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Colorimetric determination of indole-3-carbaldehyde by reaction with carbidopa and formation of aldazine in ethanolic extract of cabbage

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ABSTRACT

A colorimetric assay was developed for detection of indole-3-carbaldehyde (I3A), a bioactive compound particularly abundant in vegetables of the *Brassicaceae* family, inducing plant defense responses and ensuring the human intestinal barrier integrity. The assay is based on the selective condensation reaction between the hydrazine group of carbidopa, used as a derivatizing agent, and the aldehyde group of I3A, generating a colored aldazine with a characteristic absorbance around 415 nm. As proof of concept, among the several natural sources here reported in a quantitative data collection, the colorimetric assay was assessed in white heart cabbage ethanolic extract. I3A content was determined by LC-MS analysis, which confirmed the colored aldazine formation in matrix. The LOD (10.4 ± 0.3 μM) obtained by the calibration curve from the visible absorbance (m = 565 ± 16 M^{-1}, $_{av}$RSD % 6.5, R^2 = 0.9960) indicates that this simple, safe, and low-cost method could be beneficial also for current methodologies for identification and quantification of I3A in foods.

Introduction

Indole-3-carbaldehyde (I3A, Fig. 1) is a bioactive metabolite of tryptophan in fungi and bacteria, inducing plant defense responses and resulting particularly abundant in vegetables of the *Brassicaceae (Cruciferae)* family (Devys & Barbier, 1991; Revelou et al., 2020), with a broad therapeutic potential, acting also as a neuroprotective agent (Pappolla et al., 2021). High-performance liquid chromatography (HPLC) is the main method employed for the qualitative and quantitative analysis of I3A, also in combination with mass spectrometry and NMR (Karanikolopoulou et al., 2021).

Although the synthesis of several colored conjugates of I3A was reported (Ziarani et al., 2022; El-Sawy et al., 2017), to the best of our knowledge, I3A was never exploited to develop a colorimetric assay for I3A detection in foods. Accordingly, here we propose the first simple colorimetric assay for an affordable optical sensing of I3A, thanks to the outstanding merits of such approach also for food- and biomedical-analysis (Palladino et al., 2019; Yan et al., 2021; Zhou et al., 2021). The method is supported by a powerful chemical reaction principle, *i.e.,* the selective condensation reaction between the hydrazine group of carbidopa (CD), used as a derivatizing agent, and the aldehyde group of I3A molecules. An analogue reaction, following the formation of a benzaldazine as yellow probe, has been previously used for the detection and quantification of carbidopa in plasma, upon reaction with *p*-dimethylaminobenzaldehyde (DMAB) producing the *p*-dimethylaminobenzalazine (Fig. 1A) (Vickers & Stuart, 1973), or, more recently, in anti-Parkinson drugs upon reaction with vanillin producing the 4-hydroxy-3-methoxybenzaldazine (Fig. 1B) (Lettieri et al., 2022). Here, the reaction leads to yellow indole-3-carbaldehyde azine (I3AZ) formation (λ_{max} = 415 nm) in acidic solution (Fig. 1C) (Palladino et al., 2023), whereas the absorption spectra of aldehyde I3A is limited to the UV region (λ_{max} 296 nm) (Devys & Barbier, 1991), as well as the absorption spectra of the corresponding Schiff base (hydrazone, λ_{max} 292 nm) and the red-shifted protonated Schiff base at the imine nitrogen (λ_{max} 330 nm) (Hota & Singh, 2007).

LC-MS analysis estimated a concentration value of 1.8 ± 0.2 μM for I3A, and confirmed the I3AZ formation, in cabbage extracts. UV–Vis measurements were performed at 20.0 °C on three alcoholic extracts of cabbage leaves at 0 h and after 24 h, in presence of 1.00 ± 0.01 mM CD with 30 mM H_3PO_4 (pH 1.3) in EtOH:H_2O 9:1, spiked with I3A 0 μM (blank), 90, 180, 270, 360, 450 μM, and the absorbance values at 415 nm were linearly fitted (m = 565 ± 16 M^{-1}, LOD = 10.4 ± 0.3 μM, $_{av}$RSD % 6.5, R^2 = 0.9960). Accordingly, the colorimetric assay here developed seems to offer a simple and safer method for monitoring the

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levels of endogenous I3A in foods, and more in general, in plants (Table 1) here collected for the first time, appearing useful also to improve current methodologies for identification and quantification of I3A in foods (Karanikolopoulou et al., 2021; Suparman Inpota et al., 2020).

Materials and methods

Chemicals

For extractions, reactions, and spectroscopic analyses, chemicals were from Sigma-Aldrich (Milan, Italy). Stock solutions of reagents were obtained at room temperature by dissolving 1*H*-Indole-3-carbaldehyde (I3A, CAS Number 487–89–8) and indole-3-carbaldehyde azine (I3AZ, CAS Number 1233–49–4) 28.3 ± 0.1 mM and 99.7 ± 0.4 mM in DMSO, respectively, and Carbidopa (CD, CAS Number 28,860–95–9) 10.0 ± 0.1 mM in 300 mM H_3PO_4.

