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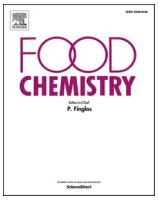

Thermal degradation of red cabbage (*Brassica oleracea* L. var. *Capitata f. rubra*) anthocyanins in a water model extract under accelerated shelf-life testing

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ABSTRACT

Red cabbage (RC) represents a source of anthocyanins acylated with hydroxycinnamic acids (HCA) that are described to enhance their stability. Nevertheless, data about their thermal degradation are still controversial. Our aim was to comprehensively analyse the degradation kinetics of individual RC anthocyanins in a model aqueous extract treated at 40 °C × 30 days to simulate severe but realistic storage conditions. Free anthocyanins and radical-scavenging capacity showed different kinetics. The results confirm the high stability of RC anthocyanins ($t_{1/2}$: 16.4–18.4 days), although HPLC analyses of each molecule displayed distinct kinetics with $t_{1/2}$ from 12.6 to 35.1 days. In particular, the sinapoyl acylation negatively affected the stability of the anthocyanins, while the forms monoacylated with glycosyl *p*-coumaric and ferulic acids exhibited higher stability. In conclusion, our results indicate that acylation is not a prerogative of stability, as this is instead more dependent on specific acylation patterns and the glycosylation of HCA.

1. Introduction

Anthocyanins (ACNs) are extensively studied plant phenolic compounds belonging to flavonoids subgroup. The structure of ACNs, based on the flavylium cation, is responsible for the intense red colour of a number of fruits, such as berries, and vegetables like black carrot, purple corn and potato. This characteristic, together with their high solubility in water, makes this class of molecules important natural pigments applicable to different food products (Giusti & Wrolstad, 2003). Anthocyanidins represent the basic aglycon structure of ACNs and consist of an aromatic ring (A) linked to a heterocyclic oxygen-bearing ring (C), which is bonded to a third aromatic ring (B). The type of sugar moiety involved in the glycosylation of anthocyanidins to give ACNs, together with the different distribution of –OH and $–OCH_3$ in the resulting structure, influence the maximum absorption wavelength (Giusti & Wrolstad, 2003; Castaneda-Ovando, Pacheco-Hernandez, Paez-Hernandez, Rodriguez, & Galan-Vidal, 2009) and chemical stability of ACNs (Sadilova, Stintzing, & Carle, 2006).

Red cabbage (*Brassica oleracea* L. var *Capitata f. rubra*, RC) is a largely consumed vegetable rich in several nutrients and bioactive molecules such as vitamins, glucosinolates and phenolic compounds, where ACNs constitute the most abundant fraction (Drozdowska, Leszczynska, Koronowicz, Piasna-Slupecka, & Dziadek, 2020). These are almost entirely represented by C_3– and C_5-glycosylated cyanidins, most of which are acylated with hydroxycinnamic acids (HCAs: sinapic, ferulic, *p*-hydroxybenzoic and caffeic acid) to the C_3-glycoside moiety (Gharaeghajlou, Hallaj-Nezhadi, & Ghasempour, 2021). RC acylated ACNs (AACNs) are known to possess higher stability than ACNs from other sources (Dyrby, Westergaard, & Stapelfeldt, 2001), especially towards basic pH (Moloney, Robbins, Collins, Kondo, Yoshida, & Dangles, 2018; Fenger, Moloney, Robbins, Collins, & Dangles, 2019). This is due to the π-stacking interaction that they can establish between the aromatic acyl groups with the pyrilum group of AACNs, a phenomenon also known as intramolecular co-pigmentation, which reduces the nucleophilic attack of water and the subsequent formation of a pseudobase or a chalcone. Since 80–85 % of ACNs are mono- or diacylated (Fang, Lin, Qu, Liang, & Wang, 2019), RC represents one of the best sources of food colourants. However, data about ACNs thermal stability are controversial. For

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instance, Wiczkowski et al. (2015) showed that stewing of RC led to a higher degradation of AACNs compared to the non-acylated forms. In addition, depending on the process, the authors suggested that a partial deacylation of mono- and diacylated AACNs could occur. On the contrary, a study by Steingass et al. (2023) has recently shown that RC AACNs exhibit high thermal stability when heated in aqueous model solutions at 80 °C for 5 h.

Although RC ACNs have been extensively studied, and the stability of some of them has been proved at neutral pH, a comprehensive study of the thermal degradation kinetics of the single RC ACNs has not been pursued so far. Thus, similarly to what has been described in other studies for other types of juices and beverages (Sanchez, Baeza, & Chirife, 2015; de Beer et al., 2018; Katz, Nagar, Okun, & Shpigelman, 2020), it was decided to carry out a study aimed at assessing the stability of anthocyanins in a RC aqueous extract under "accelerated" shelf-life testing, thus simulating the worst but realistic storage conditions this could be subjected to but over a shorter period of time.

2. Materials and method

2.1. Materials

Ethanol, hydrochloric acid, ABTS, TPTZ, sodium acetate trihydrate, iron(III) chloride hexahydrate, Trolox, gallic acid, Folin–Ciocalteu reagent, sodium carbonate, potassium persulfate, and other chemicals were purchased from Merck (Darmstadt, Germany). Methanol, acetonitrile and formic acid were of HPLC grade and obtained from Exacta + Optech Labcenter SpA (Modena, Italy). Kuromanin chloride (cyanidin-3-glucoside, Cy3G) was purchased from Extrasynthese (Genay, France). Fresh red cabbage (*Brassica oleracea* L. var. *Capitata f. rubra*) was obtained from local supermarkets (Verona, Italy).

2.2. RC extract (RCE) preparation

The whole cabbage head was used for the experiments. It was chopped into pieces of approximately 1 cm and finely ground with a Braun (Kronberg im Taunus, Germany) Multiquick MQ30 electric blender to obtain a homogeneous sample. ACNs extraction was performed following a previously reported procedure (Zanoni, Primiterra, Angeli, & Zoccatelli, 2020). 150 g of homogenised material was mixed with 300 ml of deionised water and adjusted to pH 3.3 with formic acid. This value resembles that of many beverages, such as fruit juices and soft drinks. Samples were extracted for 1 h at room temperature under continuous shaking; the extract was centrifuged at 16,000 *g* for 30 min, and the supernatant was filtered through Whatman filter paper and stored at −20 °C, until further experiments.

