Fast, sensitive, and sustainable colorimetric detection of chlorogenic acid in artichoke waste material

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ABSTRACT

Caffeoylquinic acids (CQAs) are nutraceutical polyphenols highly represented in natural sources, including artichoke waste (AW). In this study a colorimetric method for rapid and sustainable detection of a 5-CQA isomer (Chlorogenic acid) in AW extract was developed by using alkaline Tris buffer (10 mmol L^{-1}, pH 9) to generate a yellow color associated with 5- to 3-CQA isomerization reaction, as suggested by NMR and MS analyses. The strong absorbance at 360 nm was followed by standard UV–Vis methodology. The colorimetric assay was exploited for detection of 5-CQA into leaf extract from artichoke, obtaining a value of 15.2 ± 0.3 μg/mg of dry extract, in agreement with HPLC analysis (14.3 ± 0.7 μg/mg, 106 ± 2 % recovery) used as validation technique, with excellent linear correlation and precision ($R^2 = 0.9996$, $_{av}RSD\% = 3.2$ %). The method is fast and selective, offering a valuable tool for nutraceuticals identification and food waste valorization.

1. Introduction

In the recent years, the interest toward the study of plant metabolites has significantly grown, and several papers highlighted their nutraceutical properties (Cisneros-Zevallos, 2021; Hurkul et al., 2024; Nirmala et al., 2020; Talarico et al., 2024). For example, it has been amply demonstrated that health benefits associated with the Mediterranean diet are linked to bioactive compounds, such as phenols and polyphenols, contained in functional foods (Schwingshackl et al., 2020). In particular, caffeoylquinic acids (CQAs) represent a specific class of phenolic compounds constituted by one molecule of quinic acid (QA) conjugated with one or more molecules of caffeic acid (CA) (Fig. 1). In general, CQAs present different nutraceutical properties, including antioxidant, antibacterial, anti-obesity, anti-tumor, and anti-inflammatory activities (Liu et al., 2020). Additionally, CQAs have been found to be beneficial for liver, kidney, and nervous system, with several potential pharmaceutical applications (Feiden et al., 2023; Lu et al., 2020). CQAs are highly produced in some fruits, vegetables, and spices, such as coffee bean (Jaiswal et al., 2010; Silva et al., 2024),
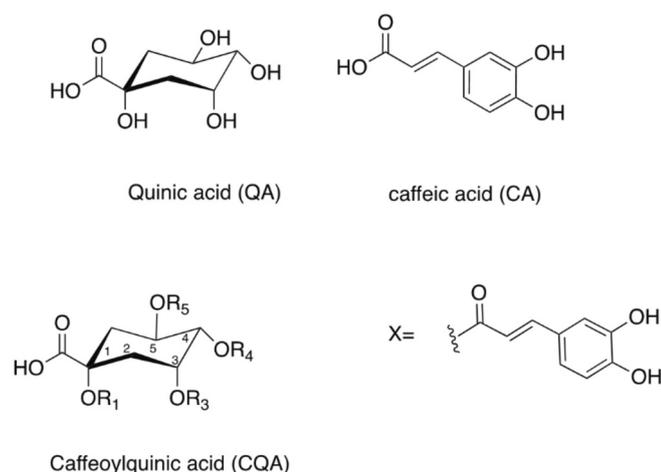
potato tubers (Islam et al., 2003), eggplant (Ma et al., 2010), and artichoke (Lu et al., 2020). The latter is a perennial plant (*Cynara cardunculus* L. var. *scolymus*) typical of the Mediterranean area, with large annual production in Africa (6.5 × 10^8 kg), Europe (6.0 × 10^8 kg), Americas (1.8 × 10^8 kg), and Asia (1.5 × 10^8 kg) (FAOSTAT, 2024). Generally, the edible part of artichoke is the inner core of the head, while leaves, stems, and bracts constitute the waste that can represent up to 80–85 % by weight of the total plant biomass. Nevertheless, artichoke by-products are a source of phenolic compounds that could be useful as dietary supplements and food additives (Pandino et al., 2013). In literature, AW is recognized as an innovative and low-cost source of polyphenols and in particular of chlorogenic acids with eventual significative antioxidant activity in intestinal cell model (Jiménez-Moreno et al., 2019), allowing AW to be suggested as dietary supplements, particularly for cancer patients undergoing radiotherapy and chemotherapy, or as an adjuvant to enhance traditional chemotherapy by reducing dosages, toxicity, and side effects of these treatments (Abdel-Khalek & Younies, 2021). Furthermore, Artichoke leaves (non-edible part) also show anti-inflammatory, antiviral and antimicrobial,

Fig. 1. Chemical structure of Quinic acid (QA), Caffeic acid (CA), and main Caffeoylquinic acids (CQAs) with IUPAC numbering system.