Indole-3-carboxaldehyde extraction from the cabbage Brassica oleracea

Fresh leaves from the internal part of the cultivated white heart cabbage (*Brassica oleracea* var. *capitata f. alba*) were cut into small pieces. Three samples of 50.07 ± 0.01 g, 50.00 ± 0.01 g, and 50.01 ± 0.01 g were extracted separately with 150 mL ethanol at 20 °C for 4 h. The extracts were filtered by using 0.22 μM cellulose acetate filters from VWR International srl (Milan, Italy) and stored in bottles at 4 °C. An analogue procedure previously reported gave for I3A 19 mg kg^{-1} fresh weight, (corresponding to 203 mg kg^{-1} dry weight before extraction) (Table 1) (Devys & Barbier, 1991).

LC-MS-DAD analysis

For the HPLC-DAD analyses the system consisted of a PU-2089 quaternary pump equipped with a degasser, an AS-950 autosampler, and an MD-2010 spectrophotometric diode array detector (all modules are Jasco International Co., Japan). The software used for the analysis was ChromNav (Jasco International). The diode array detector (DAD) acquisition was performed in the range of 200–650 nm every 0.2 s with 4 nm resolution. For the LC-ESI-Q-ToF analysis the system consisted of a HPLC 1200 Infinity, coupled with a quadrupole-time of flight mass spectrometer Infinity Q-ToF 6530 detector by a Jet Stream ESI interface (Agilent Technologies, Waldbronn, Germany). The ESI conditions were: drying and sheath gas N_2, purity>98 %, temperature 350 °C, flow 10 L min^{-1} and temperature 375 °C, flow 11 L min^{-1}, respectively, capillary voltage 4.5 KV., nebulizer gas pressure 35 psi. The fragmentor voltage was 175 V, nozzle, skimmer and octapole RF voltages were set at 1000 V, 65 V and 750 V, respectively. The high-resolution MS and MS/MS acquisition started after 5 min to avoid the peaks due to the reagent, and the acquisition range was set from 100 to 1000 *m/z* in positive mode, with acquisition rate 1.04 spectra/*sec*. For the MS/MS experiments, 30 V were applied in the collision cell to obtain CID fragmentation (collision gas N_2, purity 99.999 %). The FWHM (full width half maximum) of quadrupole mass bandpass used during MS/MS precursor isolation was 4 *m/z*. The Agilent Technologies tuning mix HP0321 was used daily to calibrate the mass axis. The eluents used for the HPLC-DAD analyses were water and acetonitrile (ACN) both HPLC grade Sigma-Aldrich (Milan, Italy), while the eluents for HPLC-ESI-Q-ToF analyses were water and ACN, both LC-MS grade Sigma-Aldrich (Milan, Italy). All eluents were added with 0.1 % v/v formic acid (FA., 98 % purity, J.T. Baker, USA). For both the systems, the chromatographic separation was performed on an analytical reversed-phase column Poroshell 120 EC—C18 (3.0 × 75 mm, particle size 2.7 μm) with a Poroshell pre-column, both Agilent Technologies. The flow rate was 0.5 mL min^{-1} and the program was: 5 % ACN with 0.1 % FA (formic acid) and 95 % H_2O with 0.1 % FA starting conditions, then to 40 % ACN with 0.1 % FA in 10.0 min, and to 98 % CAN with 0.1 % FA in 4 min. *Re*-equilibration took 8 min. During the separation, the column was kept at 30 °C. All samples were filtered through PTFE syringe filters (4 mm thickness and 0.45 μm pore diameter, Agilent Technologies) prior to injection.

An external calibration curve was built for I3A in ethanol in the range 0.1–200 μM, integrating the Extracted Ion Chromatogram corresponding to the *m/z* value of the $[M + H]^+$ ion of I3A.

UV–Visible spectroscopy

Optical measurements were performed at 20.0 °C using Evolution™ 201 UV–visible spectrophotometer (Thermo Scientific, Waltham, MA, USA). The samples were scanned in the wavelength range of 250–550 nm, 100 nm/min with a bandwidth of 1 nm and 0.6 s integration time. For each measurement, alcoholic extract of cabbage leaves was diluted 9:1 v/v with 10.0 ± 0.1 mM CD in 300 mM H_3PO_4, to obtain 1.00 ± 0.01 mM CD with 30 mM H_3PO_4 in EtOH:H_2O 9:1). The same starting

