Research Article

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# UV-A treatment of phenolic extracts impacts colour, bioactive compounds and antioxidant activity

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## Abstract

**BACKGROUND: The unintended co-extraction of chlorophylls during the recovery of polyphenols from plant sources yields green-coloured phenolic extracts with limited use in colour-sensitive foods. This study aimed at decolourizing the ethanolic extracts of sugar beet leaves using a UV-A treatment (390 nm).**

**RESULTS: Exposure of the phenolic extracts to 30 UV-A LEDs at 8.64 J $m^{-2}$ radiation dose decreased the total chlorophyll content by 69.23% and reduced the greenness parameter ($-a^*$) significantly ($P < 0.05$) from 27.33 ± 0.32 to 8.64 ± 0.16. Additionally, UV-A treatment increased the content of most individual phenolic compounds (e.g. gallic acid, ferulic acid, etc.) significantly, resulting in an increase in the overall phenolic content in the extracts from 900.56 ± 14.11 μg $g^{-1}$ fresh weight (FW) to a maximum of 975.09 ± 9.62 μg $g^{-1}$ FW at 0.67 J $m^{-2}$. However, rutin content had a significant decrease at the highest radiation dose (8.64 J $m^{-2}$). The soluble sugar content (i.e. glucose and fructose) increased simultaneously with phenolic compounds after the UV-A treatment. Although the UV treatment reduced the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, it had no significant effect on the ferrous chelating activity and the extract's ability to delay lipid oxidation in corn oil. The antioxidant activity index of the treated extract was comparable to that of butylated hydroxytoluene, a synthetic antioxidant.**

**CONCLUSION: Key findings of this study include successful decolourization of the extract, decomposition of bound polyphenols to their free form, and maintaining the antioxidant activity of the extract in the oil system after UV-A exposure.**


*Supporting information may be found in the online version of this article.*

**Keywords:** chlorophylls; phenolic compounds; sugar beet leaves; ultraviolet; polyphenol extraction; natural antioxidants

## INTRODUCTION

Extracting polyphenols from plant leaves using organic solvents (e.g., ethanol, acetone, etc.) can result in the unintended co-extraction of chlorophylls. The presence of chlorophylls imparts a characteristic green colour to the obtained phenolic extracts, posing challenges to the utilization of the extract in the development of colour-sensitive food.[1-3] Moreover, chlorophyll-containing extracts can cause lipid photo-oxidation in food products due to the generation of singlet oxygen ($^1O_2$) by chlorophylls under light exposure.[4] In this regard, there is a spotlight on finding possible methods to either avoid the recovery of chlorophylls or remove the green colour of the extracts.

To address this issue, various decolourization techniques exist to absorb the chlorophylls present in the extracts, such as the sedimentation method,[5] activated charcoal, ChloroFiltr® cartridges and liquid–liquid partitioning.[4] However, these methods are expensive, difficult to perform, involve the use of harsh chemicals and may decrease the phenolic content of the extract.[6] This highlights the need for a straightforward, safe and cheap alternative to decolourize phenolic extracts. Since UV radiation can degrade chlorophylls and induce decolourization,[6-8] it could be a possible option as a treatment to remove the colour of phenolic extracts.

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Among different UV light categories, UV-A (315–400 nm) stands out as an affordable and environmentally friendly option with minimal impact on phenolic compounds and antioxidant activity compared to UV-B (280–315 nm) and UV-C (100–280 nm).[9-11] Thus, it remains an appropriate choice for decolourizing phenolic extracts with the least possible impact on bioactive compounds. Nonetheless, the effect of UV on chlorophylls has been evaluated most commonly on plant tissues,[7,12,13] and there has not been much attention to the potential of UV as a decolourization method of phenolic extracts. Furthermore, it is unclear whether the UV-A-treated extracts would still be able to act as antioxidants in real food systems, since light exposure may transfer the bioactive compounds to their excited state, inducing prooxidant activity in the extract.[14]

The research reported here aimed to use UV-A LEDs as a post-treatment to decolourize the phenolic extracts of sugar beet leaves as a case study. The decolourization efficiency was studied by evaluating the colorimetric properties and chlorophyll content. Additionally, the UV-A irradiation impact on the phenolic profile, soluble sugar content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferrous chelating activity of the extracts was investigated. The antioxidant activity of the UV-A-treated extracts was compared to that of untreated extracts and to that of a synthetic antioxidant, butylated hydroxytoluene (BHT), in corn oil as a real food system.

## MATERIALS AND METHODS

### Chemicals and materials

Sugar beet (*Beta vulgaris* L., var. SMART DJERBA-KWS) leaves were obtained from a local farm in Padova, Italy. All the chemicals were of analytical grade and bought from Sigma (St Louis, MO, USA). The corn oil was purchased from a local market in Padova, Italy.