Caffeoylquinic acids	Acronym	R_1	R_3	R_4	R_5
1-O-caffeoiylquinic acid	1-CQA	X	H	H	H
3-O-caffeoylquinic acid	3-CQA	H	X	H	H
4-O-caffeoylquinic acid	4-CQA	H	H	X	H
5-O-caffeoiylquinic acid (CGA)	5-CQA	H	H	H	X
1,3-O-dicaffeoiylquinic acid	1,3-CQA	X	X	H	H
1,4-O-dicaffeoiylquinic acid	1,4-CQA	X	H	X	H
1,5-O-dicaffeoiylquinic acid	1,5-CQA	X	H	H	X
3,4-O-dicaffeoiylquinic acid	3,4-CQA	H	X	X	H
3,5-O-dicaffeoiylquinic acid	3,5-CQA	H	X	H	X
4,5-O-dicaffeoiylquinic acid	4,5-CQA	H	H	X	X

hepatoprotective and antihyperglycemic properties (Zayed & Farag, 2020).

Numerous studies focused on the extraction and quantification of such compounds by means of high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and capillary electrophoresis (Esposito Salsano et al., 2022; Gatea et al., 2015; Giusti et al., 2017; Kremr et al., 2017; La Barbera et al., 2017). However, these methodologies require the use of sophisticated and expensive laboratory equipment, lengthy sample preparations, and qualified personnel. Accordingly, the development of rapid, sensitive, and affordable methods for detecting phenols and polyphenols in different matrices is considered crucial, especially for agricultural waste recycling and reuse. In fact, wastes deriving from food production contain large amount of polyphenols (Cuffaro, Bertini, et al., 2023; Cuffaro, Bertolini, et al., 2023, Espro et al., 2021) since during plant development polyphenols tend to accumulate in the peripheral parts of the plant (Vanoh et al., 2021). Colorimetric methods represent a suitable alternative for the routine examination of food samples, due to their simplicity and the broad availability of low-cost spectrophotometers. Currently, the Folin-Ciocâlteu (FC) colorimetric assay is widely used to quantify the total phenol and polyphenol content (Grosso et al., 2024; Raposo et al., 2024; Srisuk, 2016), but it does not allow to discriminate among the subclasses of phenolic compounds. Moreover, due to the heavy metals' composition, FC reagent is highly harmful for humans and dangerous to the environment. Other assays are employed to quantify natural

antioxidants but still requiring expensive or toxic chemicals (Borah & Tamuly, 2022; Re et al., 1999; Yang et al., 2024). Several colorimetric methods for quantitative analyte estimation have been recently developed by our group and applied both to drug and food analysis based on the exploitation of analyte's functional groups to generate a colored compound detected by means of visible spectroscopy (Lettieri et al., 2022; Lettieri et al., 2023; Palladino et al., 2019; Palladino et al., 2024). It was demonstrated that dissolving chlorogenic acid (5-O-caffeoyil-quinic acid, 5-CQA), that is the most abundant CQA isomer in plant sources, in tetramethylammonium hydroxide at pH 9 favored the formation of the isomer 3-O-caffeoylquinic acid (3-CQA) (Clifford et al., 1989), giving rise to a yellow color solution. This was the starting point for the development of our colorimetric assay in microwell plates for high-throughput measurements of a specific class of phenolic compounds, discriminating 5-CQA and 1,5-CQA from CA, by using a common alkaline aqueous buffer, avoiding specific measures for safe reagents' handling, storage, transport, and disposal, toward more sustainable analytical protocols. We focused on these two isomers because they are the only CQAs usually detected in artichoke waste, i.e. the matrix here analyzed (Colombo et al., 2024; Pandino et al., 2013). The calibration curves for 5-CQA were easily constructed in buffer, reporting excellent analytical performances in terms of linear correlation, precision, and sensitivity. Subsequently, the assay was successful exploited for detection of 5-CQA from leaves of artichoke cultivar *Terom*, a CQAs-rich matrix suitable for nutraceutical applications, using the standard addition method, and validated by classic HPLC methodology. Further investigations by NMR and mass spectrometry (MS) have been devoted to the chromogen characterization in artichoke waste. The strong correlation between the developed colorimetric method and the conventional HPLC analysis indicates the reliability and high accuracy of the assay in quantifying CQAs in artichoke matrix among several phenols and polyphenols. More in general, the scope of this study was to present a novel colorimetric method for the selective, rapid, and sustainable detection of CQAs, nutraceutical (poly)phenols, in food waste matrix, here represented by the case study artichoke leaf extract, to contribute to the valorization of plant by-products, promoting their utilization as a potential source of bioactive compounds in various applications, obviating the necessity for the use of large instruments, thus aligning with the principles of the circular economy and sustainable chemistry (Olas, 2024).