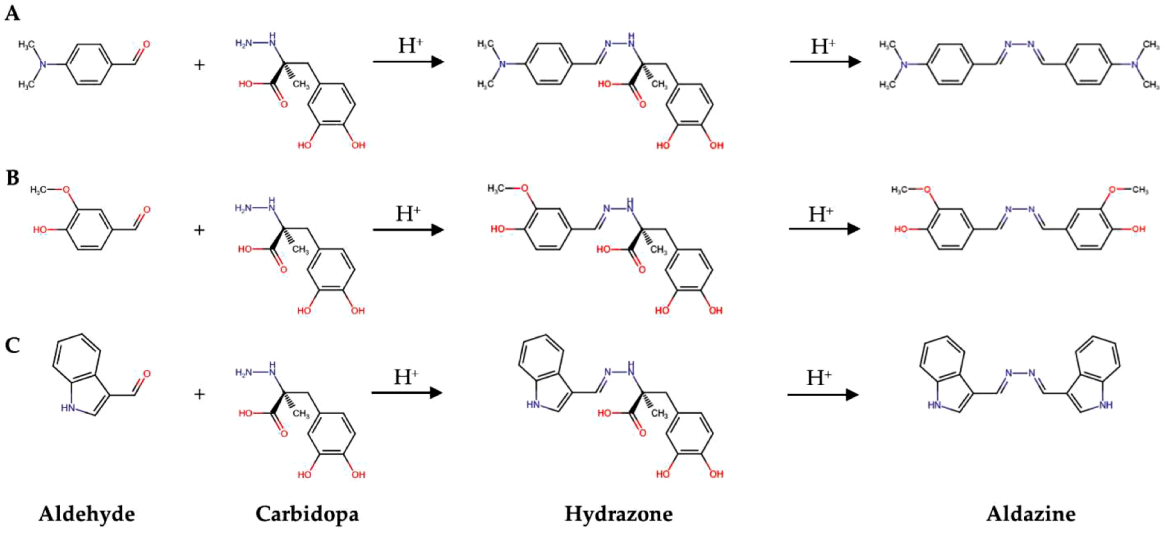

Fig. 1. Synthesis of colored aldazines in hydroalcoholic solution via hydrazone intermediates. (A) Reaction of p-dimethylaminobenzaldehyde (DMAB) with carbidopa (CD), producing the p-dimethylaminobenzalazine (Vickers & Stuart, 1973). (B) Reaction of 4-hydroxy-3-methoxybenzaldehyde (vanillin) with CD, producing the 4-hydroxy-3-methoxybenzaldazine (Lettieri et al., 2022). (C) Reaction of indole-3-carbaldehyde (I3A) with CD, producing the indole-3-carbaldehyde azine (I3AZ) (Palladino et al., 2023).

Table 1
Levels of I3A from plant tissues.

Species	Tissue	[I3A] ppm[1]	Solvents[2]	Method[3]	Ref.
White cabbage (*Brassica oleracea* var. *capitata*)	Leaves	5.5 ± 0.6 fw	B	LC-MS	This work
		19 fw, 203	C	TLC	Devys & Barbier, 1991
		245.9 ± 0.3	F	LC-MS	Revelou et al., 2020
Purple broccoli (*Brassica oleracea* var. *italica*)	Florets	30.63 ± 0.15			
White cauliflower (*Brassica oleracea* var. *botrytis*)	Florets	25.48 ± 0.15			
Green broccoli (*Brassica oleracea* var. *italica*)	Florets	25.24 ± 0.03			
Red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*)	Leaves	21.93 ± 0.10			
Radish (*Raphanus raphanistrum sativus*)	Roots	3.95 ± 0.13			
Turnip (*Brassica rapa* var. *rapa*)	Roots	2.08 ± 0.03			
Collards (*Brassica oleracea* var. *viridis*)	Leaves	5–27 fw	B/C/E	HPLC	Wall et al., 1988
Monodora brevipes	Seeds	25	C/E/J	TLC	Etse et al., 1989
Cotton (*Gossypium hirsutum*)	Green bracts	14.1	A/B	HPLC	Hong et al., 1985
	Brown bracts	14.3			
Cichorium calvum	Aerial parts	9.09	B	HPLC	Michalska et al., 2014
Wedelia chinensis	Whole plant	8.0	A/B/E/G	HPLC	Lin et al., 2007
Lactuca aculeata	Roots	7.97	B	HPLC	Michalska & Kisiel, 2010
Water hyacinth (*Pontederia crassipes*)	Roots	7.5	A/B/C	HPLC	Costa et al., 2021
Lactuca altaica	Roots	6.41	B	HPLC	Michalska et al., 2010
Ligularia subspicata	Whole plants	5.84	A/B/F/G	HPLC	Muhammad et al., 2021
Phyllanthus virgatus	Whole plants	5.70	B/C/E	TLC	Huang et al., 1998
Plantago asiatica	Aerial parts	3.8	A/C/D/F/G/H/	LC-MS	Ahn et al., 2018
Nitraria tangutorum	Fruits	3.2	B	HPLC	Jiang et al., 2021
Vernonia tufnelliae	Whole plants	3.0	B	HPLC	Bitchagno et al., 2022
Sweet potato (*Ipomoea batatas*)	Leaves	2.8	E/D/G	HPLC	Zhang et al., 2016
Judean sage (*Salvia Judaica*)	Aerial parts	2.7	A/B/C/D/E/H/J	TLC	Al-Qudah et al., 2020
Lactarius Subplinthogalus	Body	2.6	A/B/G	TLC	Wang et al., 2004
Golden Angel (*Leucosceptrum japonicum*)	Whole plants	2.0	A/C/J/K	HPLC	Murata et al., 2009
London rocket (*Sisymbrium irio*)	Whole plants	1.8	A/B/C/D/E/H/J	CC	Al-Qudah & Abu Zarga, 2010
Rashomon-kazura (*Meehania urticifolia*)	Whole plants	1.7	A/C/J	HPLC	Murata et al., 2011
Stenochlaena palustris	Leaves	1.6	C/E/H	TLC	Liu et al., 1998
Taraxacum formosanum	Aerial parts	1.6 fw	A/C/D/E	CC	Leu-et al., 2003
Piptostigma fugax	Roots	1.5	A/C/F/G/J	GCMS–	Achenbach & Schwinn, 1995
Japanese hops (*Humulus japonicus*)	Aerial parts	1.4	A/C/D/F/G/H	LC-MS	Yang et al., 2018
Phellodendron amurense var. *wilsonii*	Leaves	1.3	A/C/E	TLC	Wu et al., 2003

[1] Quantity expressed as mg kg^{-1} (ppm) of dry weight tissue if not specified as fresh weight (fw).
[2] Extraction/partition solvents. A (Water), B (Ethanol), C (Methanol), D (n-Butanol), Chloroform (E), F (Dichloromethane), G (Ethyl acetate), H (n-Hexane), I (Cyclohexane), J (Petroleum ether).
[3] Main isolation/quantification methods. Column Chromatography (CC), Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS), Gas Chromatography-Mass Spectrometry (GC–MS).