### Ultrasound-assisted extraction of polyphenols

The phenolic extracts were obtained according to the method reported by Ebrahimi *et al.*[15] with some minor modifications. The extraction was carried out in triplicate at room temperature using an ultrasonic homogenizer (HD 2200.2, Bandelin, Berlin, Germany) equipped with a probe (titanium, diameter: 6 mm; KE 76, Bandelin, Berlin, Germany). First, 1 g of ground fresh sugar beet leaves was added to a centrifuge tube containing 10 mL of 70% ethanol. The ultrasonic probe was submerged to a depth of 2 mm in the resulting mixture, and the ultrasonication was performed for 10 min while the test tube was placed in an ice bucket to prevent overheating. The output power, frequency and amplitude were 0.2 kW, 20 kHz and 25%, respectively, with a pulsation occurring for 10 s every 60 s. The mixture obtained from ultrasonication was centrifuged at 4 °C and 4000 × *g* for 10 min. The supernatant was passed through Whatman No. 1 filter papers (GE Healthcare, Chalfont Saint Giles, UK) and cellulose acetate syringe filters (0.45 µm, Test Scientific, Perugia, Italy) and stored at −18 °C until the subsequent analyses.

### Treatment of extracts using UV-A LEDs

The phenolic extracts were treated using an illuminator comprising through-hole UV-A LEDs (UV5TZ-390-30, Bivar, Inc. Thomas, Irvine, CA, USA) with emission angle, wavelength and luminous intensity of 30°, 390 nm and 0.04 W, respectively. An amount of 2 mL of the extract in plastic Petri dishes (diameter: 35 mm, height: 11 mm; Thermo Fisher Scientific, Waltham, MA, USA) was exposed to UV-A irradiation with different numbers of LEDs, durations and voltages (Table 1) in triplicate at 25 °C. The illuminator was set at a distance of 0.5 cm from the extracts in a chamber to avoid visible light interference (Fig. 1). The intensity of irradiation in each condition was measured using a radiometer (ILT IL-1700, International Light Technology, MA, USA) to calculate the radiation dose in each exposure time using Eqn (1)[16]:

$$RD\left(\mathrm{Jm^{-2}}\right) = I\left(\mathrm{Wm^{-2}}\right) \times T(\mathrm{s}) \tag{1}$$

where RD is radiation dose, $I$ is intensity and $T$ is exposure time.

### Chlorophyll content of extracts

The concentration of chlorophylls was determined spectrophotometrically using the method described by Ebrahimi *et al.*[17] The contents of chlorophylls a and b were calculated using Eqns (2) and (3), respectively. The depletion of total chlorophylls was calculated based on the sum of chlorophylls a and b in each extract using Eqn (4).

$$\mathrm{chl_a}\left(\mu\mathrm{g\,g^{-1}}\right) = \frac{(13.36(A_{664.1} - A_{750}) - 5.19(A_{648.6} - A_{750})) \times V_{ex}(\mathrm{mL})}{W_{ex}(\mathrm{g})} \tag{2}$$

$$\mathrm{chl_b}\left(\mu\mathrm{g\,g^{-1}}\right) = \frac{(27.43(A_{648.6} - A_{750}) - 8.12(A_{664.1} - A_{750})) \times V_{ex}(\mathrm{mL})}{W_{ex}(\mathrm{g})} \tag{3}$$

$$\text{Depletion of total chlorophylls}(\%) = \frac{C_1}{C_0} \times 100 \tag{4}$$

where $\mathrm{chl_a}$ represents chlorophyll a, $\mathrm{chl_b}$ is chlorophyll b, $A$ is the absorbance at 664.1, 648.6 and 750 nm, $V_{ex}$ is the volume of solvent in the extraction, $W_{ex}$ is the weight of sample in

**Table 1.** The specifications of different conditions of UV-A treatment

| | UV-A irradiation condition | | Radiation dose (J m$^{-2}$) in each exposure time | | | | |
|---|---|---|---|---|---|---|---|
| Extract code | Number of UV-A LEDs | Voltage (V) | 10 min | 20 min | 30 min | 45 min | 60 min |
| L10 | 9 | 10 | 0.06 | 0.11 | 0.17 | 0.25 | 0.33 |
| L15 | 9 | 15 | 0.47 | 0.94 | 1.40 | 2.11 | 2.81 |
| L20 | 9 | 20 | 0.90 | 1.80 | 2.70 | 4.05 | 5.40 |
| H10 | 30 | 10 | 0.11 | 0.22 | 0.33 | 0.50 | 0.67 |
| H15 | 30 | 15 | 0.77 | 1.54 | 2.30 | 3.46 | 4.61 |
| H20 | 30 | 20 | 1.44 | 2.88 | 4.32 | 6.48 | 8.64 |