2. Materials and methods

2.1. Materials and chemicals

Solvents used for extraction procedures and HPLC, such as HPLC-grade water (H_2O), HPLC-grade methanol (MeOH), HPLC-grade acetonitrile (ACN), HPLC-grade ethanol (EtOH), HPLC-grade acetic acid (AcOH, 100 %) were all purchased from Merck (Merck srl, Milan, Italy). The commercial pure standards of 5-O-caffeoylquinic acid (5-CQA) and 1,5-dicaffeoylquinic acid (1,5-CQA) were purchased from Toronto Chemicals (Toronto, Canada). Tris (hydroxymethyl)aminomethane hydrochloride and DMSO-d_6 were purchased from Merck. Transparent flat-bottom 96-well microplates were purchased from Sarstedt (Nümbrecht, Germany).

2.2. Samples preparation

AW is constituted by artichoke leaves from cultivar *Terom* collected in Tuscan area in February 2022. After collection, the sample was dried at 25 °C and kept in the dark for 60 days. The dried AW was manually crushed to obtain a homogeneous AW powder and stored at −20 °C until analysis. The polyphenolic extraction was performed according to the following procedure (Lavecchia et al., 2019) using AW powder brought to 20 °C. Specifically, 4 g of AW powder were macerated with an EtOH/H_2O solution (80:20 *v*/v), stirring for 2 h by magnetic stirring at 20 °C

and 700 rpm. Then, the suspension was centrifuged (1000*g* force), and supernatant filtered by 45 μm diameter filter, and finally evaporated. The resulting extract was dissolved in 1 mL of MeOH: H$_2$O (1:1 v/v) solution and injected for HPLC analysis using 5-CQA and 1,5-CQA, as reference standards.

2.3. Analysis of phenolic compounds by HPLC

HPLC analysis of samples was performed using a Shimadzu HPLC Nexera series (model CBM–40D) instrumentation equipped with a binary pump (LC-40D XR), a degassing unit (DGU-405), and a diode array detector (SPD-M40) (Shimadzu, OR, USA). The chromatograms were analyzed by the Shimadzu LabSolutions software LC-GC. A Phenomenex Gemini reverse-phase C18 column (250 × 4.6 mm, 5 μm particle size; Phenomenex, Castel Maggiore, Italy) was used as stationary phase. HPLC analysis of samples was performed using a slightly modified method previously reported (Jiménez-Moreno et al., 2019). The mobile phase was a mixture of H$_2$O:AcOH (98:2 v/v) (A) and ACN 100 % (B). Gradient elution was applied with the linear gradient progressing from 5 % (B) to 25 % (B) in 30 min; then to 50 % (B) for 10 min (40 min total time); for further 10 min it changed to 100 % (B) (50 min total time). Finally, 5 min re-equilibration were applied (60 min total time) to reach its initial composition, left 5 % (B). The flow rate was 1 mL/min, and the injected volume was 20.0 μL. Linear calibration curves for 5-CQA and 1,5-CQA were carried out in duplicate, at the above established conditions, in the range 250–100 μg/mL. Good linearities with correlation coefficients (R^2) of 0.999 for 5-CQA and 0.995 for 1,5 CQA, respectively, were obtained.

2.4. Analysis of 5-CQA and AW by optical assay

Solutions of standards and AW sample were freshly prepared in the Tris 10 mmol L^{-1}, pH 9 (Tris buffer) and immediately analyzed. Optical measurements were carried out in 96-well microplate at 25.0 °C by using in a Molecular Devices SPECTRO starNano (200–1000 nm) UV–Vis spectrophotometer by properly diluting and mixing the sample with Tris buffer. The absorbance has been measured, in the full range 250–800 nm, against a negative control, prepared without the addition of standards or matrix AW solutions. Further measurements of the yellow solution have been carried out at λ$_{max}$ 360 nm. For the AW extract analysis, the standard addition method was applied. Each well of the plate was filled with 0.1 mL of AW (1 mg/mL concentration) and 0.1 mL 5-CQA standard solutions in Tris buffer, or Tris buffer without 5-CQA (blank), for a total volume of 0.2 mL. Thus the original AW solution was diluted 1:1 with a final AW concentration in the well of 0.5 mg/mL.

2.5. NMR analysis

^{1}H and ^{13}C NMR spectra and COSY and HSQC bidimensional spectra were recorded on a Bruker Avance III HD (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer or with a Jeol CZR 500 MHz (frequency of 500 MHz for ^{1}H and 100 MHz for ^{13}C), using the residual solvent peak as internal reference; chemical shifts (δ) values are given in parts per million (ppm) and coupling constants (*J*) in Hertz. Chemical shifts (d) are reported in parts per million and coupling constants (*J*) are reported in hertz (Hz). The following abbreviations were used to explain multiplicities: singlet (s), doublet (d), triplet (t), double doublet (dd), broad (br), and multiplet (m). ^{1}H NMR, ^{1}H—^{1}H correlation spectroscopy, ^{13}C NMR and ^{1}H—^{13}C correlation spectroscopy spectra of 5-CQA solutions are reported in the Supplementary material.