cabbage extract was diluted 9:1 (v/v) with 300 mM H_3PO_4 and used as blank. The solutions were kept for 24 h at 20 °C. The samples were analyzed in quartz cuvettes with an optical path length of 10.0 mm. After blank correction (unspiked cabbage extract), colorimetric data from I3A-CD reaction were fitted using the linear equation (1): $A = (A_{415nm} - A_{550nm})_{24h} - (A_{415nm} - A_{550nm})0\ h = m \times C$, where A represents the sample absorbance due to formation of a yellow I3A azine by reaction of I3A with constant CD content, m represents the slope of the calibration curve, C represents the I3A concentration (90, 180, 270, 360, 450 μM). Data were baseline-corrected by subtracting the absorbance from 550 nm to correct baseline offsets caused by complex matrices (Torrini et al., 2022). The assay reproducibility is reported as mean relative standard deviation% ($_{av}$RSD%).

Results and discussion

Analysis of pH- and solvent-dependent color of indole-3-carbaldehyde azine solutions

Recently, we reported that the color development from the reaction of an aldehyde with CD was proportional to ethanol content (Lettieri et al., 2022). Accordingly, the best conditions for color development in the assay for the detection of I3A analyte were determined by investigating the hydroalcoholic solutions of commercially available indole-3-carbaldehyde azine. In fact, the condensation of two molecules of I3A and one of CD gave the azine reported in Fig. 2, where the protonation of the azine group determines a pH-dependent color change of 50.0 ± 0.1 μM azine solution in water:ethanol mixtures in presence of 30 mM H_3PO_4, being yellow at pH 1.3, due to the large absorbance at 415 nm, and transparent at pH 5.9, with the large absorbance at 350 nm, irrespective of the ethanol content that is 10 % in Fig. 2A, 50 % in Fig. 2B, and 90 % in Fig. 2C. However, due to the increase of UV–Vis absorbance with ethanol percentage, the latter condition (90 % EtOH, 30 mM H_3PO_4, pH 1.3) was chosen for quantitative detection of I3A by using azine formation as a colorimetric reporter. The proper description of the mechanisms behind such solvent effect would require structural and computational investigations that are out of the scope of this paper.

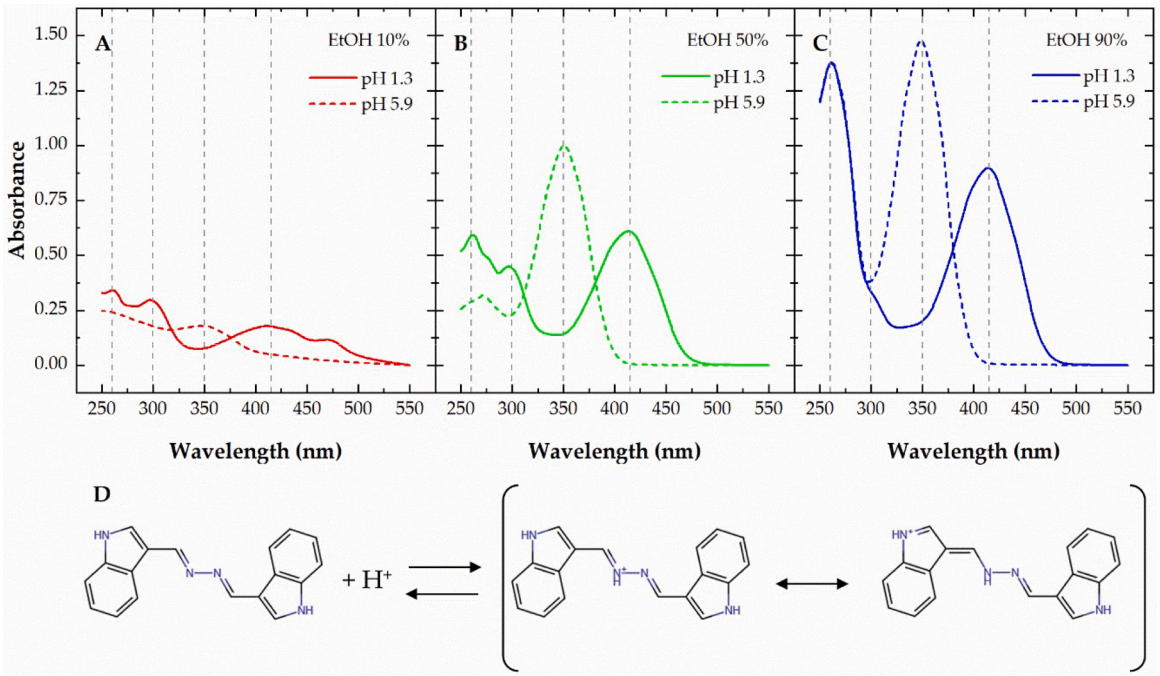

Fig. 2. UV–Vis spectra of 50.0 ± 0.1 μM azine solutions in 10 % (A, red), 50 % (B, green), and 90 % (C, blue) EtOH in presence of H_3PO_4 30 mM at pH 1.3 (solid line) and 5.9 (dashed line). Scheme of Indole-3-carbaldehyde azine protonation and charge delocalization (D).