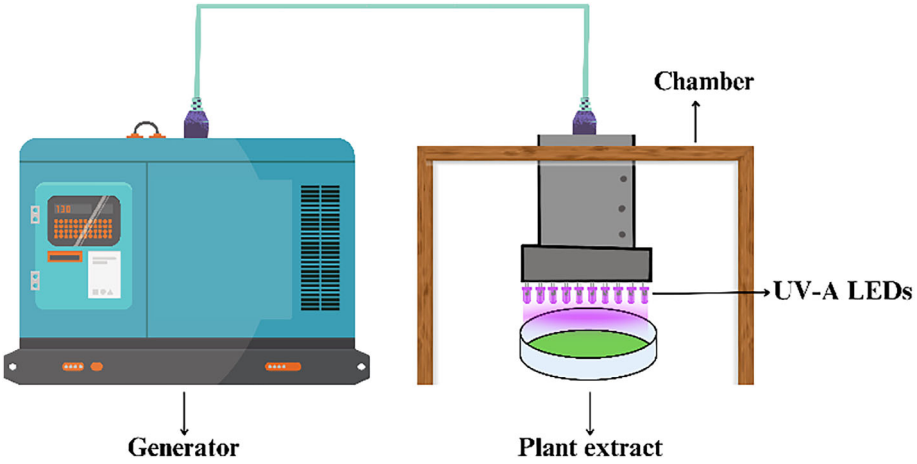


**Figure 1.** Schematic of the UV-A illuminator and exposure of extract to it.

the extraction, $C_0$ is the total chlorophyll content in the untreated extract and $C_1$ is total chlorophyll content in the UV-treated extract.

### Colorimetric properties of extracts

The colorimetric characteristics of the extracts were assessed according to Dal-Bó and Freire.[18] The CIELab parameters were measured with a D65 illuminant and a 10° observation angle using a Chroma Meter CR-300 colorimeter (Konica-Minolta, Tokyo, Japan). Before the trials, the device was calibrated on a standard white plate ($L^* = 84.1$, $a^* = 0.32$, $b^* = 0.33$; Konica-Minolta, Tokyo, Japan). The negative value of $a^*$ was considered the greenness parameter ($-a^*$).

### Calculation of kinetic rate constant (*k*) and half-life values

The kinetics of the decrease in green colour due to UV-A exposure was studied according to Nisha *et al.*[19] Based on the linear regression of the data, a first-order kinetic model (Eqn (5)) and a second-order kinetic model (Eqn (6))[20] were selected to calculate the reaction rate constant for the degradation of the $-a^*$ value and total chlorophylls, respectively:

$$\ln\frac{A_t}{A_0} = -kt \tag{5}$$

$$\frac{1}{C_t} = \frac{1}{C_0} + kt \tag{6}$$

where $A_t$ is the $-a^*$ value at time $t$, $A_0$ is the initial $-a^*$ value, $C_t$ is the total chlorophylls at time $t$, $C_0$ is the initial total chlorophylls, $k$ is the rate constant ($min^{-1}$) and $t$ is the UV-A exposure time (min).

The half-life values of $-a^*$ and chlorophyll degradation were calculated using Eqns (7) and (8), respectively[21]:

$$t_{1/2} = \frac{\ln 2}{k} \tag{7}$$

$$t_{1/2} = \frac{1}{k(C_0)} \tag{8}$$

where $t_{1/2}$ is the half-life, $C_0$ is the initial chlorophyll concentration and $k$ is the rate constant ($min^{-1}$).

### Phenolic profile of extracts

The individual phenolic compounds were detected using a high-pressure liquid chromatography (HPLC) system (1260 Infinity II Prime LC, Agilent, Santa Clara, CA, USA) equipped with a diode array detector and a Poroshell 120 EC-C18 column (2.7 μm i.d., 3.0 × 150 mm; Agilent, Santa Clara, CA, USA). The column and guard column were maintained at a constant temperature of 30 °C. Extracts and standards were eluted using a mobile phase composed of MilliQ water acidified with 0.1% formic acid (designated as solvent A) and acetonitrile acidified with 0.1% formic acid (designated as solvent B) at a flow rate of 0.3 mL $min^{-1}$. The solvent gradient, expressed in volumetric ratios of solvents A and B, was as follows: 15–40% B for 40 min and 40–100% B for 5 min. After each analysis, the column was conditioned for 10 min with the mobile phase composition set at 100% A. The injection volume was 1.5 μL, and the detection wavelengths used for monitoring phenolic compounds were 280, 310 and 370 nm. Stock solutions of standards (1000 mg $L^{-1}$) were prepared in ethanol. Calibration curves for each compound were established at concentrations ranging from 3 to 200 mg $L^{-1}$ to determine the final concentrations of detected phenolic compounds. The sum of the individual phenolic compounds was considered as the total phenolic content.

