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Corresponding author:

Marta Silva

marta.sdds@gmail.com

Author Affiliation:

REQUIMTE-LAQV.

Fate of Brassicaceae Microgreen Bioactive Compounds After Gastrointestinal Digestion and Colonic Fermentation: A Systematic Review

Silva, M; Araújo, AM; Ramos, H; Ribeiro, Mafalda; Viegas, O; Faria, MA; Ferreira IMPLVO.

ADMINISTRATIVE INFORMATION**Support** - - Programa Regional do Norte, NORTE2030-FEDER-01786100, STrengthS4WineChain project, co-funded by the European Union.

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Review Stage at time of this submission - Formal screening of search results against eligibility criteria.**Conflicts of interest** - None declared.**INPLASY registration number:** INPLASY202550090**Amendments** - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 29 May 2025 and was last updated on 6 July 2026.**INTRODUCTION**

Review question / Objective This systematic review of the literature was performed to synthesise current evidence from in vitro models on the impact of gastrointestinal digestion and colonic fermentation on glucosinolates, isothiocyanates and phenolic compounds of Brassicaceae microgreens.

Condition being studied Levels and bioaccessibility of glucosinolates, isothiocyanates and phenolic compounds of Brassicaceae microgreens after in vitro digestion and/or colonic fermentation.

METHODS

Search strategy The search strategy employed four main concepts. Concepts 1 and 2 define the plant selection criteria to ensure the inclusion of studies on Brassicaceae microgreens, while excluding other growth stages such as sprouts or mature plants. Concept 3 relates to the type of bioactive compounds of interest, based on their most common structural features. Concept 4 targets the subjection of Brassicaceae microgreens to digestion and colonic fermentation, aiming to understand the bioaccessibility and biotransformation of their bioactive compounds.

Concept 1: Microgreen

Concept 2: Brassica, Brassicaceae, Crucifer, Radish, Broccoli, Cabbage, Kale, Mizuna, Pak

choi, Bok choi, Cauliflower, Romanesco, Collard greens, Collard, Rapeseed, Turnip, Kohlrabi, Tatsoi
 Concept 3: Glucosinolate, Isothiocyanate, Nitrile, Epithionitrile, Indole, Phenolic, Phenolic Compound, Polyphenol, Flavonoid, Phenolic Acid, Flavonol, Hydroxybenzoic Acid, Hydroxycinnamic Acid, Lignin, Lignan, Stilbene, Ellagitannin, Coumarin, Furanocoumarin, Anthocyanin

Concept 4: Digest, Bioaccessible, Bioavailable, Absorption, Pharmacokinetics, Microbiota, Microbiome, Colonic, Faecal

A comprehensive search was conducted on three databases: PubMed, Web of Science and Scopus.

Participant or population Brassicaceae microgreens.

Intervention Digestion and/or colonic fermentation studies performed in in vitro models.

Comparator A comparator was not mandatory for study inclusion.

Study designs to be included Experimental analytical chemistry studies with quantitative data, using in vitro gastrointestinal digestion and/or colonic fermentation models.

Eligibility criteria The following exclusion criteria will be considered:

- Sprouts, baby leaves, mature vegetables or other developmental stages other than microgreens; or of other microgreens families.
- No digestion or colonic fermentation.
- No reference to glucosinolates, isothiocyanates or phenolic compound levels, or of their metabolites/catabolites; or to their bioaccessible percentages.
- In vivo studies, bioactivity studies (cells, preclinical, other), in silico analyses, review articles, opinion articles, commentaries, book chapters, conference communications, abstracts only, notes, theoretical studies, editorials, papers published in any other language than Portuguese, English or Spanish, papers whose full texts are not available.

Information sources The articles were retrieved from three databases (PubMed, Web of Science and Scopus) up to January 2026.

Main outcome(s) Quantitative data (or percentage of bioaccessibility) of glucosinolates, isothiocyanates, phenolic compounds, and/or their respective metabolites/catabolites, after digestion and/or colonic fermentation.

Quality assessment / Risk of bias analysis All articles were independently reviewed selected by four authors. In case of doubt, the decision to

include or exclude an article was discussed among all the authors. All authors collaboratively revised and approved the data collection strategy. The data from each article was independently collected by four authors. The remaining authors carefully reviewed the collected data to verify its accuracy and completeness and resolved any disagreements through discussion based on the pre-established criteria.

Strategy of data synthesis Relevant data will be manually collected from articles, reported data in tables, figures, text or supplementary material within the final eligible records. Considering data is often published in the graph format, WebPlotDigitizer version 5 (Automeris) will be used to extract numerical data from plots. Data will be recorded using a pre-designed form, including: (1) microgreens identification, including species, variety and cultivar; (2) preparation and processing methods of the sample; (3) gastrointestinal digestion or colonic fermentation model; (4) study type (in vitro or in vivo); (5) duration of the gastrointestinal digestion or colonic fermentation process and enzymes used; (6) experimental details, such as fraction analysed (gastric, intestinal, bioaccessible, residual, among others); (7) sample size; (8) quantification of bioactive compounds, including standard deviations, units, and the weight basis (fresh weight or dry weight); (9) extraction solvents; (10) analytical methods and reference standards; (11) study limitations; and (12) key study conclusions. To facilitate comparison between studies and extract relevant conclusions, extracted values will be standardized to the same units, milligrams of compound(s) per 100 grams in dry weight (dw) of microgreens that were digested, therefore conversions were applied as necessary. Bioaccessibility percentages will be calculated whenever they are not reported by the original studies, using the following formula: Bioaccessibility (%) = (content in the bioaccessible fraction / initial content) x 100.

When the total compound content is not reported, it was calculated as the sum of individual compounds or classes of compounds (only for chromatographic methods). For those, the respective standard deviation will be calculated using the following formula: $\sqrt{(\sum((x_i - \bar{X})^2)/(k - 1))}$; where x_i represents each individual sample mean ($i = 1$ to k); $\bar{X}$ is the overall mean of all the sample means; and k is the number of samples means.

Subgroup analysis Subgroups were defined based on:

- Brassicaceae microgreens (which were grouped per microgreen variety/cultivar).

- Digestion and colonic fermentation fractions (before vs after).
- Bioactive compounds (phenolic compounds; glucosinolates and isothiocyanates).
- Analytical method (spectrophotometric and chromatographic techniques).

Sensitivity analysis Not applicable.

Language restriction Only articles written in English, Portuguese and/or Spanish.

Country(ies) involved Portugal.

Keywords Microgreens; digestion; colonic fermentation; phenolic compounds; glucosinolates.

Dissemination plans This systematic review will be submitted for publication in a peer-reviewed journal relevant to the field.

Contributions of each author

Author 1 - Marta Silva.

Email: marta.sdds@gmail.com

Author 2 - Ana Margarida Araújo.

Email: ana.margarida.c.araujo@gmail.com

Author 3 - Helena Ramos.

Email: ahelenaramos@gmail.com

Author 4 - Mafalda Ribeiro.

Email: mafaldaifribeiro@gmail.com

Author 5 - Olga Viegas.

Email: olgaviegas@fcna.up.pt

Author 6 - Miguel A. Faria.

Email: mfaria@ff.up.pt

Author 7 - Isabel M.P.L.V.O. Ferreira.

Email: isabel.ferreira@ff.up.pt