2.6. MS analysis

MS analyses were performed using a Q Exactive Plus Orbitrap-based high resolution-mass spectrometer (Thermo, San Jose, CA, USA), equipped with an ionization electrospray (ESI) source and a hybrid-

quadrupole analyzer at the Center for Instrument Sharing of Pisa University (CISUP). ESI-MS spectra were recorded by direct injection and the working conditions were as follows: negative polarity, spray voltage 3.2 kV, capillary temperature 290 °C, and S-lens RF level 50. The sheath and the auxiliary gases were set at 28 and 4 (arbitrary units), respectively. Full MS spectra are reported in the Supplementary material.
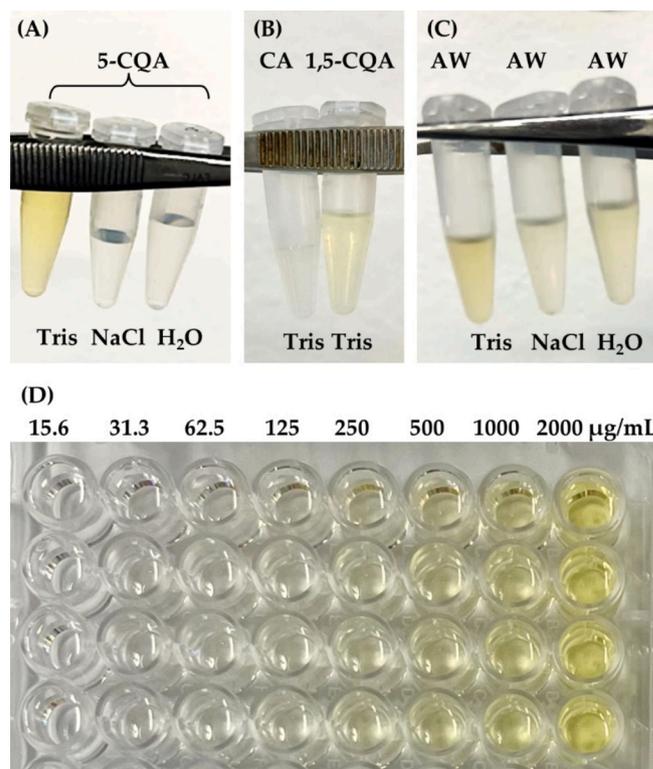
3. Results and discussion

3.1. Development of the colorimetric microwell assay

As already reported in literature, 5-CQA develops an intense yellow color in basic aqueous solutions, simultaneously carrying out transesterification and isomerization reactions, obtaining the 3-CQA isomer (Fig. 1) (Clifford et al., 1989; Xie et al., 2011). Thus, the first step in our approach was to verify the development of a yellow color by naked eye and acquiring the visible spectra (80–500 nm) for a 5-CQA standard solution and for an AW extract, rich in CQAs (Fig. 2).

Fig. 2A shows 5-CQA (200 μg/mL, 0.56 mmol L^{-1}) dissolved at room temperature in Tris buffer (never used before), in NaCl 10 mmol L^{-1}, and in H$_2$O, respectively.