### DPPH radical scavenging activity of extracts

The DPPH radical scavenging activity of extracts was evaluated according to Danielski and Shahidi.[22] Inhibition of DPPH radical was determined using Eqn (9):

$$\text{Inhibition of DPPH radical}(\%) = \frac{A_0 - A_1}{A_0} \times 100 \tag{9}$$

where $A_0$ and $A_1$ represent the absorbance of the blank and the extracts, respectively, at 515 nm.

### Ferrous chelating activity of extracts

The ferrous ($Fe^{2+}$) chelating activity of the extracts was measured using the method described by Bayram *et al.*[23] with some modifications. The test mixture was 0.8 mL of sodium citrate buffer (pH 6.0, 0.1 mol $L^{-1}$), 0.2 mL of extract or 70% ethanol as blank, 60 μL of 10 $mmol^{-1}$ ferrous chloride solution and 60 μL of 50 mmol $L^{-1}$ ferrozine solution. After a 10 min incubation, the absorbance was measured at 562 nm. The inhibition of




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$Fe^{2+}$–ferrozine complex formation was calculated according to Eqn (10):

$$\text{Chelating activity}(\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (10)$$

where $A_0$ and $A_1$ represent the absorbance of the blank and the extracts, respectively.

### Detection of water-soluble sugars in extracts using HPLC

An HPLC method was used to measure the content of water-soluble sugars in the extracts to assess the effect of UV-A on the glycosidic phenolic compounds. Firstly, 5 mL of each phenolic extract was evaporated under a nitrogen flow and resuspended in 2.5 mL of 0.1 N sulfuric acid to extract glucose and fructose. The samples were shaken for 1 h at room temperature and then filtered with 0.22 μm cellulose acetate syringe filters (Test Scientific, Perugia, Italy). The analysis was performed using a Jasco HPLC apparatus (Jasco Corporation, Tokyo, Japan) equipped with a refractive index detector (RI-2031 Plus, Jasco Corporation, Tokyo, Japan). An Aminex HPX 87H column (ion exclusion column, i.d., 300 × 7.8 mm; Biorad, Hercules, CA, USA) was used to analyse the samples. The column temperature was controlled at 65 °C using a column compartment (Jasco CO-2060, Jasco Corporation, Tokyo, Japan). The elution of extracts and standards was performed under a 0.6 mL $min^{-1}$ flow rate using an isocratic pump (PU-2080 Plus, Jasco Corporation). The mobile phase was 0.0025 N sulfuric acid, and the injection volume of the sample was 20 μL. To calculate the final concentration of glucose and fructose, calibration curves of each standard were prepared using concentrations ranging from 0.25 to 4 mg $mL^{-1}$.

### Antioxidant activity of extracts in corn oil

To assess the antioxidant activity of the extracts in a real sample, corn oil was chosen as a model food due to its significant content of linoleic acid, rendering it susceptible to oxidation.[24] The extracts were added to the corn oil in a concentration of 10% (v/v). Another sample was prepared by adding 0.02% (w/v) of BHT to the corn oil as a benchmark. Then, the mixtures were homogenized at 1000 × *g* while in an ice bucket, using a homogenizer (T 25 digital ULTRA-TURRAX®, IKA-Werke GmbH and Co. KG, Staufen, Germany) for 5 min. The oxidative stability of the oils was analysed using the Rancimat method according to Cisneros-Yupanqui *et al.*[25] The oxidative stability was expressed as the induction period (IP) (h), and the antioxidant activity index (AAI) of extracts was calculated using Eqn (11):

$$\text{AAI} = \frac{\text{IPof oil with antioxidants}}{\text{IPof oil without antioxidants}} \quad (11)$$

### Statistical analysis

All the analyses were conducted in triplicate. The collected data were analysed using IBM SPSS Statistics (Version 20.0, SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was performed on the data, and Tukey's test was employed for comparisons, with significance and confidence levels set at 0.05 and 95%, respectively. A heatmap based on Pearson correlation was generated using Origin Pro 2022 (OriginLab, Northampton, MA, USA).

## RESULTS AND DISCUSSION

### Degradation of green colour in extracts

According to Fig. 2(a),(b), the contents of chlorophyll a and b in H20, after 60 min, were significantly lower than those of other samples ($P < 0.05$), showing the high decolourization efficiency of 30 UV-A LEDs at 20 V. This treatment condition resulted in the highest depletion rate in total chlorophyll content, decreasing 69.23% of the initial total chlorophyll content after 60 min (Fig. 2(d)). However, this degradation mostly happened in chlorophyll a, resulting in a decrease in its yield from 256.46 ± 0.49 to 29.23 ± 2.12 μg $g^{-1}$ in extract H20.

