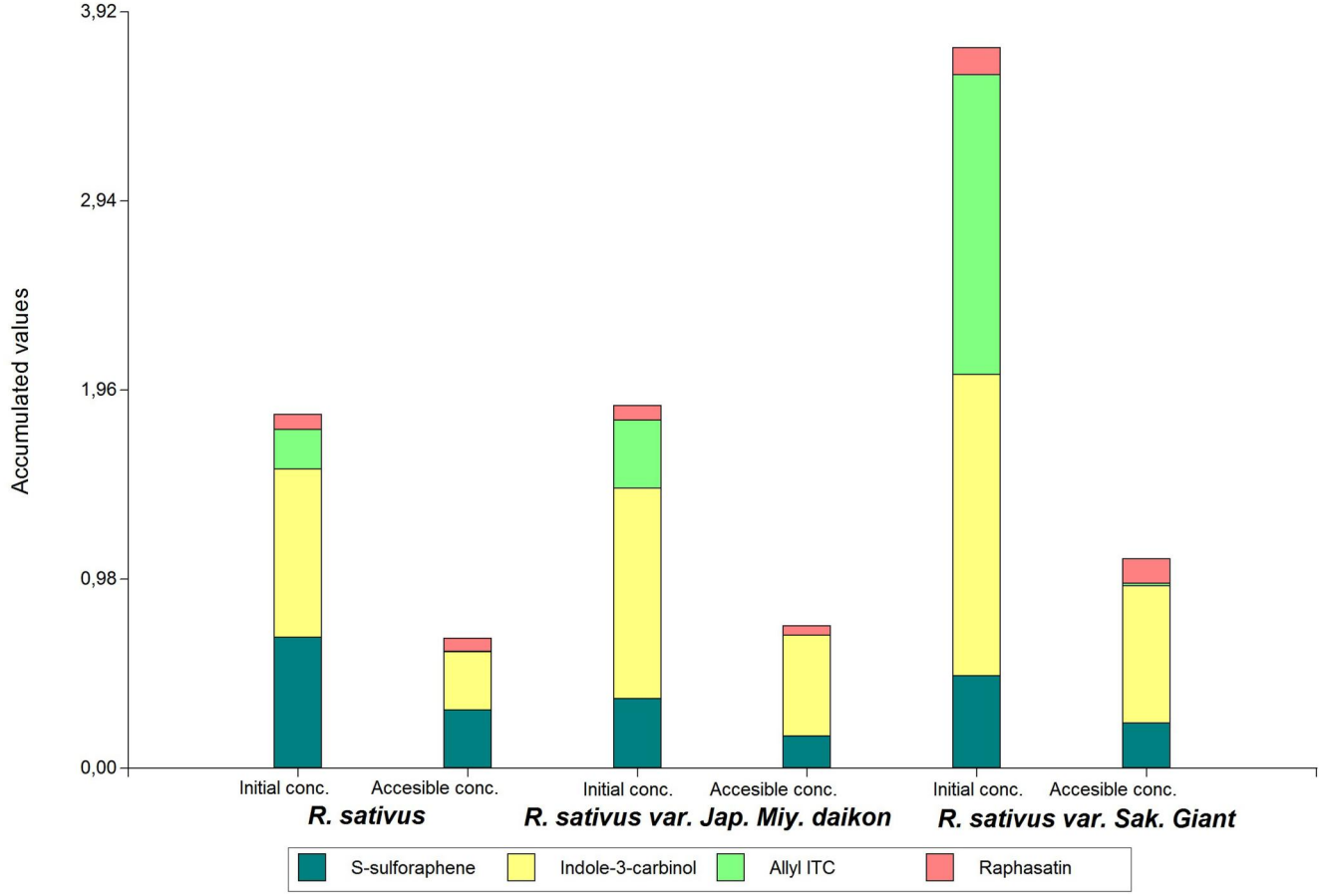

FIGURE 1 Accumulated concentration values for each ITCs and I3C considering their initial concentrations in the radish taproots versus the accessible concentration after the in vitro digestion protocol. ITCs, isothiocyanates.

bioactive properties have been associated with these two compounds (Barillari et al., 2005; Blažević & Mastelić, 2009; Hanlon et al., 2007; Kim et al., 2015; Li et al., 2015).

For I3C, although there was a significant statistical difference between white radish (var. Japanese Miyashige Daikon) and giant radish (var. Sakurajima Giant) when compared to red radish (Table 2), these percentage values did not fluctuate much (35%–45%), showing average bioaccessibility values. It has been discussed that at gastric pH, I3C can form dimers and condensation products such as diindolylmethane or indolo[3,2-b]carbazole (Anderton et al., 2003; Oliviero et al., 2018), which would explain I3C lower bioaccessibility amounts compared to raphasatin and sulforaphene.

On the other hand, AITC was the least bioaccessible compound (3%–4%). This low bioaccessibility could be improved if radish was incorporated along with other food components such as proteins or lipids. In previous reports, in vivo studies have shown that AITCs bioaccessibility is higher when it is ingested with milk, corn oil, or meat. This effect was explained by the presence of lipids in the food matrix that may have enhanced ITCs availability due to the incorporation of ITCs into mixed micelles (Oliviero et al., 2018).