Immediately after addition of Tris buffer, 5-CQA solution resulted in an intense yellow color. On the contrary, the solutions of 5-CQA in NaCl 10 mmol L^{-1} or in H$_2$O were colorless, underling the importance of a basic environment for the color development. To further confirm this effect, other molecules structurally similar to 5-CQA were evaluated at 200 μg/mL. In particular CA, missing the quinic acid unit, and 1,5-CQA, differing from 5-CQA only for an additional substitution in position 1 (Fig. 1), were dissolved in Tris buffer. We observed that CA solution was colorless, while 1,5-CQA solution developed a yellow color, similarly to 5-CQA (Fig. 2B), and an absorption peak at 360 nm (Fig. S1).
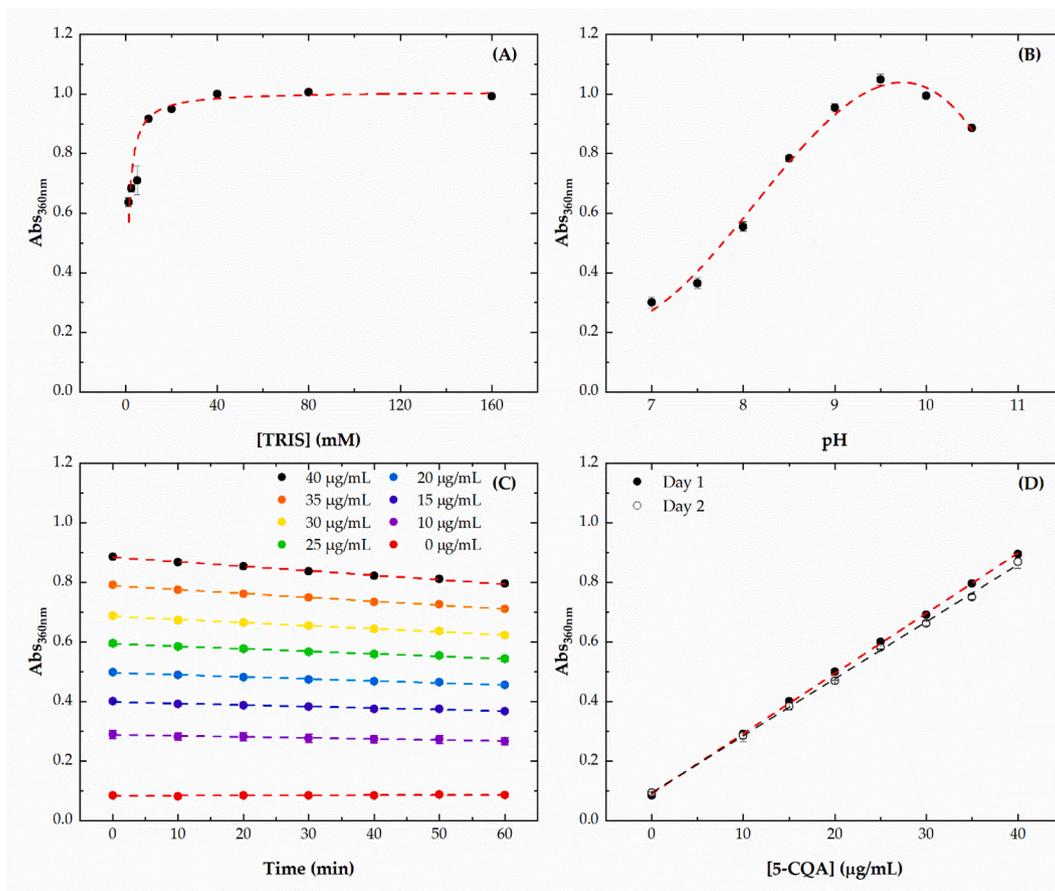