The decrease in chlorophyll content of the extract after the UV-A treatment in the present case is consistent with the results obtained by Yasuda *et al.*,[1] where they proved that UV-A can degrade the content of chlorophyll a in ethanolic solutions. This decrease may be attributed to the transfer of electrons surrounding the porphyrin ring in the core of chlorophylls, due to light exposure, leading to the instability of these compounds.[26] The light-induced electron migration in chlorophylls may cause their decomposition into smaller compounds and the formation of chlorophyll radicals. This can be explained by the generation of singlet oxygen ($^1O_2$) and hydroxyl radicals ($^{\bullet}OH$) because of photosensitive reactions.[27]

Photodecomposition of chlorophylls after UV irradiation may cause the generation of pheophytins, chlorophyllide and pheophorbide.[1] Not all these compounds have adverse effects, and they could be beneficial. For instance, pheophytins have antioxidant activity and can slow the rate of oil autooxidation, although they are less effective than chlorophylls.[28-30] Chlorophyllide, another chlorophyll derivative, also has notable antioxidant activity.[31]

Figure 2(c) shows the changes in the greenness parameter (−*a** value) among different extracts. The extract treated with 30 UV-A LEDs at 20 V had significantly lower greenness values than the others ($P < 0.05$), indicating successful chlorophyll degradation. The results demonstrate that higher UV radiation doses led to greater decolourization. Yasuda *et al.*[1] found that the colour of chlorophyll a dissolved in high ethanol concentrations (≥60%) rapidly changes from green to pale brown when exposed to UV-A (366 nm). The 70% ethanol used in this study likely contributed to similar results. Additionally, Yasuda *et al.*[1] reported that the decolourization rate is inversely proportional to ethanol concentration, highlighting the solvent's crucial role in chlorophyll a decolourization.

Photos of extracts exposed to different conditions of UV-A light are shown in Fig. 2(e),(f). The colour of untreated extracts (i.e. at time 0) was dark green before UV-A irradiation. However, the greenness of extracts treated with high radiation doses showed instability when treated with 30 UV-A LEDs, turning to light yellow. The unstable green colour of chlorophyll solutions under light has been previously reported by Wang *et al.*[32] In the supporting information, the changes in the *b** value (Fig. S1(a)), *L** value (Fig. S1(b)), the total colour difference (Fig. S1(c)) and carotenoid content (Fig. S2) in the extracts during the UV exposure period are provided.

Table 2 provides the rate constants (*k*), correlation coefficients ($R^2$) and half-lives ($t_{1/2}$) for the greenness parameter and total chlorophyll content under various conditions of UV-A exposure. The data indicate that higher voltage and an increased number of UV-A LEDs lead to higher *k* and shorter $t_{1/2}$. This suggests that chlorophyll degradation occurs more rapidly under intensive