However, such behavior is similar to acidochromism reported for an analogous molecule (Ray et al., 2013). Here, the solvent-dependent azine group protonation (Fig. 2D) should be responsible of the strong bathochromic and hyperchromic shifts within the UV–Vis spectra due to the delocalization of the positive charge over the aromatic rings, influencing the HOMO-LUMO electronic transitions.

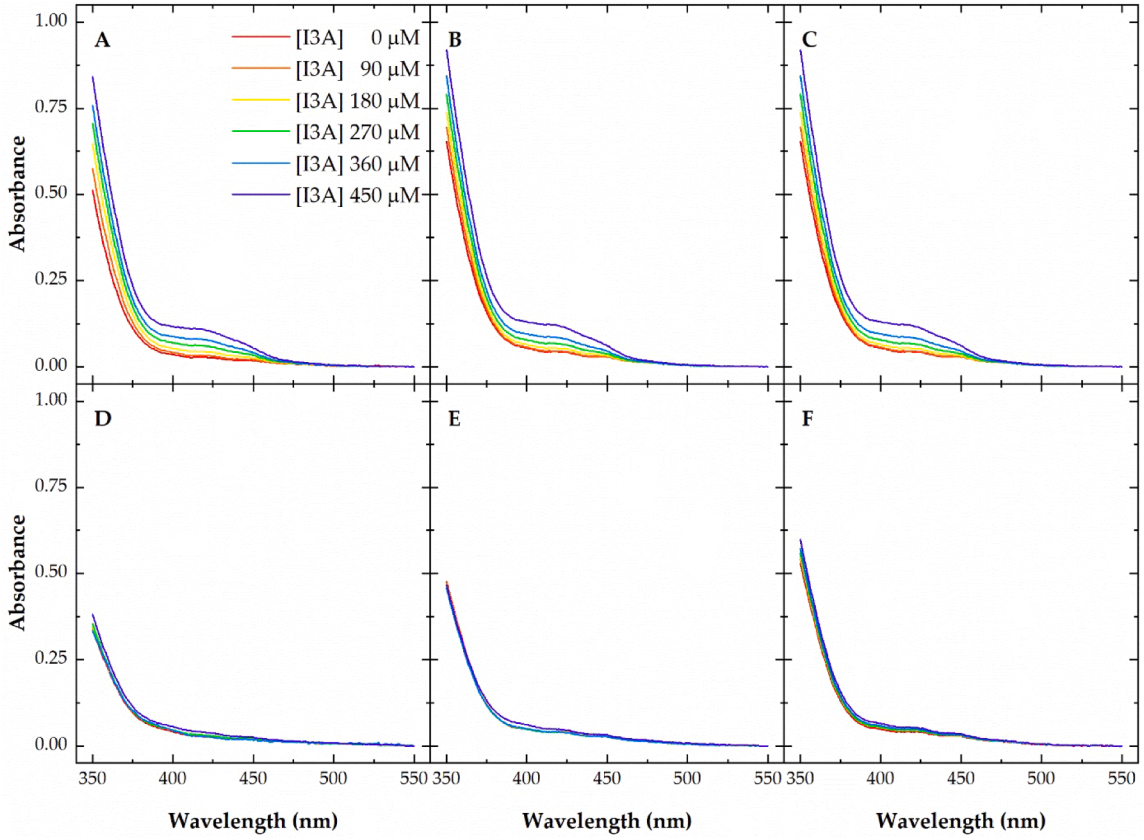

Fig. 3. Visible spectra of three cabbage extracts (EtOH:H_2O 9:1) spiked with 0, 90, 180, 270, 360, 450 μM I3A, in presence of 1.00 mM CD and 30 mM H_3PO_4 pH 1.3 at 20 °C after 24 h (A, B, C) and 0 h (D, E, F).

LC-MS analysis of I3A in real matrices

LC-MS analysis calculated a concentration of I3A in the cabbage extract 1.8 ± 0.2 μM, corresponding to 5.5 ± 0.6 mg kg^{-1} (ppm) of fresh weight (Table 1). Supplementary materials report the ion chromatograms relative to $[M-H]^+$ ion of I3A for different solutions (Fig. S1), including the unspiked cabbage extract (Fig, S1A), the cabbage extract added with 2.5 μM I3A (Fig. S1B), the standard solution added with 2.5 μM I3A (Fig. S1C), and its tandem mass spectra for $[M-H]^+$ ion fragmentation (Fig. S1D). Azine LC-MS analysis is described in Fig. S2. Both the commercial I3AZ (Fig. S2A) and the I3AZ obtained by reaction of 400 μM I3A with 1.0 mM CD added to the cabbage extract (Fig. S2B) present two chromatographic peaks with the same retention times acquired for the molecular ion with raw formula of the expected azine ($C_{18}H_{14}N_4$). Moreover, the tandem mass spectra acquired for the same ion for each peak (Fig. S3A, S3B, S3C, S3D) are identical, and the corresponding UV–Vis spectra (Fig. S4A, S4B, S4C, S4D) are very similar to the one reported in Fig. 2. All together, these results emphasize the existence of isomers for I3AZ as recently reported (Palladino et al., 2023).