3.2.2 | Effect of the digestive stage on ITCs and indole quali-quantitative amounts

Regarding the ITCs and I3C levels, considering the initial amounts before digestion compared to the digested radish samples, it should be noted that highly bioaccessible compounds are not necessarily associated with high concentration values after digestion. This is related to the phytochemical's initial concentration, as well as their extractability and stability in the digestive fluids (Núñez-Gómez et al., 2023). Therefore, to claim about potential bioavailability and subsequent health properties, it is important to analyze the remaining ITCs and I3C quantitative levels in the gut. In this sense, Figure 1 shows GLS breakdown products concentration before and after in vitro digestion. The initial concentrations represent ITCs and I3C levels obtained from myrosinase hydrolysis and chemical extraction, hence corresponding to the potential GLS breakdown products from the vegetable matrix, whereas the accessible fraction shows the obtained ITCs and I3C originated from the digestion process. In the last case, the extractability of GLS is influenced by the digestive conditions (enzymes, salts, and pH), and once GLS are liberated from the matrix, they transform into different sulfur compounds (Abellán

et al., 2021; Núñez-Gómez et al., 2023; Oliviero et al., 2018). As can be seen in Figure 1, the initial analytes levels were higher than those found in the accessible fraction, therefore showing that the digestive process indeed limits the compounds extractability from the vegetable matrix and/or the phytochemicals' stability. These results are in agreement with other reports that also found lower GLS breakdown products in digestive fluids (Abellán et al., 2021; Oliviero et al., 2018). Nevertheless, physiological conditions appeared to affect differently each phytochemical. As shown in Table S3, the remaining amounts of I3C and S-Sulforaphene were the highest in all samples, except for giant radish (var. Sakurajima Giant). Based on the above, I3C and S-Sulforaphene become promising phytochemicals that could have potentially beneficial effects on radish consumption, due to their bioaccessibility and their considerable remaining amounts after digestion. Thus, being able to exert diverse in situ biological activities as already discussed or being available to be absorbed in the enterocytes to exert systemic activities.

On the other hand, considering raphasatin, even though it showed the highest bioaccessible percentages, its remaining level in the digested samples was distinctively low. This has to do with raphasatin initial concentration in the taproot matrix, which was also low, as explained in the previous section. In this case, to favor raphasatin formation and stabilization in the digestive fluids, this compound could be incorporated into designed delivery systems to maintain the integrity and concentration of this compound throughout the entire digestion process.

Finally, AITC showed the lowest levels in all the accessible fractions. We have not found comparable data about AITC digestion. However, we hypothesize that under digestive conditions, this compound may have been transformed into other degradation products. ITCs are electrophilic compounds that can react with different compounds containing thiol, hydroxyl, and amino groups. In vitro, studies have shown that ITCs can potentially react with amino acids, peptides, and proteins to form a vast range of thiocarbamates, dithiocarbamates, and thiourea derivatives, and this reactivity may reduce the ITCs final bioaccessibility and bioavailability (Oliviero et al., 2018).

In general, even though digestive conditions limit GLS extraction and the hydrolysis products formation compared with a chemical extraction, digestion was not a limiting step for the subsequent bioavailability of ITCs and I3C in radish, except for AITCs which were highly affected by digestive conditions.

4 | CONCLUSIONS

Results showed that the ITCs and indole profile in the radish's taproots after aqueous myrosinase hydrolysis followed by DLLME were distinctively different from other previously reported. Moreover, a richer GLS breakdown products profile can be obtained; hence, accurate information about health-promoting ITCs upon radish intake can be estimated. ITCs bioaccessible percentages were also calculated. Notably, raphasatin showed the highest bioaccessible percentages, despite its low quantitative yields. On the other hand, I3C and S-Sulforaphene become promising phytochemicals that could have potentially beneficial effects on radish consumption, due to their bioaccessibility and their considerable remaining amounts after digestion. Overall, these results showed that when considering radish taproots, ITCs and I3C intake are highly dependent on the GLS myrosinase enzymolysis conditions. From this data, an accurate profile of GLS breakdown products from radishes upon consumption could be obtained. Particularly, the analytical approach here employed to assess radishes' functional potential stands out from others because besides obtaining better recoveries, it considers the implications of the human physiological environment once the vegetable is consumed, and consequently, the data obtained is closer to a real situation allowing a better bioaccessibility interpretation. More in vivo studies should be performed to confirm the outcome of our results.

AUTHOR CONTRIBUTIONS

Daniela Ramirez: Conceptualization; Data curation; Formal analysis; Methodology; Writing – original draft; Writing – review & editing. **Vanesa Beretta**: Data curation; Investigation; Writing – review & editing. **Carolina Torres-Palazzolo**: Data curation; Investigation; Methodology; Writing – review & editing. **Alejandra B. Camargo**: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Visualization; Writing – review & editing.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts to declare.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ETHICS STATEMENT

None declared.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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