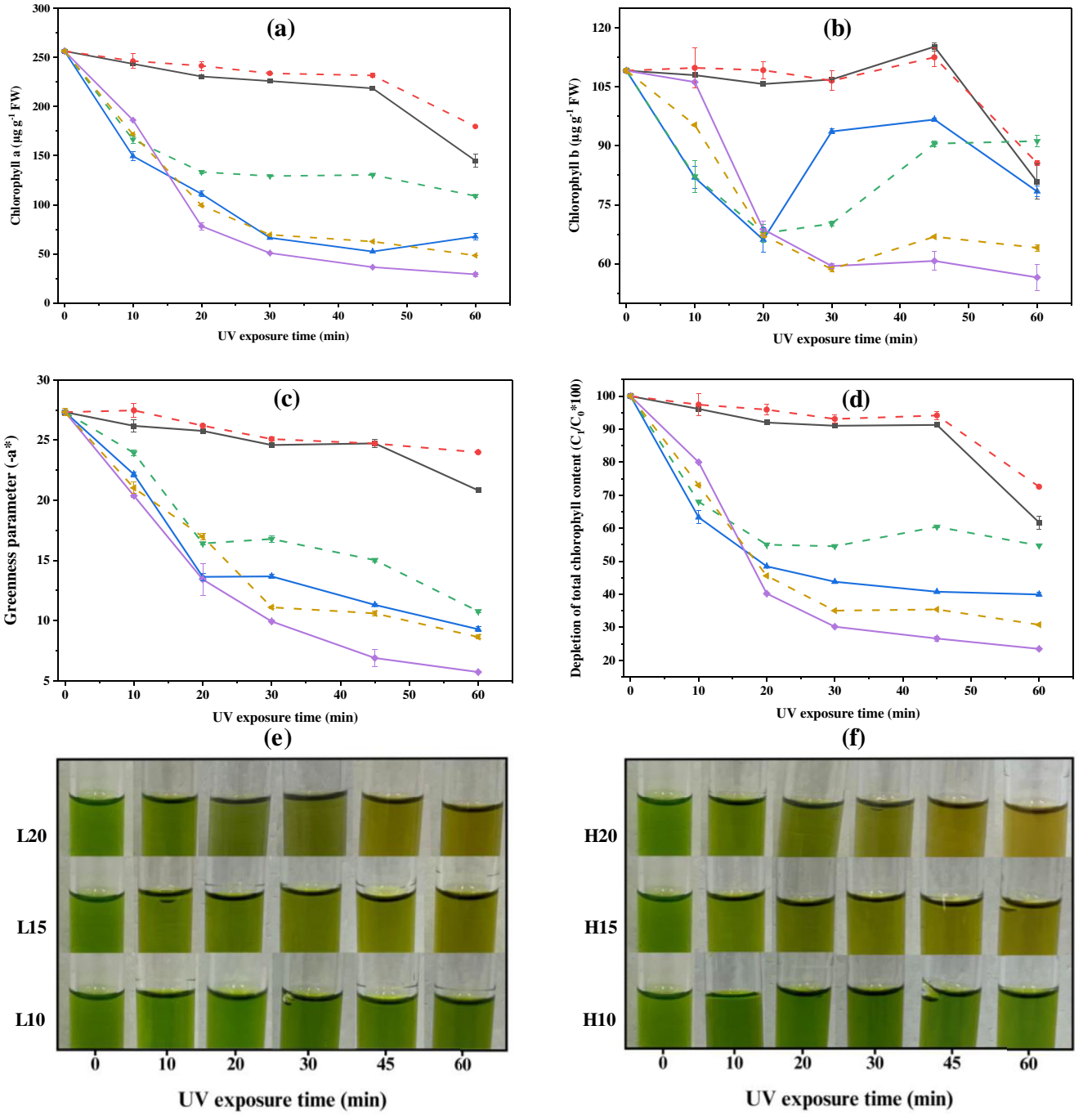


**Figure 2.** Changes in the content of chlorophyll a (a), chlorophyll b (b), greenness parameter ($-a^*$ value) (c), depletion of total chlorophyll content (i.e. sum of chlorophylls a and b) (d), and pictures of the extracts treated with 9 (e) and 30 (f) UV-A LEDs at different times and voltages. (●: L10, ▼: L15, ◀: L20, ■: H10, ▲: H15, ♦: H20.) Results are indicated as mean ± standard deviation ($n = 3$). Some error bars are beneath the data points. Dashed lines and solid lines show the UV-A treatment with 9 and 30 LEDs, respectively.

conditions. These findings are consistent with the observed downward trends in Fig. 2(c),(d), which illustrate the diminishing greenness and total chlorophyll content over time with increased UV-A exposure. This correlation between the experimental parameters and the degradation rates underscores the significant impact of UV-A intensity on chlorophyll stability.

Based on $-a^*$ values, the chlorophyll content and the kinetic data, only the extracts treated with 30 LEDs were chosen for assessing antioxidant activity, phenolic profile, etc., due to their superior efficiency in colour removal.

### Effect of UV-A on bioactive compounds in extracts

UV radiation can impact the content of phenolic compounds,[9] making it important to evaluate the phenolic profile, and corresponding antioxidant activity, of the extracts after the UV treatment. Table 3 presents phenolic composition, DPPH radical

**Table 2.** Reaction rate constants ($k$), correlation coefficients ($R^2$) and half-lives ($t_{1/2}$) for greenness parameter and total chlorophyll content in the extracts

| | Greenness parameter ($-a^*$)[a] | | | Total chlorophyll content[b] | | |
|---|---|---|---|---|---|---|
| Extract code | $k$ (min$^{-1}$) | $R^2$ | $t_{1/2}$ (min) | $k$ (min$^{-1}$) | $R^2$ | $t_{1/2}$ (min) |
| L10 | $2.40 \times 10^{-3}$ | 0.93 | 288.81 | $1.81 \times 10^{-5}$ | 0.95 | 151.12 |
| L15 | $1.44 \times 10^{-2}$ | 0.92 | 48.14 | $7.78 \times 10^{-5}$ | 0.88 | 35.16 |
| L20 | $1.93 \times 10^{-2}$ | 0.93 | 35.91 | $1.02 \times 10^{-4}$ | 0.96 | 26.82 |
| H10 | $4.52 \times 10^{-3}$ | 0.98 | 153.35 | $2.96 \times 10^{-5}$ | 0.96 | 92.41 |
| H15 | $1.74 \times 10^{-2}$ | 0.90 | 39.84 | $6.75 \times 10^{-5}$ | 0.93 | 40.52 |
| H20 | $2.69 \times 10^{-2}$ | 0.97 | 25.77 | $1.47 \times 10^{-4}$ | 0.95 | 18.61 |

[a] Reaction follows first-order kinetics.
[b] Reaction follows second-order kinetics.