Fig. 2. Yellow color development by (A) 5-CQA in Tris buffer 10 mmol L^{-1} pH 9, NaCl 10 mmol L^{-1}, and H$_2$O; (B) CA and 1,5-CQA in Tris buffer 10 mmol L^{-1} pH 9; (C) AW in Tris buffer 10 mmol L^{-1} pH 9, NaCl 10 mmol L^{-1} and H$_2$O; (D) 5-CQA 15.6–2000 μg/mL in Tris buffer 10 mmol L^{-1} pH 9. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Accordingly, the assay is able to discriminate CQAs from CA. Then, similarly, the AW extract (10 g/L), with an estimated concentration of 5-CQA around 200 µg/mL, as deduced from HPLC analysis (see Paragraph 3.2), was dissolved in Tris buffer, NaCl 10 mmol L^{-1}, and H$_2$O. Also in this case, the AW Tris buffer resulted in a more intense yellow solution, compared to H$_2$O and 10 mmol L^{-1} NaCl (Fig. 2C). Preliminary observation by naked eye of yellow color formation in Tris buffer 10 mmol L^{-1} pH 9 is possible above 125 µg/mL, as reported in Fig. 2D where 5-CQA increases from 15.6 µg/mL to 2000 µg/mL.
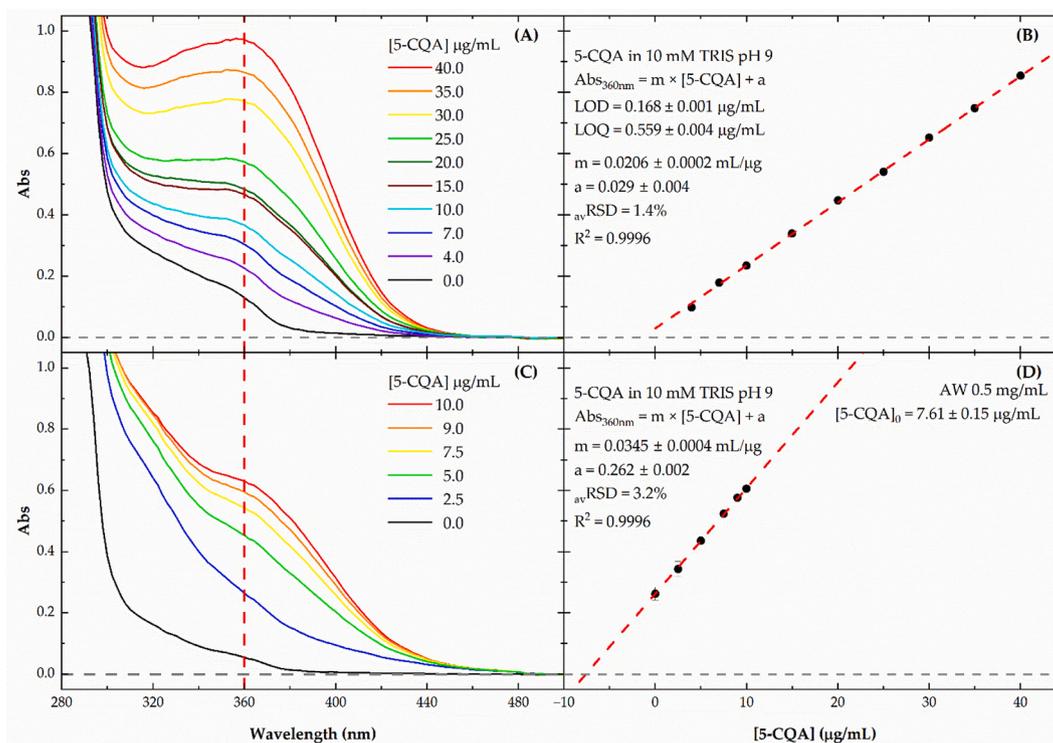
The robustness of the approach was tested in the following experiments, showing that the assay does not suffer in the presence of small changes of experimental conditions around the ones reported above (Tris buffer 10 mmol L^{-1} pH 9). This was achieved by verifying the effect of buffer concentration, pH, and reaction time on yellow color development (Fig. 3). In particular, the absorbance recorded at 360 nm for 40 µg/mL 5-CQA in Tris buffer at 20 °C showed a large increase in dependence of buffer concentration up to 10 mmol L^{-1}, but reaching a plateau around 40 mmol L^{-1}, with small absorbance diffence (3.5 %) between 10 mmol L^{-1} and 20 mmol L^{-1} Tris buffer (Fig. 3A). The same 5-CQA concentration showed a large dependence on pH for color development at 10 mmol L^{-1} Tris buffer solutions, as expected. The maximum absorbance at 360 nm was recorded around pH 9.5, but giving limited absorbance diffence (3.8 %) between pH 9.0 and 10.0 (Fig. 3B). Moreover, reaction time was analyzed for 5-CQA (0–40 µg/mL) in Tris buffer 10 mmol L^{-1} pH 9.0 (0–60 min), finding a minor linear decrease for the absorbance over 10 min (2.3 %) over the entire concentration range for 5-CQA (0–40 µg/mL) (Fig. 3C). Finally, an interday analysis for 5-CQA in 10 mmol L^{-1} Tris buffer pH 9.0 at 20 °C showed very good reproducibility with an accettable mean difference (4.8 %)

over the range 0–40 µg/mL, appearing in most case within the little error bars (Fig. 3D).

Accordingly, the ability of CQAs to develop a yellow color in Tris buffer, opened the way for a real application of the colorimetric assay to complex matrices, using 5-CQA as target molecule. A full spectrum in the interval 280–500 nm was acquired for 5-CQA in Tris buffer as reported in Fig. 4A, where an absorption peak can be clearly observed at 360 nm. The absorbance recorded at 360 nm is used for the calibration curve (Fig. 4B). The yellow color development increased with the 5-CQA concentration, as expected. The limit of detection (LOD, $3 \times SD_{blank}/m$) and quantitation (LOQ, $10 \times SD_{blank}/m$), were 0.168 ± 0.001 µg/mL and 0.559 ± 0.004 µg/mL, respectively. The reproducibility estimated as average relative standard deviation ($_{av}RSD\%$) was excellent (1.4 %) as well as the linearity in the explored 5-CQA concentration range (4.0–40 µg/mL) as indicated by a correlation coefficient R^2 of 0.9996. The assay was further applied to AW material derived from artichoke leaf extract, a 5-CQA rich matrix with interest in nutraceutical applications, using the standard addition method. AW was analyzed for 5-CQA content by means of the optical microplate reader. AW extracts aliquotes were spiked with 5-CQA solution in Tris buffer, obtaining a total volume of 200 µL/well (see Materials and method). The absorption spectra were recorded (Fig. 4C) and the peak absorbance (360 nm) value plotted against the spiked 5-CQA concentrations (Fig. 4D). The linearity observed was excellent with a R^2 of 0.9996, as well as the reproducibility ($_{av}RSD$ 3.2 %). The estimated 5-CQA concentration in AW was as 15.2 ± 0.3 µg/mg of dry AW extract.