Colorimetric analysis of azine formation in real matrices

Three cabbage extracts in EtOH were filtered and spiked with I3A 0 μM (blank), 90, 180, 270, 360, 450 μM (see Materials and methods) in presence of CD 1.00 ± 0.01 mM (EtOH:H_2O 9:1, H_3PO_4 30 mM, pH 1.3). The visible spectrum of each solution was acquired at 20.0 °C from 350 nm to 550 nm, showing a peak at about 415 nm after 24 h (Fig. 3A, B, C), which is absent for the unspiked extract, and the extracts at the beginning of the reaction (Fig. 3D, E, F). The absorbance values at 415 nm, obtained subtracting the Abs_{415nm} values at t 0 h from the Abs_{415nm} after 24 h (see Materials and methods), were linearly fitted, completing the calibration curve reported in Fig. 4 ($m = 565 \pm 16$ M^{-1}, LOD = 10.4 ± 0.3 μM, $_{av}$RSD% 6.5, $R^2 = 0.9960$). Accordingly, the endogenous value of I3A in these cabbage extracts, as calculated by LC-MS analysis, is below the LOD (=3 × SD_{blank}/m) and the unspiked cabbage extract can be used as blank with good approximation.

Conclusions

We have developed a simple colorimetric assay for detection of bioactive indole-3-carbaldehyde in food matrix based on the selective condensation reaction at room temperature between the hydrazine group of carbidopa, used as a derivatizing agent, and the aldehyde group of I3A, generating a yellow-colored indole-3-carbaldehyde azine in acidic hydroalcoholic solution with a characteristic visible absorbance band with a λ_{max} at 415 nm. The colorimetric assay principle was assessed in white heart cabbage ethanolic extracts, obtaining a linear calibration curve ($m = 565 \pm 16$ M^{-1}, $_{av}$RSD % = 6.8 %, LOD = 10.4 ± 0.3 μM, $R^2 = 0.9960$) by spiking known amount of I3A in real cabbage extract. The azine formation in real matrix was confirmed by LC-MS analysis. The advantage of the method is to be very simple, cheap, and could be beneficial also for current methodologies for identification and quantification of I3A in foods.

CRediT authorship contribution statement

Pasquale Palladino: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Lorenzo Attanasio:** Investigation. **Simona Scarano:** Writing – review & editing. **Ilaria Degano:** Investigation, Writing – review & editing. **Maria Minunni:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

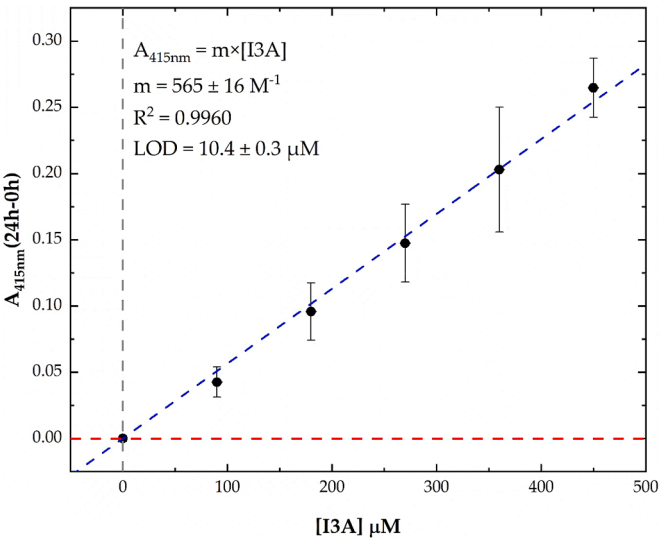

Fig. 4. Calibration curve of indole-3-carbaldehyde azine formed in cabbage extracts (EtOH:H_2O 9:1) spiked with 0, 90, 180, 270, 360, 450 μM I3A, in presence of 1.00 mM CD and 30 mM H_3PO_4 pH 1.3. Each point represents the mean value ± SD (3 extracts) of the absorbance at 415 nm after 24 h minus 0 h at 20 °C, considering the 0 μM I3A as blank ($m = 565 \pm 16$ M^{-1}, $_{av}$RSD % = 6.8 %, LOD = 10.4 ± 0.3 μM, $R^2 = 0.9960$).

Data availability

Data will be made available on request.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2024.100643.

References

Achenbach, H., & Schwinn, A. (1995). Aporphinoid alkaloids and terpenoids from *Piptostigma fugax*. *Phytochemistry, 38*(4), 1037–1048. https://doi.org/10.1016/0031-9422(94)00675-J

Ahn, J. H., Jo, Y. H., Kim, S. B., Turk, A., Oh, K. E., Hwang, B. Y., Lee, K. Y., & Lee, M. K. (2018). Identification of antioxidant constituents of the aerial part of *Plantago asiatica* using LC–MS/MS coupled DPPH assay. *Phytochemistry Letters, 26*, 20–24. https://doi.org/10.1016/j.phytol.2018.05.006

Al-Qudah, M. A., & Abu Zarga, M. H. (2010). Chemical constituents of *Sisymbrium irio* L. from Jordan. *Natural Product Research, 24*(5), 448–456. https://doi.org/10.1080/14786410903388025

Al-Qudah, M. A., Tashtoush, H. I., Khlaifat, E. F., Ibrahim, S. O., Saleh, A. M., Al-Jaber, H. I., Zarga, M. H. A., & Abu Orabi, S. T. (2020). Chemical constituents of the aerial parts of *Salvia judaica* Boiss. from Jordan. *Natural Product Research, 34*(20), 2981–2985. https://doi.org/10.1080/14786419.2019.1597349

Bitchagno, G. T. M., Schüffler, A., Gross, J., Krumb, M., Tane, P., & Opatz, T. (2022). Sesquiterpene Lactones from *Vernonia tufnelliae*: Structural Characterization and Biological Evaluation. *Journal of Natural Products, 85*(7), 1681–1690. https://doi.org/10.1021/acs.jnatprod.2c00055