**Table 3.** Phenolic profile, DPPH radical scavenging and ferrous chelating activity of different extracts before and after UV-A exposure with 30 LEDs for 60 min

| | | | Content (μg g$^{-1}$ FW) | | | |
|---|---|---|---|---|---|---|
| Component | Detection $\lambda$ (nm) | Retention time (min) | Untreated extract | H10 | H15 | H20 |
| Gallic acid | 280 | 7.468 | 4.85 ± 0.04$^{a}$ | 6.05 ± 0.03$^{b}$ | 6.42 ± 0.03$^{c}$ | 6.99 ± 0.28$^{d}$ |
| Protocatechuic | 280 | 9.341 | 11.01 ± 0.03$^{a}$ | 11.06 ± 0.02$^{ab}$ | 11.07 ± 0.02$^{b}$ | 11.07 ± 0.03$^{b}$ |
| (−)-Epigallocatechin | 280 | 10.163 | 35.29 ± 0.69$^{a}$ | 37.27 = ± 0.40$^{b}$ | 39.45 ± 0.20$^{c}$ | 42.22 ± 0.20$^{d}$ |
| Catechin | 280 | 11.023 | 38.97 ± 0.14$^{a}$ | 40.19 ± 0.12$^{b}$ | 40.78 ± 0.24$^{c}$ | 41.85 ± 0.12$^{d}$ |
| Hydroxybenzoic acid | 280 | 11.298 | 6.03 ± 0.30$^{a}$ | 6.82 ± 0.09$^{b}$ | 6.91 ± 0.18$^{b}$ | 7.26 ± 0.18$^{b}$ |
| Vanillic acid | 280 | 12.473 | 13.90 ± 0.63$^{a}$ | 15.48 ± 0.13$^{b}$ | 14.39 ± 0.32$^{a}$ | 14.39 ± 0.32$^{a}$ |
| Syringic acid | 280 | 12.854 | 7.19 ± 0.06$^{a}$ | 7.47 ± 0.03$^{b}$ | 7.35 ± 0.03$^{b}$ | 7.16 ± 0.03$^{a}$ |
| Epicatechin | 280 | 13.027 | 16.82 ± 1.41$^{a}$ | 20.36 ± 0.28$^{b}$ | 17.98 ± 0.42$^{a}$ | 16.16 ± 0.28$^{a}$ |
| (−)-Epigallocatechin-3-gallate | 280 | 13.366 | 244.75 ± 15.65$^{a}$ | 276.04 ± 8.94$^{b}$ | 270.45 ± 1.12$^{ab}$ | 253.69 ± 11.18$^{ab}$ |
| *m*-Coumaric acid | 280 | 20.158 | 64.99 ± 1.49$^{a}$ | 73.26 ± 1.01$^{b}$ | 77.23 ± 0.34$^{c}$ | 84.54 ± 1.22$^{d}$ |
| *o*-Coumaric acid | 280 | 23.616 | 36.10 ± 0.47$^{a}$ | 36.89 ± 0.46$^{a}$ | 37.13 ± 0.57$^{a}$ | 36.83 ± 0.05$^{a}$ |
| Chlorogenic acid | 310 | 10.801 | 14.04 ± 0.10$^{a}$ | 14.72 ± 0.09$^{b}$ | 14.71 ± 0.09$^{b}$ | 15.07 ± 0.09$^{c}$ |
| Caffeic acid | 310 | 12.527 | 5.32 ± 0.18$^{a}$ | 5.73 ± 0.07$^{b}$ | 6.25 ± 0.03$^{c}$ | 6.66 ± 0.03$^{d}$ |
| *p*-Coumaric acid | 310 | 17.088 | 7.25 ± 0.04$^{a}$ | 7.27 ± 0.01$^{a}$ | 7.25 ± 0.07$^{a}$ | 7.27 ± 0.01$^{a}$ |
| Ferulic acid | 310 | 19.129 | 41.64 ± 0.35$^{a}$ | 46.49 ± 0.15$^{b}$ | 48.36 ± 0.19$^{c}$ | 50.35 ± 0.65$^{d}$ |
| Quercetin-3-*O*-rutinoside (rutin) | 370 | 19.474 | 328.71 ± 9.35$^{bc}$ | 346.31 ± 1.72$^{c}$ | 311.68 ± 11.30$^{ab}$ | 298.17 ± 6.14$^{a}$ |
| Quercetin | 370 | 30.775 | 23.70 ± 0.04$^{a}$ | 23.68 ± 0.01$^{a}$ | 23.65 ± 0.00$^{a}$ | 23.67 ± 0.01$^{a}$ |
| Total phenolic content (SUM) | — | — | 900.56 ± 14.11$^{a}$ | 975.09 ± 9.62$^{c}$ | 941.08 ± 12.02$^{b}$ | 923.35 ± 4.35$^{ab}$ |
| DPPH radical scavenging activity (%) | — | — | 35.02 ± 1.15$^{c}$ | 27.70 ± 1.21$^{b}$ | 24.53 ± 0.65$^{ab}$ | 23.54 ± 3.11$^{a}$ |
| Ferrous chelating activity (%) | — | — | 36.42 ± 1.95$^{a}$ | 39.75 ± 1.51$^{a}$ | 42.71 ± 6.98$^{a}$ | 39.91 ± 4.87$^{a}$ |