Fig. 3. Absorbance at 360 nm in Tris buffer at 20 °C. Absorbance dependence on (A) buffer concentration (1.25–160 mmol L^{-1}) at pH 9.0 for 40 µg/mL 5-CQA; (B) pH (7.0–10.5) at 10 mmol L^{-1} buffer for 40 µg/mL 5-CQA; (C) reaction time for 5-CQA (0–40 µg/mL) in Tris buffer 10 mmol L^{-1} pH 9.0 (0–60 min); (D) Interday analysis of 40 µg/mL 5-CQA in Tris buffer 10 mmol L^{-1} pH 9.0. Day 1 (black circles) and Day 2 (white circles).

Fig. 4. UV–Visible spectra (A) and calibration curve (B) for 5-CQA in Tris buffer at 20 °C, and UV–Visible spectra (C) and standard addition curve (D) for 5-CQA in Tris buffer at 20 °C in presence of AW 0.5 mg/mL.

3.2. Assay validation by HPLC analysis

HPLC analysis was applied to evaluate the phenolic content in AW extract, consisting in dry leaves of artichoke (see Matrial and Mehtods) as validating method for the colorimetric assay. Considering the high level of similiarity in their molecular structure, we could considered a total amount of 14.31 ± 0.07 μg/mg for CQAs in the AW extract. This value from the conventional reference quantification method was compared to the result obtained by the colorimetric assay for 5-CQA derivatives which resulted to be 15.2 ± 0.3 μg/mg showing excellent agreement, corresponding to 106 ± 2 % recovery (Table 1). Basically, the colorimetric assay provides the quantitative estimation of 5-CQA and 1,5-CQA as chlorogenic acid equivalent (CGAE) in the AW extract and this data were confirmed by HPLC analysis.

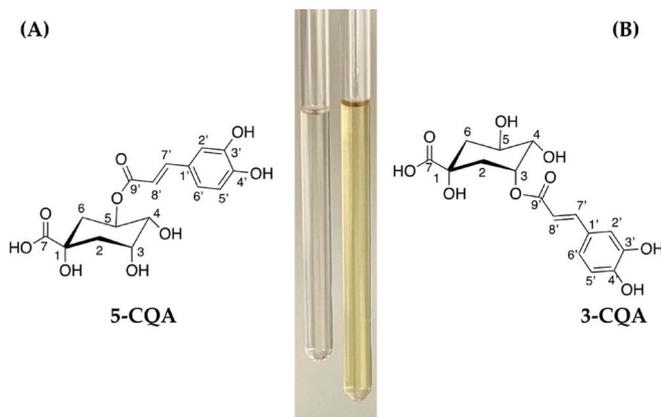## 3.3. Characterization of the CQAs solutions by NMR and MS

3.3.1. NMR analysis

Two solutions of 5-CQA were prepared in DMSO-d_6, appearing colorness (Fig. 5A), or dissolved into Tris buffer solution (10 mmol L^{-1} pH 9), further evaporated at 25 °C, and resuspended in DMSO-d_6, appearing yellow (Fig. 5B). The NMR analysis was conducted on both samples, performing ^{1}H (Fig. S2), COSY (Fig. S3), ^{13}C (Fig. S4), and HSQC (Fig. S5) spectra. Comparing the ^{1}H spectra of both samples, a noticeable difference in the H-6 and H-2 chemical shift was observed (Table 2). In

Table 1
Caffeoylquinic acids detection in AW extract.

Caffeoylquinic acid	HPLC	Colorimetry	
	μg/mg of dry extract[1]	μg/mg of dry extract	Recovery (%)
5-CQA	8.98 ± 0.03		
1,5-CQA	5.33 ± 0.06		
CGAE	14.31 ± 0.07	15.2 ± 0.3	106 ± 2

[1] Mean ± SD (n = 3).

Fig. 5. NMR tubes containing 5-CQA dissolved in DMSO-d_6 (A), or dissolved in Tris buffer solution (10 mmol L^{-1} pH 9), evaporated at 25 °C, and resuspended in DMSO-d_6, forming 3-CQA (B). Chemical structures of 5-CQA (A) and 3-CQA (B) are reported with numbering of carbon atoms corresponding to spectral assignment in Table 2.

particular, in solution A (Fig. S2A), all the H-6-protons and one of the H-2-proton exhibit one common multiplet signal at 2.03–1.92 ppm and the other H2 proton results at 1.80–1.75 ppm. For the solution B (Fig. S2B), the chemical shift of H-6 and H-2 resulted as three separate signals (2.07–1.98 1× H-6); 1.85–1.77 (H-2); 1.63–1.60 (1× H-6) attributable to those of the 3-CQA isomer, further confirmed by bidimensional analysis (Figs. S3-S5), as well as the expected ^{1}H shift values exchange between the isomers for the H-3 and H-5 signals upon caffeic acid moiety migration, in agreement with previous NMR analyses (Marković et al., 2016; Morishita et al., 1984; Xie et al., 2011). Analogously, the yellow color development by 1,5-CQA in Tris buffer at pH 9 is again attributed to the isomerization to 1,3-CQA due to caffeic acid moiety migration from position 5 to position 3.