Costa, M. F., Luiz, M. M., de Souza, L. C., Tempone, A. G., Lago, J. H. G., & Nascimento, I. R. (2021). Phenylnaphthalic anhydrides from water hyacinth (*Pontederia crassipes* Mart. *Phytochemistry Letters, 46*, 1–5. https://doi.org/10.1016/j.phytol.2021.09.003

Devys, M., & Barbier, M. (1991). Indole-3-carboxaldehyde in the cabbage *Brassica oleracea*: A systematic determination. *Phytochemistry, 30*(2), 389–391. https://doi.org/10.1016/0031-9422(91)83690-M

El-Sawy, E., Abo-Salem, H., & Mandour, A. (2017). 1H-Indole-3-carboxaldehyde: Synthesis and reactions. *Egyptian Journal of Chemistry, 60*(5), 723–751. https://doi.org/10.21608/ejchem.2017.1097.1053

Etse, J. T., Gray, A. I., Thomas, D. W., & Waterman, P. G. (1989). Terpenoid and alkaloid compounds from the seeds of *Monodora brevipes*. *Phytochemistry, 28*(9), 2489–2492. https://doi.org/10.1016/S0031-9422(00)98011-7

Hong, Y. K., Gilbert, R. D., & Fornes, R. E. (1985). Quantitative determination of indole-3 carboxyaldehyde in aqueous extracts of cotton bracts by HPLC. *Textile Research Journal, 55*(1), 17–19. https://doi.org/10.1177/004051758505500104

Hota, P. K., & Singh, A. K. (2007). Bacteriorhodopsin analogue from indolic chromophores. *Letters in Organic Chemistry, 4*(4), 300–305. https://doi.org/10.2174/157017807781024228

Huang, Y. L., Chen, C. C., Hsu, F. L., & Chen, C. F. (1998). Tannins, Flavonol Sulfonates, and a Norlignan from *Phyllanthus virgatus*. *Journal of Natural Products, 61*(10), 1194–1197. https://doi.org/10.1021/np970336v

Jiang, S., Zhang, Y., Zhao, X., Shao, Y., Wei, W., Tao, Y., & Yue, H. (2021). A new flavonol acylglycoside from the fruits of *Nitraria tangutorum* Bobr. *Natural Product Research, 35*(21), 3652–3657. https://doi.org/10.1080/14786419.2020.1721487

Karanikolopoulou, S., Revelou, P. K., Xagoraris, M., Kokotou, M. G., & Constantinou-Kokotou, V. (2021). Current methods for the extraction and analysis of isothiocyanates and indoles in cruciferous vegetables. *Analytica, 2*(4), 93–120. https://doi.org/10.3390/analytica2040011

Lettieri, M., Scarano, S., Palladino, P., & Minunni, M. (2022). Colorimetric determination of carbidopa in anti-Parkinson drugs based on 4-hydroxy-3-methoxybenzaldazine formation by reaction with vanillin. *Analytical and Bioanalytical Chemistry, 414*(23), 6911–6918. https://doi.org/10.1007/s00216-022-04256-4

Leu, Y. L., Shi, L. S., & Damu, A. G. (2003). Chemical constituents of *Taraxacum formosanum*. *Chemical and Pharmaceutical Bulletin, 51*(5), 599–601. https://doi.org/10.1248/cpb.51.599

Lin, F. M., Chen, L. R., Lin, E. H., Ke, F. C., Chen, H. Y., Tsai, M. J., & Hsiao, P. W. (2007). Compounds from *Wedelia chinensis* synergistically suppress androgen activity and growth in prostate cancer cells. *Carcinogenesis, 28*(12), 2521–2529. https://doi.org/10.1093/carcin/bgm137

Liu, H., Orjala, J., Rali, T., & Sticher, O. (1998). Glycosides from *Stenochlaena palustris*. *Phytochemistry, 49*(8), 2403–2408. https://doi.org/10.1016/S0031-9422(98)00352-5

Michalska, K., & Kisiel, W. (2010). Sesquiterpene lactones from roots of *Lactuca aculeata*. *Biochemical Systematics and Ecology, 38*(4), 830–832. https://doi.org/10.1016/j.bse.2010.04.005

Michalska, K., Beharav, A., & Kisiel, W (2014). Chemotaxonomic value of magastigmane glucosides of *Cichorium calvum*. *Natural Product Communications, 9*(3). https://doi.org/10.1177/1934578X1400900306, 1934578X1400900306.

Michalska, K., Szneler, E., & Kisiel, W. (2010). *Lactuca altaica* as a rich source of sesquiterpene lactones. *Biochemical Systematics and Ecology, 38*(6), 1246–1249. https://doi.org/10.1016/j.bse.2010.12.010

Muhammad, I., ul Hassan, S. S., Cheung, S., Li, X., Wang, R., Zhang, W. D., Yan, S. K., Zhang, Y., & Jin, H. Z. (2021). Phytochemical study of *Ligularia subspicata* and valuation of its anti-inflammatory activity. *Fitoterapia, 148*, Article 104800. https://doi.org/10.1016/j.fitote.2020.104800

Murata, T., Arai, Y., Miyase, T., & Yoshizaki, F. (2009). An alkaloidal glycoside and other constituents from *Leucosceptrum japonicum*. *Journal of Natural Medicines, 63*, 402–407. https://doi.org/10.1007/s11418-009-0342-8