2.3. Thermal treatment kinetics study

Thirty tubes containing 30 ml each of RCE were enriched with 0.01 % (w/v) sodium azide to avoid microbial growth and placed in an oven (Memmert, Schwabach, Germany) at 40 °C for 30 days. Samples were collected every three days (10 samplings) and subsequently analysed. The pH of the samples was also monitored. The 0th-order and the 1st-order reaction rate constants (k) were estimated by fitting experimental data with the equations:

$$0^{th} order: C_t = C_0 - k \times t \quad (1)$$

from where the half-life ($t_{1/2}$) was calculated according to:

$$t_{1/2} = C_0/2k \quad (2)$$

$$1^{st} - order: ln(C_t/C_0) = -kt \quad (3)$$

from where $t_{1/2}$ was calculated according to:

$$t_{1/2} = ln(2)/k \quad (4)$$

where t and k represent the days after storage and rate constant ($days^{-1}$) respectively, while C_0 represents the initial concentration of the target molecules, and C_t the concentration at time t.

2.4. Differential pH assay

The concentration of total free ACNs was determined using the pH-differential method described by Lee et al. (2005) with some modifications. Two dilutions of RCE were prepared using HCl (pH = 1.0) and NaOH (pH = 4.5) separately, and absorbance of samples was measured at a wavelength of 520 and 700 nm using a Jasco V730 spectrophotometer (Jasco Europe Srl, Lecco, Italy). The ACNs concentration was quantified with the following equation (1):

$$\text{Anthocyanin concentration } \left(\frac{mg}{l}\right) = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l} \quad (5)$$

where $A = (A_{520} - A_{700})_{pH=1.0} - (A_{520} - A_{700})_{pH=4.5}$, MW is the molecular weight of cyanidin 3-glucoside (449.2 g mol^{-1}), DF is the dilution factor, *l* is the optical path (1 cm), and ε is the molar extinction coefficient of cy3G (26900 $Lmol^{-1} \cdot cm^{-1}$) (Chandrasekhar, Madhusudhan, & Raghavarao, 2012).

2.5. HPLC-DAD-MS analysis

RC extract was analysed by HPLC-DAD using an Extrema LC-4000 system (Jasco Europe, Italy) equipped with a C-18 silica Zorbax Eclipse Plus column (i.d. 4.6 x 250 mm, 5 µm particle size, Agilent Technologies, Palo Alto, CA, USA). The mobile phases were as follows: (A) 5 % formic acid (pH 3.2) and (B) acetonitrile, and the analyses were carried out applying the following multistep gradient: from 5 % B to 20.5 % B in 72 min, to 100 % B in 3 min followed by a 3 min plateau, to 5 % B in 1 min and an 11 min plateau. The total time of analysis was 90 min, flow rate 0.75 ml/min.

ACNs identification was performed using an HP 1260L liquid chromatography equipped with a DAD detector (Agilent Technologies, Palo Alto, CA, USA) and an API (Atmospheric Pressure Ionisation) interface/ESI (ElectroSpray Ionization) using the same column and conditions described for HPLC-DAD analysis. MS operating conditions were as follows: N_2 flow of 10.5 L/min; gas temperature of 350 °C; capillary voltage of 3500 V; nebuliser pressure of 1811 Torr. The acquisition of the spectra was performed in positive ionisation, with a fragmentation energy of 150 eV. Tentative identification was carried out by comparison of retention times, UV–Vis and mass spectra of analytical standards, when available. In the other cases, attribution was achieved by comparing mass spectra and elution order with the ones reported elsewhere (Wiczkowski, Szawara-Nowak, & Topolska, 2013; Mizgier, Kucharska, Sokol-Letowska, Kolniak-Ostek, Kidon, & Fecka, 2016; Fang et al., 2019; Izzo, Rodriguez-Carrasco, Pacifico, Castaldo, Narvaez, & Ritieni, 2020; Steingass et al., 2023). The content of the main ACNs was calculated by HPLC-DAD and expressed as relative area percentage. All the analyses were performed in triplicate.

2.6. Total phenolic content (Folin-Ciocalteau)

The total polyphenols content (TPC) was determined by the Folin-Ciocalteu method, as described before (Zanoni et al., 2020). Briefly, 150 µl of Folin-Ciocalteau reagent (previously diluted at 1:15 with water) was added to 5 µl of each sample and incubated for 3 min. Finally, 40 µl of 20 % (w/v) sodium carbonate solution was added and left to react for 30 min in the dark. The absorbance was measured at 750 nm with a Tecan Infinite 200 Pro microplate reader (Tecan Trading AG, Männedorf, Switzerland). Water was used as blank and gallic acid (GA) served as a standard. Results were expressed as mg/l of GA equivalents.

2.7. Radical-scavenging capacity (ABTS)

The radical-scavenging capacity of RCE was evaluated by the ABTS method, as described by Bellumori et al. (2021). A methanolic 7.4 mM ABTS and a 2.46 mM potassium persulfate aqueous solution were mixed in a 1:1 ratio and allowed to stand for 12 h at room temperature in the dark. This working solution was diluted with methanol to reach an absorbance of 0.75 ± 0.02 at 734 nm. A new solution was prepared for each assay. Twenty µl of each sample was placed in microplate wells, and 200 µl of the working solution was added. The decrease in absorbance was monitored at 734 nm using a Tecan Infinite 200 Pro microplate reader (Tecan Trading AG, Männedorf, Switzerland). Results were expressed as percentage of inhibition, calculated with the following equation:

$$\%\ inibition = \frac{A_{blk} - A_x}{A_{blk}} \bullet 100 \quad (6)$$

where A_{blk} and A_x are the absorbances of the blank and the samples, respectively.

2.8. Ferric reduction antioxidant power (FRAP)

Ferric reducing antioxidant power assay was performed according to the method described by Benzie and Strain (1996). The working reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ dissolved in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution in 10:1:1 ratio. Ten µl of each sample was mixed with 188 ml of working reagent and 1 ml of water. The mixture was incubated at 37 °C for 10 min, and the absorbance was measured at 593 nm using a microplate reader Tecan Infinite 200 Pro. Water was used as blank, Trolox served as a standard, and the results were expressed as mM of TE (Trolox Equivalents).