Table 2
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR parameters of 5-CQA dissolved in (A) or (B).[1]

Solvent	^{1}H, δ/ ppm	^{13}C, δ/ ppm	Assignment
A	12.4	175.5	**C**OOH
		166.3	**C**OOR
	9.59	148.8	**C**-4'-OH
	9.17	146.1	**C**-3'-OH
	7.41	145.5	**C**-7'-H
		126.1	**C**-1'
	7.02	114.8	**C**-2'-H
	6.99–6.96	121.9	**C**-6'-H
	6.76	116.2	**C**-5'-H
	6.14	115.3	**C**-8'-H
		74	**C**-1
	5.08–5.06	70.9	**C**-5-H
	4.92, 4.76	71.4, 68.5	**C**-3-OH, **C**-4-OH
	3.92–3.91	71.4	**C**-3-H
	3.57–3.54	68.5	**C**-4-H
	2.03–1.91	37.7	**C**-6-H
	2.03–1.91; 1.80–1.75	36.6	**C**-2-H
B		176.6	**C**OOH
		166.3	**C**OOR
		148.3,	**C**-4'-OH
		145.6,	**C**-3'-OH
	7.44	144.6,	**C**-7'-H
		125.6	**C**-1'
	7.05	114.7	**C**-2'-H
	6.97–6.98	121.2	**C**-6'-H
	6.76	116.3	**C**-5'-H
	6.22	115.3	**C**-8'-H
		75.6	**C**-1
	5.15–5.13	72.1	**C**-3-H
	4.02	71.1	**C**-5-H
	3.43	73	**C**-4-H
	2.03–1.98, 1.63–1.60	38.6	**C**-6-H
	1.85–1.77	38.6	**C**-2-H

[1] (A) DMSO-d_6. (B) Tris buffer solution (10 mmol L^{-1} pH 9), evaporated at 25 °C, and resuspended in DMSO-d_6 forming 3-CQA.

3.3.2. MS analysis

Two solutions of 5-CQA were prepared for MS mass exact analysis by Orbitrap analyzer in H_2O (Fig. S6), appearing colorness, or in 10 mmol L^{-1} Tris buffer at pH 9 (Fig. S7), giving a yellow solution. Both solutions displayed the same molecular and diagnostic fragment ions, as expected, but the relative abundance was different. In particular, the $C_{16}H_{18}O_9$ [M-H]$^-$ 353.0875 ion *m/z* was found in both solutions (Figs. S6A and S7A). 191.055 m/z ion, due to the cleavage of an ester bond and quinic acid formation, is also present in both samples (Figs. S6B and S7B). The caffeic acid fragmentation ion at 179.034 m/z is present in both samples but the signal intensity is slightly higher for the 5-CQA in Tris buffer (Fig. S7B). Finally, the ion fragmentation at 135.045 m/z, due to the neutral loss of CO_2 (44 Da) from the caffeic acid ion, is evident only in the sample of 5-CQA in Tris buffer (Fig. S7B). Equivalent fragmentations were previously reported by Xie et al., who attributed the difference in abundance signals to the occurrence of the isomerization reaction from 5-CQA to 3-CQA (Xie et al., 2011).

4. Conclusions

This study presents a novel colorimetric method for the selective, rapid, and sustainable detection of CQAs in food waste matrix. 5-CQA and 1,5-CQA exhibited a yellow color when dissolved in Tris buffer 10 mmol L^{-1} at pH 9, attributed to 5- to 3-CQA isomerization process, while caffeic acid solution remains colorless, as confirmed by NMR and MS analysis. The colorimetric assay was applied to standard solutions, resulting in excellent sensitivity, outstanding linearity, and high reproducibility, as well as to 5-CQA-rich artichoke leaf extract. The strong correlation between the developed colorimetric method and the conventional HPLC analysis indicates the reliability and high accuracy of the assay in quantifying CQAs in artichoke matrix among several

phenols and polyphenols. The study contributes to the valorization of artichoke by-products, promoting their utilization as a potential source of bioactive compounds in various applications.

CRediT authorship contribution statement

Doretta Cuffaro: Writing – review & editing, Investigation, Data curation. **Pasquale Palladino:** Writing – review & editing, Investigation, Data curation, Conceptualization. **Maria Digiacomo:** Writing – review & editing, Investigation, Data curation. **Simone Bertini:** Writing – review & editing. **Maria Minunni:** Writing – review & editing, Investigation, Data curation. **Marco Macchia:** Writing – review & editing, Funding acquisition.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.141505.

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