Murata, T., Miyase, T., & Yoshizaki, F. (2011). New phenolic compounds from *Meehania urticifolia*. *Journal of Natural Medicines, 65*, 385–390. https://doi.org/10.1007/s11418-010-0501-y

Palladino, P., Brittoli, A., Pascale, E., Minunni, M., & Scarano, S. (2019). Colorimetric determination of total protein content in serum based on the polydopamine/protein adsorption competition on microplates. *Talanta, 198*, 15–22. https://doi.org/10.1016/j.talanta.2019.01.095

Palladino, P., Rainetti, A., Lettieri, M., Pieraccini, G., Scarano, S., & Minunni, M. (2023). Quantitative colorimetric sensing of Carbidopa in anti-Parkinson drugs based on selective reaction with indole-3-carbaldehyde. *Sensors, 23*, 9142. https://doi.org/10.3390/s23229142

Pappolla, M. A., Perry, G., Fang, X., Zagorski, M., Sambamurti, K., & Poeggeler, B. (2021). Indoles as essential mediators in the gut-brain axis. Their role in Alzheimer's disease. *Neurobiology of Disease, 156*, Article 105403. https://doi.org/10.1016/j.nbd.2021.105403

Ray, D., Dalapati, S., & Guchhait, N. (2013). Spectral properties of a simple azine Schiff base and its sensing ability towards protic environment through hydrogen bonding interaction. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 115*, 219–226. https://doi.org/10.1016/j.saa.2013.06.013

Revelou, P. K., Kokotou, M. G., & Constantinou-Kokotou, V. (2020). Determination of indole-type phytonutrients in cruciferous vegetables. *Natural Product Research, 34*(17), 2554–2557. https://doi.org/10.1080/14786419.2018.1543680

Suparman Inpota, P., Phonchai, A., Wilairat, P., & Chantiwas, R. (2020). Rapid measurement of indole levels in *Brassica* vegetables using one millilitre binary organic extraction solvent and capillary electrophoresis-UV analysis. *Phytochemical Analysis, 31*(4), 522–530. https://doi.org/10.1002/pca.2916

Torrini, F., Renai, L., Scarano, S., Del Bubba, M., Palladino, P., & Minunni, M. (2022). Colorimetric selective quantification of anthocyanins with catechol/pyrogallol moiety in edible plants upon zinc complexation. *Talanta, 240*, Article 123156. https://doi.org/10.1016/j.talanta.2021.123156

Vickers, S., & Stuart, E. K. (1973). Spectrofluorometric determination of Carbidopa [L-(−)-α-Hydrazino-3,4-dihydroxy-α-methylhydrocinnamic Acid] in Plasma. *Journal of Pharmaceutical Sciences, 62*(9), 1550–1551. https://doi.org/10.1002/jps.2600620940

Wall, M. E., Taylor, H., Perera, P., & Wani, M. C. (1988). Indoles in edible members of the *Cruciferae*. *Journal of Natural Products, 51*(1), 129–135. https://doi.org/10.1021/np50055a020

Wang, Y., Yang, S. P., Wu, Y., & Yue, J. M. (2004). Alkaloids from the fungus *Lactarius subplinthogalus*. *Natural Product Research, 18*(2), 159–162. https://doi.org/10.1080/14786410310001608073

Wu, T. S., Hsu, M. Y., Kuo, P. C., Sreenivasulu, B., Damu, A. G., Su, C. R., & Chang, H. C. (2003). Constituents from the leaves of *Phellodendron amurense* var. *wilsonii* and their bioactivity. *Journal of Natural Products, 66*(9), 1207–1211. https://doi.org/10.1021/np030034v

Yan, T., Zhang, G., Chai, H., Qu, L., & Zhang, X. (2021). Flexible biosensors based on colorimetry, fluorescence, and electrochemistry for point-of-care testing. *Frontiers in Bioengineering and Biotechnology, 9*, Article 753692. https://doi.org/10.3389/fbioe.2021.753692

Yang, H. H., Oh, K. E., Jo, Y. H., Ahn, J. H., Liu, Q., Turk, A., Jang, J. Y., Hwang, B. Y., Lee, K. Y., & Lee, M. K. (2018). Characterization of tyrosinase inhibitory constituents from the aerial parts of *Humulus japonicus* using LC-MS/MS coupled online assay. *Bioorganic & Medicinal Chemistry, 26*(2), 509–515. https://doi.org/10.1016/j.bmc.2017.12.011

Zhang, L., Tu, Z. C., Yuan, T., Wang, H., Xie, X., & Fu, Z. F. (2016). Antioxidants and α-glucosidase inhibitors from *Ipomoea batatas* leaves identified by bioassay-guided approach and structure-activity relationships. *Food Chemistry, 208*, 61–67. https://doi.org/10.1016/j.foodchem.2016.03.079

Zhou, Y., Huang, X., Hu, X., Tong, W., Leng, Y., & Xiong, Y. (2021). Recent advances in colorimetry/fluorimetry-based dual-modal sensing technologies. *Biosensors and Bioelectronics, 190*, Article 113386. https://doi.org/10.1016/j.bios.2021.113386

Ziarani, M. G., Hasani, S., Mohajer, F., Varma, R. S., & Rafiee, F. (2022). The molecular diversity of 1 *H*-indole-3-carbaldehyde derivatives and their role in multicomponent reactions. *Topics in Current Chemistry, 380*(4), 24. https://doi.org/10.1007/s41061-022-00379-5