2.9. Statistical analysis

All analyses were performed in triplicate, and results were expressed as mean values ± standard error of the mean. Regression analysis was performed using the SigmaPlot software (Systat Software Inc., ver. 12.5, Palo Alto, CA, USA). Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Effects of thermal treatment on total free ACNs, radical-scavenging capacity and ferric-reducing powder

RC extract was analysed through different assays to quantify the free ACNs and TPC content and to evaluate the antioxidant capacity in terms of scavenging capacity of the ABTS radical and reducing power of a ferric complex (FRAP assay). Since Folin-Ciocalteu reacts with –OH residues responsible for the radical-scavenging capacity of phenolic compounds, TPC is frequently used in combination with these assays. Collected data are summarised in Table 1.

Samples of this extract were incubated at 40 °C to simulate a severe controlled storage temperature condition and collected every 3 days for a total of 30 days of treatment. The conditions adopted for the this shelf-life test were chosen on the basis of other studies conducted on similar matrices, such as plant juices (Sanchez et al., 2015) in which thermal degradation of ACNs was accelerated increasing temperature up to 37–40 °C. This range realistically represents the worst storage conditions that food products may encounter. Fig. 1 illustrates the variation in the content of ACNs (A), total polyphenols (B), radical-scavenging

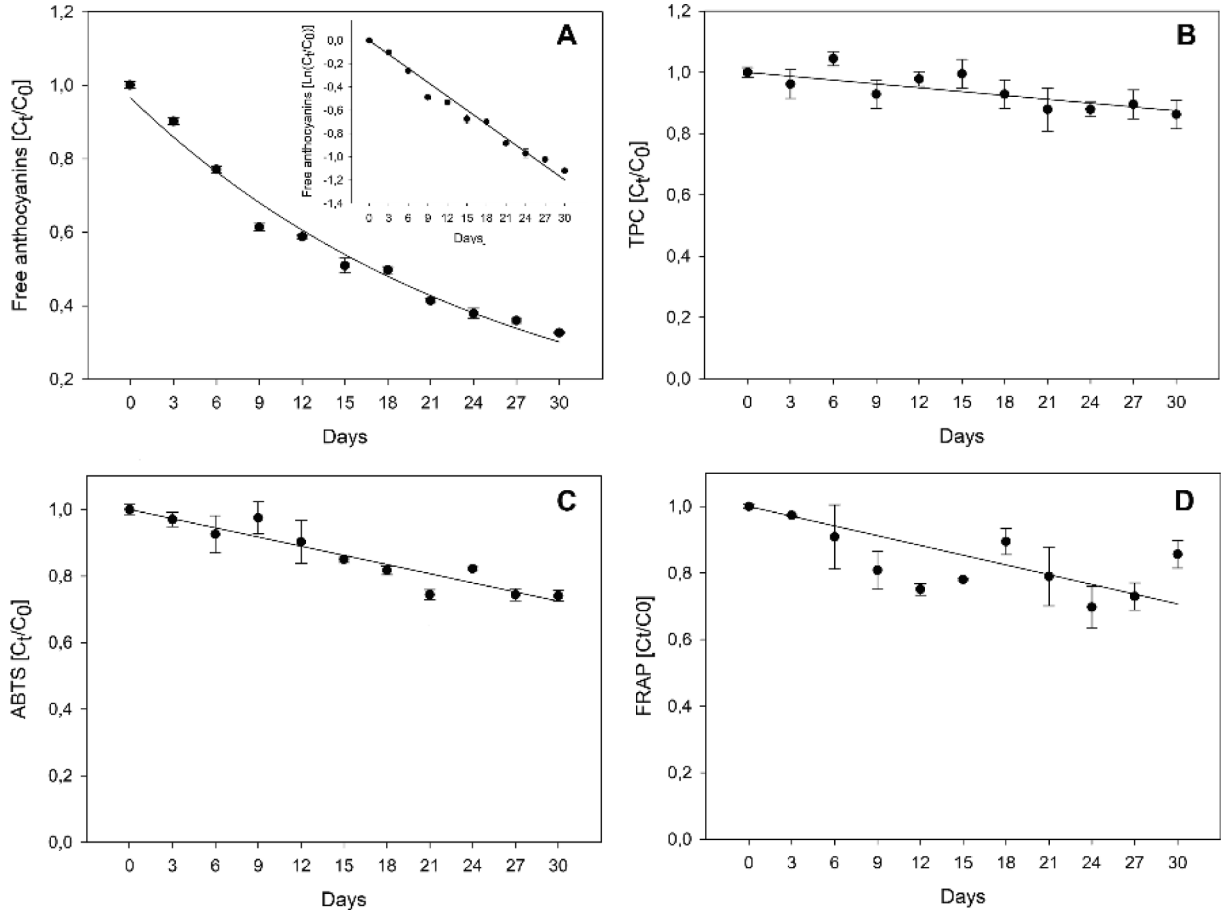

Fig. 1. Time-dependent degradation of ACNs (A), TPC (B), ABTS (C), and FRAP (D). Inset: log-transformed data.

capacity (C), and ferric-reducing power (D) during the thermal treatment experiment described above. More specifically, Fig. 1A shows a decrease of ACNs, leading to 33 % of the starting value after 30 days. As shown by the R^2 value in Table 1, the observed data trend appears to follow an apparent 1st-order degradation kinetics as already observed (Dyrby et al., 2001). This is supported by the linear fitting of the log-transformed data (Fig. 1A, inset).

These results are in agreement with the literature data since it is known that ACNs degradation under isothermal heating follows 1st order kinetics (Patras, Brunton, O'Donnell, & Tiwari, 2010). The half-life ($t_{1/2}$) of RC ACNs was calculated to be included between 16.4 and 18.4 days (95 % confidence level). This value is higher than those of several ACNs-rich juices obtained from different sources (Sanchez et al., 2015), even though experimental conditions might slightly differ, e.g. in terms of starting concentration and pH.

The time-dependent analysis of TPC, ABTS and FRAP data led to different results compared to total free ACNs. In particular, the data obtained by these three assays did not show a clear-cut exponential pattern. The data showed oscillations around an apparent linear - i.e. 0th-order degradation trend. It is tempting to speculate that these oscillations are due to the presence, in the extracts, of different molecules that may take part in the same colourimetric reactions at different times. For example, some degradation products that accumulate at the initial stages of the experiment might acquire scavenging potential that could be exerted again at later times with different rates, as already observed (Chen, Du, Li, & Li, 2020).

3.2. Free ACNs quali-quantitative profile

The chromatographic profile at 520 nm of the extract is reported in Fig. 2. The profile at 320 nm, showing the separation of HCAs is provided as Supplementary material (see Fig. S1). These are mostly sinapic acid derivatives. All the ACNs, the object of study, were previously identified in other studies. The relative abundance of these ACNs was similar to what Fang et al. (2019) previously described, indicating the monoacylated and di-glycosylated forms as the predominant. In fact, in the present work, the three monoacylated ACNs Cy3(p-coum)diG5G, Cy3(sin)diG5G and Cy3(fer)diG5G (peaks 9 and 10) together account for almost 50 % of the total free ACNs. On the other side, in the study by Wiczkowskiet al. (2013), non-acylated forms were indicated as the most representative. As already stated by other authors (Strauch et al., 2019), these discrepancies in the ACNs patterns may be explained by other factors such as genotype, maturation and cultivation conditions.

3.3. Kinetics of degradation of RC ACNs

The HPLC-DAD analyses of the RCE samples collected during the storage allowed for quantifying the concentration of the thirteen most abundant ACNs (black-labelled in Fig. 2) and outlining their degradation kinetics (Fig. 3). As expected, for all the ACNs loss seems to follow a 1st order kinetic ($R^2 > 0.990$, $p < 10^{-4}$). However, their rate showed marked differences, with a $t_{1/2}$ ranging from a minimum of 12.6 to a maximum of 35.1 days. The non-acylated Cy3diG5G, representing 17 % of free ACNs, was characterised by a $t_{1/2}$ between 18.21 and 19.70 days (95 % confidence interval). According to some authors (Giusti & Wrolstad, 2003; Steingass et al., 2023), this ACN form was supposed to be the most unstable due to the lack of acyl groups that could protect the pyrilum group from the nucleophile attack of water. Nonetheless, as a matter of fact, some AACNs showed lower stability, as observed in previous studies (Sadilova, Carle, & Stintzing, 2007; Wiczkowski et al., 2015): ACNs possessing the lowest stability were those conjugated to sinapic acid, with the diacylated Cy3(sin)(sin)diG5G being the most unstable ($t_{1/2}$ of 12.6–13.0 days). With respect to the other di-glycosylated and diacylated ACNs, Cy3(*p*-coum)(sin)diG5G and Cy3(fer)(sin)diG5G, which represent the two predominant ones, showed significantly higher half-life times (21.1–22.7 days and 20.2–22.8 days, respectively). The most stable ACNs were the monoacylated Cy3(G-fer)diG5G and Cy3(G-*p*-coum)diG5G, showing half-lives beyond 32 days, while the sinapoylated homologue, i.e. Cy3(G-sin)diG5G (peak #6), displayed, also in this case, lower stability (24.4–27.6 days). These ACNs are characterised by having a hexose unit bound to the *p*-hydroxyl group (highlighted in red in Fig. 2) of the HCA constituting the C_3-glycosylation moiety as recently indicated by Steingass et al. (2023), while earlier observations mentioned generic tri-glycosylated forms (Wiczkowski et al., 2013, 2015; Fang et al., 2019). Among the diacylated and glycosyl-HCA-bearing AACNs, Cy(G)(*p*-coum)(sin)diG5G was more stable than Cy(G)(fer)(sin)diG5G (25.9–30.2 days Vs 22.6–25.8 days). The degree of stability of AACNs in the extracts may be explained by considering the type of glycosylation and acylation moieties. In particular, the half-life seems to be inversely associated with the antioxidant capacity of the different HCAs. These phenolic compounds are characterised by a strong antioxidant capacity that decreases following the order sinapic acid ≫ferulic acid > p-coumaric acid (Mizgier et al., 2016). As a confirmation, the results of Wiczkowski et al.(2013) highlighted the stronger reactivity of RC AACNs conjugated with sinapic acid when tested by different assays to quantify antioxidant capacity. The reactivity of sinapoyl residue could explain why Cy3(G-*p*-coum)diG5G, and Cy3(*p*-coum)(sin)diG5G, showed similar half-life, despite these two molecules are characterized by different susceptibility to water addition, with Cy3(*p*-coum)(sin)diG5G much less susceptible. The discrepancy from the data we obtained could be again explained considering the higher tendency of sinapoyl acyl to oxidation. In addition, the results of Table 2 suggest that also the glycosylation pattern might affect the stability of the ACNs. In particular, the diglycosylated ACNs were less stable than their homologues characterised by a glycosyl-HCA. Indeed, Cy3(*p*-coum)diG5G displayed a definitely lower $t_{1/2}$ than Cy3(G-*p*-coum)diG5G, and Cy3(*p*-coum)(sin)diG5G and Cy(fer)(sin)diG5G ($t_{1/2}$ of 21.1–22.7 and 20.2–22.8 days, respectively) showed to be less stable than Cy(G)(*p*-coum)(sin)diG5G and Cy(G)(fer)(sin)diG5G ($t_{1/2}$: 25.9–30.2 and 22.6–25.8 days, respectively). Unfortunately, the co-elution of Cy3(sin)diG5G and Cy3(fer)diG5G in peak #10 did not allow us to formally compare the stability of these AACNs with the glycosyl-HCA-bearing homologues eluting in peaks #5 and #6. Still, the $t_{1/2}$ of peak #10 was lower, which agrees with the previous findings. It is noteworthy that Cy3(sin)diG5G is present in both peaks #2 and #10, which are characterized by different stability. This is probably due to a different position of sinapoyl acylation in Cy3(sin)diG5G structure that affects its susceptibility to oxidation, as already described (Denish et al., 2021).

The presence of a hexose bound to the –OH group of the HCA, as mentioned before, could help explain the higher stability of these ACNs. In this view, the glycosylation would limit the abstraction of H atoms, thus partially protecting the HCAs from oxidation and explaining why these ACNs are characterised by greater $t_{1/2}$. Nevertheless, Cy3(sin)diG5G and Cy3(G-sin)diG5G seem not to follow this trend since the former exhibited slightly higher stability ($t_{1/2}$: 19.4–22.6 days vs. 14.3–15.7 days).

Wiczkowski et al.(2015) suggested that the deacylation of ACNs might have occurred during the stewing and the storage of red cabbage leaves upon fermentation since the authors observed an increase in the relative amount of the non-acylated Cy3diG5G during processing and storage. In our hands, no evident deacylation phenomena have occurred

Table 1
Initial free ACNs content and antioxidant capacity of RCE and regression data.

		0th-order	1st-order	p
	t_0	R^2	R^2	<0.05
Free anthocyanins	251 ± 3 mg/L CyGE	–	0.974	<0.05
TPC	507 ± 2 mg/L GAE	0.643	–	<0.05
ABTS	66 ± 2 % inhibition	0.891	–	<0.05
FRAP	8.2 ± 0.2 mM TE	0.326	–	<0.05

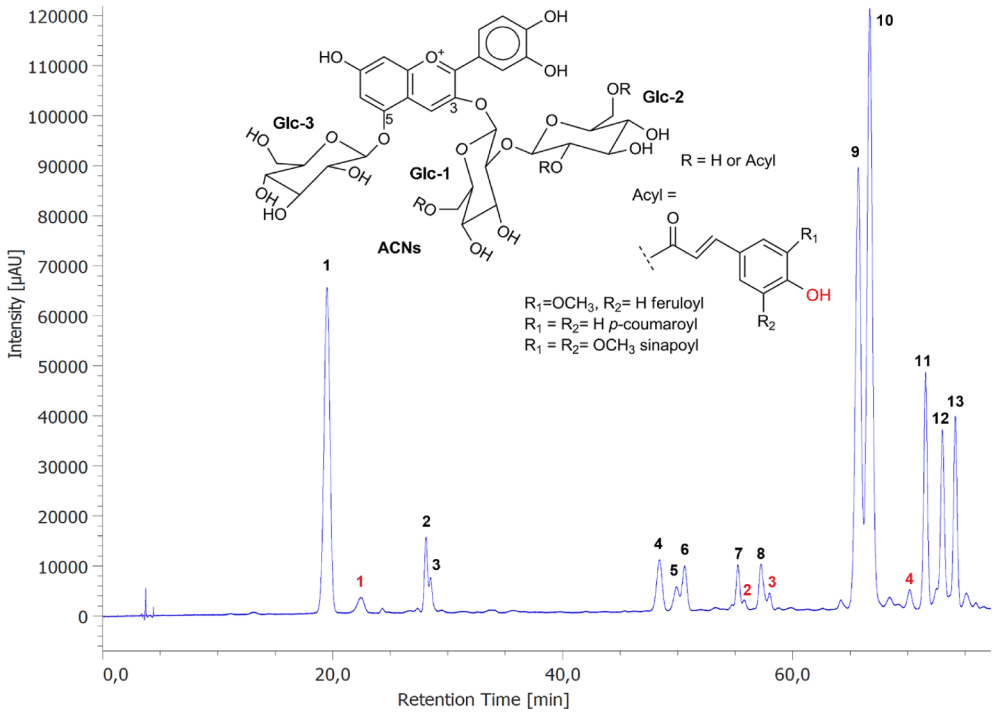

Fig. 2. HPLC-DAD analysis of RCE (520 nm). Peaks labelled in black were identified and quantitatively analysed, while peaks in red were only identified. Identifications and quantitative data are summarised in Table 2. Inset: generic structure of RC ACNs as described in (Fierri et al., 2023). For ACNs #3-8 and #3, the –OH group (highlighted in red) is bound to a hexose unit. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

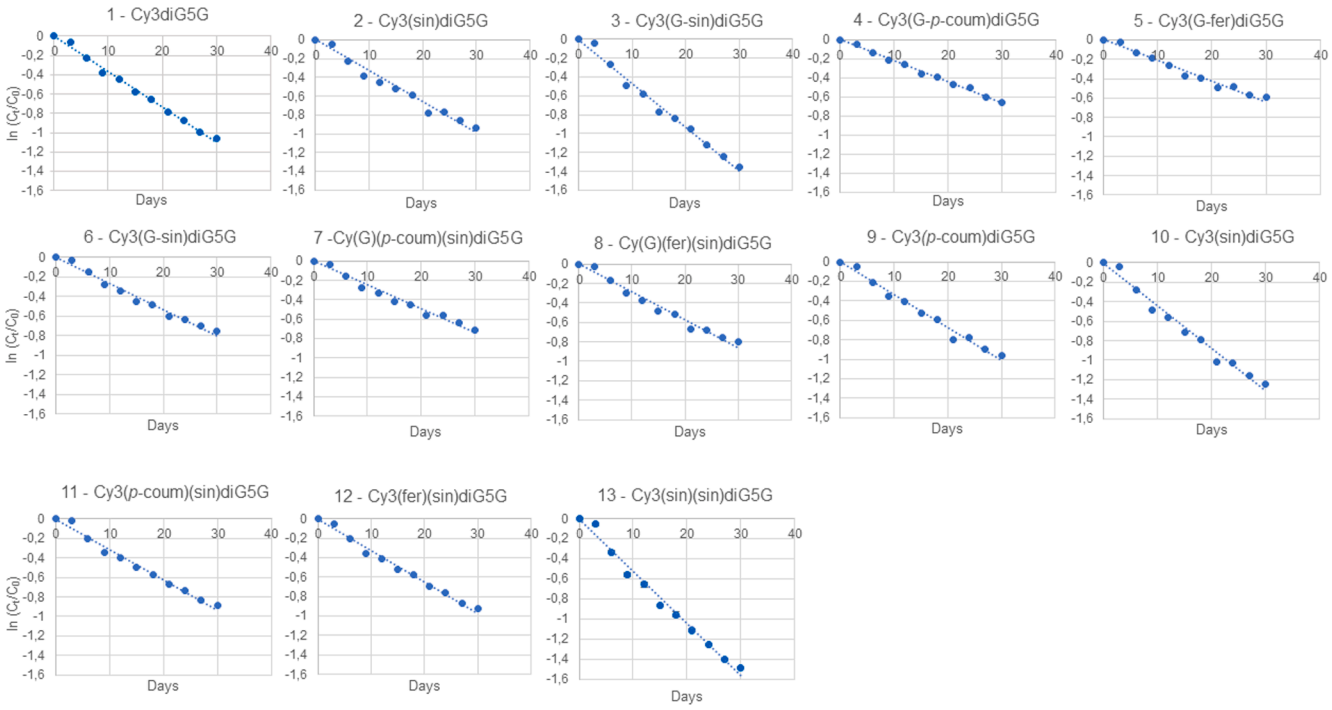

Fig. 3. Time-dependent degradation plots of the single ACNs measured by HPLC-DAD (520 nm).

since no fluctuations were visible in the degradation kinetics of the thirteen plots of Fig. 3, even if we cannot exclude that such phenomena might have taken place to small extents.

The experimental model could explain these different results: indeed, while we first extracted ACNs from RC leaves that were later thermally treated, the authors processed the plant material and subsequently extracted ACNs for HPLC analyses. It is possible that the thermal treatment of the tissues might have modified the extractability of the phenolic compounds, leading to different ACNs concentrations. In addition, the pH of these plant tissues is well above the pH of our extract (Husted & Schjoerring, 1995), further contributing to explaining the different observed results. Steingass et al. (2023) have recently shown

Table 2
MS data and kinetic parameters related to the degradation of individual anthocyanins during storage at 40 °C for 30 days.

Peak	ACN name	Abbreviation	$[M]^+$ (*m/z*)	Relative quantity (%)	k ($days^{-1}$) x 10^{-3}	$t_{1/2}$ (days) *	R^2	p
1	Cyanidin-3-diglucoside-5-glucoside	Cy3diG5G	773	16.93 ± 0.03	36.6 ± 0.6	[18.2; 19.7]	0.998	< 0.0001
2	Cyanidin-3-(sinapoyl)-diglucoside-5-glucoside	Cy3(sin)diG5G	979	2.08 ± 0.01	32 ± 1	[19.4; 22.6]	0.990	< 0.0001
3	Cyanidin-3-(glucosyl-sinapoyl)-diglucoside-5-glucoside	Cy3(G-sin)diG5G	1141	1.01 ± 0.01	46 ± 1	[14.3; 15.7]	0.997	< 0.0001
4	Cyanidin-3-(glucosyl-*p*-coumaroyl)-diglucoside-5-glucoside	Cy3(G-*p*-coum)diG5G	1081	2.49 ± 0.04	21.2 ± 0.4	[30.2; 32.8]	0.999	< 0.0001
5	Cyanidin-3-(glucosyl-feruloyl)-diglucoside-5-glucoside	Cy3(G-fer)diG5G	1111	1.10 ± 0.01	21.2 ± 0.7	[30.4; 35.1]	0.994	< 0.0001
6	Cyanidin-3-(glucosyl-sinapoyl)-diglucoside-5-glucoside	Cy3(G-sin)diG5G	1141	1.90 ± 0.05	26.6 ± 0.7	[24.4; 27.6]	0.995	< 0.0001
7	Cyanidin-3-(glucosyl)(*p*-coumaroyl)(sinapoyl)-diglucoside-5-glucoside	Cy(G)(*p*-coum)(sin)diG5G	1287	1.47 ± 0.01	24.7 ± 0.8	[25.9; 30.2]	0.991	< 0.0001
8	Cyanidin-3-(glucosyl)(feruloyl)(sinapoyl)-diglucoside-5-glucoside	Cy(G)(fer)(sin)diG5G	1317	1.90 ± 0.02	28.7 ± 0.8	[22.6; 25.8]	0.994	< 0.0001
9	Cyanidin-3-(*p*-coumaroyl)-diglucoside-5-glucoside	Cy3(*p*-coum)diG5G	919	21.43 ± 0.09	33.7 ± 0.8	[19.5; 21.7]	0.995	< 0.0001
10	Cyanidin-3-(synapoil)-diglucoside-5-glucoside (Cyanidin-3-(feruloyl)-diglucoside-5-glucoside)	Cy3(sin)diG5G (Cy3(fer)diG5G)	979 (949)	28.87 ± 0.02	44 ± 1	[14.9; 16.7]	0.995	< 0.0001
11	Cyanidin-3-(*p*-coumaroyl)(sinapoyl)-diglucoside-5-glucoside	Cy3(*p*-coum)(sin)diG5G	1125	7.8 ± 0.1	31.0 ± 0.8	[21.1; 22.7]	0.995	< 0.0001
12	Cyanidin-3-(*p*-feruloyl)(sinapoyl)-diglucoside-5-glucoside	Cy3(fer)(sin)diG5G	1155	6.06 ± 0.04	32.2 ± 0.9	[20.2; 22.8]	0.995	< 0.0001
13	Cyanidin-3-(sinapoyl)(sinapoyl)-diglucoside-5-glucoside	Cy3(sin)(sin)diG5G	1185	6.4 ± 0.1	52 ± 1	[12.6; 13.0]	0.996	< 0.0001
1	Cyanidin-3-glucoside-5-glucoside	Cy3G5G	611					
2	Cyanidin-3-(caffeoyl)-diglucoside-5-glu	Cy3(caf)diG5G	935					
3	Cyanidin-3-(glucosyl)(sinapoyl)(sinapoyl)-diglucoside-5-glucoside	Cy3(G)(sin)(sin)diG5G	1347					
4	Cyanidin-3-(sinapoyl)-glucoside-5-glucoside	Cy3(sin)G5G	817					

ACNs indicated in red were identified but not quantified.

that after five hours of heat treatment of an RC water extract at 80 °C, 68 % of the initial ACNs were retained. The authors described that the non-acylated anthocyanin was more prone to thermal degradation than the mono- and diacylated ACNs, which displayed similar stability. The higher temperature conditions (80 °C Vs 40 °C in our study) might explain the discrepancies with our results. In our hands, the degradation kinetics of ACNs seem to be more associated with specific patterns of acylation and glycosylation rather than being dependent on the mono or diacylation with HCAs. We can reasonably exclude that the degradation of ACNs might have been affected by polyphenol oxidases since the pH of the extract was out of the activity range of these enzymes, which have been described to catalyse the oxidation of phenolic compounds above pH 6.0 (Fujita, Binsaari, Maegawa, Tetsuka, Hayashi, & Tono, 1995). On the contrary, ascorbic acid, a potent antioxidant that is known to negatively affect ACNs stability, might have a role in explaining the different results obtained by Steingass et al. (2023) and our group. RC is very rich in ascorbic acid (Drozdowska et al., 2020), and due to its typical instability, this might have been degraded during the heating at 80 °C while still active at 40 °C in the first days of incubation (Herbig & Renard, 2017). In this sense, depending on the incubation temperature, it might contribute differently to the degradation of ACNs.

In addition to the results above, no trace of aglycone or specific deglycosylation phenomena has been observed along the treatment, suggesting that the degradation of the AACNs might have probably occurred via the cleavage of the core flavonoid heterocycle. Indeed, as described previously (Sun, Bai, Zhang, Liao, & Hu, 2011) for cyanidin-3-sophoroside, the degradation pathway is influenced by pH. In particular, a very low pH, i.e. 1.5, would first catalyse the deglycosylation and subsequently the fragmentation of the flavonoid to protocatechuic acid and phloroglucinaldehyde, while at pH 3.5, very close to our conditions, cyanidins are supposed to be first degraded to chalcones glycosides and later deglycosylated and fragmented. We cannot confirm that chalcones are crucial intermediates of RC ACNs degradation, not having targeted these molecules in our analysis. What is clear is that the degradation is accelerated when ACNs bear "unprotected" acyl residues.

4. Conclusions

This study represents the first formal analysis of the degradation kinetics of RC anthocyanins at accelerated conditions. Our results confirm that the thirteen analysed ACNs degrade following an apparent 1st order kinetics as described for other plant sources, even if the radical-scavenging capacity and ferric-reducing power of the extract followed different trends. The analysis of the kinetics revealed that although the stability of RC ACNs was, on the whole, greater than other plant sources, di-acylated ACNs, supposed to be more stable than the monoacylated and non-acylated forms, showed modest stability unless characterised by a glycosylated HCA. On the contrary, the stability seems to be inversely dependent on the radical scavenging capacity and, more in general, on the reactivity of the acyl group. Further studies focused on ACNs bearing glycosyl HCAs will help elucidate their stronger stability, considering also the possible role of other compounds like ascorbic acid and free HCAs as a function of temperature and time.

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CRediT authorship contribution statement

Laura De Marchi: Conceptualization, Investigation, Data curation. **Laura Salemi:** Investigation, Data curation. **Maria Bellumori:** Investigation, Writing – review & editing. **Roberto Chignola:** Formal analysis,

Data curation, Writing – review & editing. **Federica Mainente:** Data curation. **Diana Vanessa Santisteban Soto:** Investigation. **Ilaria Fierri:** Investigation, Writing – review & editing. **Marco Ciulu:** Writing – review & editing. **Gianni Zoccatelli:** Conceptualization, Supervision, Writing – original draft, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Zoccatelli Gianni reports financial support was provided by Regione del Veneto. The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.138272.

References

Bellumori, M., De Marchi, L., Mainente, F., Zanoni, F., Cecchi, L., Innocenti, M., Mulinacci, N., & Zoccatelli, G. (2021). A by-product from virgin olive oil production (pate) encapsulated by fluid bed coating: Evaluation of the phenolic profile after shelf-life test and in vitro gastrointestinal digestion. *International Journal of Food Science and Technology, 56*(8), 3773–3783. https://doi.org/10.1111/ijfs.15068

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Castaneda-Ovando, A., Pacheco-Hernandez, M. D., Paez-Hernandez, M. E., Rodriguez, J. A., & Galan-Vidal, C. A. (2009). Chemical studies of anthocyanins: A review. *Food Chemistry, 113*(4), 859–871. https://doi.org/10.1016/j.foodchem.2008.09.001

Chandrasekhar, J., Madhusudhan, M. C., & Raghavarao, K. S. M. S. (2012). Extraction of anthocyanins from red cabbage and purification using adsorption. *Food and Bioproducts Processing, 90*(C4), 615–623. https://doi.org/10.1016/j.fbp.2012.07.004

Chen, J. Y., Du, J., Li, M. L., & Li, C. M. (2020). *Degradation kinetics and pathways of red raspberry anthocyanins in model and juice systems and their correlation with color and antioxidant changes during storage* (p. 128). Lwt-Food Science and Technology.

de Beer, D., Pauck, C. E., Aucamp, M., Liebenberg, W., Stieger, N., van der Rijst, M., & Joubert, E. (2018). Phenolic and physicochemical stability of a functional beverage powder mixture during storage: Effect of the microencapsulant inulin and food ingredients. *Journal of the Science of Food and Agriculture, 98*(8), 2925–2934. https://doi.org/10.1002/jsfa.8787

Denish, P. R., Fenger, J. A., Powers, R., Sigurdson, G. T., Grisanti, L., Guggenheim, K. G., Laporte, S., Li, J. L., Kondo, T., Magistrato, A., Moloney, M. P., Riley, M., Rusishvili, M., Ahmadiani, N., Baroni, S., Dangles, O., Giusti, M., Collins, T. M., Didzbalis, J., Yoshida, K., Siegel, J. B., & Robbins, R. J. (2021). Discovery of a natural cyan blue: A unique food-sourced anthocyanin could replace synthetic brilliant blue. *Science Advances, 7*(15). https://doi.org/10.1126/sciadv.abe7871

Drozdowska, M., Leszczynska, T., Koronowicz, A., Piasna-Slupecka, E., & Dziadek, K. (2020). Comparative study of young shoots and the mature red headed cabbage as antioxidant food resources with antiproliferative effect on prostate cancer cells. *Rsc Advances, 10*(70), 43021–43034. https://doi.org/10.1039/d0ra07861a

Dyrby, M., Westergaard, N., & Stapelfeldt, H. (2001). Light and heat sensitivity of red cabbage extract in soft drink model systems. *Food Chemistry, 72*(4), 431–437. https://doi.org/10.1016/S0308-8146(00)00251-X

Fang, S., Lin, F. B., Qu, D. F., Liang, X. R., & Wang, L. P. (2019). Characterization of Purified Red Cabbage Anthocyanins: Improvement in HPLC Separation and Protective Effect against H2O2-Induced Oxidative Stress in HepG2 Cells. *Molecules, 24*(1). https://doi.org/10.3390/molecules24010124

Fenger, J. A., Moloney, M., Robbins, R. J., Collins, T. M., & Dangles, O. (2019). The influence of acylation, metal binding and natural antioxidants on the thermal stability of red cabbage anthocyanins in neutral solution. *Food & Function, 10*(10), 6740–6751. https://doi.org/10.1039/c9fo01884k

Fierri, I., De Marchi, L., Chignola, R., Rossin, G., Bellumori, M., Perbellini, A., Mancini, I., Romeo, A., Ischia, G., Saorin, A., Mainente, F., & Zoccatelli, G. (2023). Nanoencapsulation of Anthocyanins from Red Cabbage (Brassica oleracea L. var. Capitata f. rubra) through Coacervation of Whey Protein Isolate and Apple High Methoxyl Pectin. *Antioxidants (Basel), 12*(9). https://doi.org/10.3390/antiox12091757.

Fujita, S., Binsaari, N., Maegawa, M., Tetsuka, T., Hayashi, N., & Tono, T. (1995). Purification and Properties of Polyphenol Oxidase from Cabbage (Brassica-Oleracea L). *Journal of Agricultural and Food Chemistry, 43*(5), 1138–1142. https://doi.org/10.1021/jf00053a005

Ghareaghajlou, N., Hallaj-Nezhadi, S., & Ghasempour, Z. (2021). Red cabbage anthocyanins: Stability, extraction, biological activities and applications in food systems. *Food Chemistry, 365*. https://doi.org/10.1016/j.foodchem.2021.130482

Giusti, M. M., & Wrolstad, R. E. (2003). Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal, 14*(3), 217–225. https://doi.org/10.1016/S1369-703x(02)00221-8

Herbig, A. L., & Renard, C. M. G. C. (2017). Factors that impact the stability of vitamin C at intermediate temperatures in a food matrix. *Food Chemistry, 220*, 444–451. https://doi.org/10.1016/j.foodchem.2016.10.012

Husted, S., & Schjoerring, J. K. (1995). Apoplastic Ph and Ammonium Concentration in Leaves of Brassica-Napus L. *Plant Physiology, 109*(4), 1453–1460. https://doi.org/10.1104/pp.109.4.1453

Izzo, L., Rodriguez-Carrasco, Y., Pacifico, S., Castaldo, L., Narvaez, A., & Ritieni, A. (2020). Colon Bioaccessibility under In Vitro Gastrointestinal Digestion of a Red Cabbage Extract Chemically Profiled through UHPLC-Q-Orbitrap HRMS. *Antioxidants, 9*(10). https://doi.org/10.3390/antiox9100955

Katz, I. H., Nagar, E. E., Okun, Z., & Shpigelman, A. (2020). The Link between Polyphenol Structure, Antioxidant Capacity and Shelf-Life Stability in the Presence of Fructose and Ascorbic Acid. *Molecules, 25*(1). https://doi.org/10.3390/molecules25010225

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of Aoac International, 88*(5), 1269–1278. https://doi.org/10.1093/jaoac/88.5.1269

Mizgier, P., Kucharska, A. Z., Sokol-Letowska, A., Kolniak-Ostek, J., Kidon, M., & Fecka, I. (2016). Characterization of phenolic compounds and antioxidant and anti-inflammatory properties of red cabbage and purple carrot extracts. *Journal of Functional Foods, 21*, 133–146. https://doi.org/10.1016/j.jff.2015.12.004

Moloney, M., Robbins, R. J., Collins, T. M., Kondo, T., Yoshida, K., & Dangles, O. (2018). Red cabbage anthocyanins: The influence of D-glucose acylation by hydroxycinnamic acids on their structural transformations in acidic to mildly alkaline conditions and on the resulting color. *Dyes and Pigments, 158*, 342–352. https://doi.org/10.1016/j.dyepig.2018.05.057

Patras, A., Brunton, N. P., O'Donnell, C., & Tiwari, B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology, 21*(1), 3–11. https://doi.org/10.1016/j.tifs.2009.07.004

Sadilova, E., Carle, R., & Stintzing, F. C. (2007). Thermal degradation of anthocyanins and its impact on color and in vitro antioxidant capacity. *Molecular Nutrition & Food Research, 51*(12), 1461–1471. https://doi.org/10.1002/mnfr.200700179

Sadilova, E., Stintzing, F. C., & Carle, R. (2006). Thermal degradation of acylated and nonacylated anthocyanins. *Journal of Food Science, 71*(8), C504–C512. https://doi.org/10.1111/j.1750-3841.2006.00148.x

Sanchez, V., Baeza, R., & Chirife, J. (2015). Comparison of monomeric anthocyanins and colour stability of fresh, concentrate and freeze-dried encapsulated cherry juice stored at 38 degrees C. *Journal of Berry Research, 5*(4), 243–251. https://doi.org/10.3233/Jbr-150106

Steingass, C. B., Burkhardt, J., Baumer, V., Kumar, K., Mibus-Schoppe, H., Zinkernagel, J., Esquivel, P., Jimenez, V. M., & Schweiggert, R. (2023). Characterisation of acylated anthocyanins from red cabbage, purple sweet potato, and Tradescantia pallida leaves as natural food colourants by HPLC-DAD-ESI(+)-QTOF-MS/MS and ESI(+)-MS(n) analysis. *Food Chemistry, 416*, Article 135601. https://doi.org/10.1016/j.foodchem.2023.135601

Sun, J. X., Bai, W. B., Zhang, Y., Liao, X. J., & Hu, X. S. (2011). Identification of degradation pathways and products of cyanidin-3-sophoroside exposed to pulsed electric field. *Food Chemistry, 126*(3), 1203–1210. https://doi.org/10.1016/j.foodchem.2010.12.002

Wiczkowski, W., Szawara-Nowak, D., & Topolska, J. (2013). Red cabbage anthocyanins: Profile, isolation, identification, and antioxidant activity. *Food Research International, 51*(1), 303–309. https://doi.org/10.1016/j.foodres.2012.12.015

Wiczkowski, W., Szawara-Nowak, D., & Topolska, J. (2015). Changes in the content and composition of anthocyanins in red cabbage and its antioxidant capacity during fermentation, storage and stewing. *Food Chemistry, 167*, 115–123. https://doi.org/10.1016/j.foodchem.2014.06.087

Zanoni, F., Primiterra, M., Angeli, N., & Zoccatelli, G. (2020). Microencapsulation by spray-drying of polyphenols extracted from red chicory and red cabbage: Effects on stability and color properties. *Food Chemistry, 307*. https://doi.org/10.1016/j.foodchem.2019.125535