Results are reported as mean ± standard deviation (n = 3).
Different characters in each row show a significant difference ($P \leq 0.05$) among the samples, as assessed by one-way ANOVA and Tukey's honestly significant difference tests.

scavenging activity and ferrous chelating activity of extracts before and after UV-A treatment with 30 LEDs for 60 min. The evaluated phenolic compounds were selected based on the most abundant phenolic compounds in sugar beet leaves according to the literature.[33-36]

The content of individual phenolic compounds in most cases increased significantly after UV-A exposure ($P < 0.05$). This increase may be attributed to the effect of UV-A treatment on the scission of glycoside bonds in the soluble conjugate forms of polyphenols (i.e. glycosides). These forms of phenolic compounds are bound to carbohydrates through glycosidic bonds.[37,38] Therefore, a simultaneous increase in the content of carbohydrates is also expected after UV-A treatment. This was confirmed by the significant increase in the content of soluble sugars (i.e. glucose and fructose; Fig. 3) in the UV-A-treated extracts. Another possible reason for the increase in the content of the phenolic compounds could be the upregulation of genes associated with their biosynthesis due to UV treatment.[39]

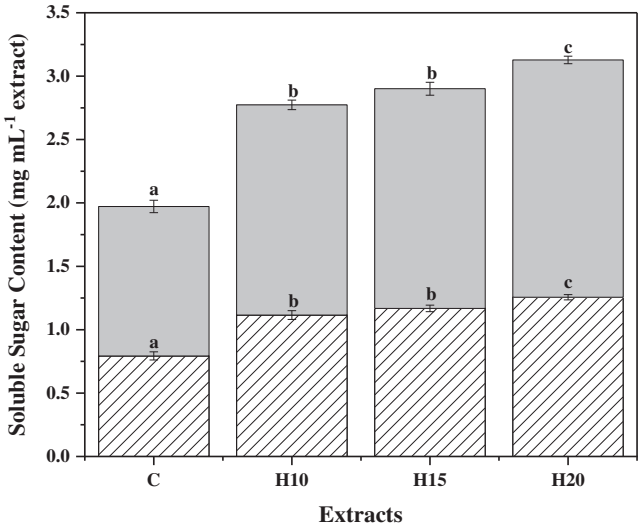


**Figure 3.** Glucose and fructose content in the different extracts before and after UV treatment for 60 min. The hatched white and grey bars show the content of glucose and fructose, respectively. Results are indicated as mean ± standard deviation ($n = 3$). Different characters show a significant difference ($P \leq 0.05$) in each compound, as assessed by one-way ANOVA and Tukey's honestly significant difference tests.

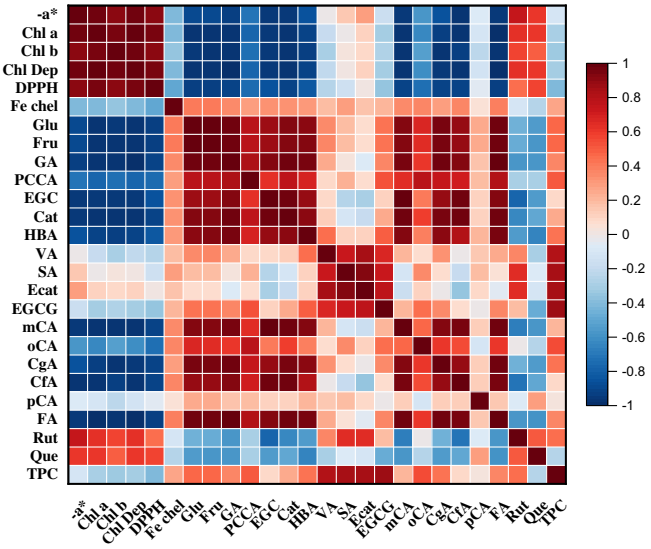


**Figure 4.** Heatmap showing the Pearson correlation among greenness parameter, chlorophyll depletion, DPPH radical scavenging activity, ferrous chelating activity and phenolic contents. The darker the colour, the more significant the correlation. Chl a: chlorophyll a; Chl b: chlorophyll b; Chl Dep: depletion of total chlorophyll content; DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; Fe chel: ferrous chelating activity; Glu: glucose; Fru: fructose; GA: gallic acid; PCCA: protocatechuic; EGC: (−)-epigallocatechin; Cat: catechin; HBA: hydroxybenzoic acid; VA: vanillic acid; SA: syringic acid; Ecat: epicatechin; EGCG: (−)-epigallocatechin-3-gallate; mCA: *m*-coumaric acid; oCA: *o*-coumaric acid; CgA: chlorogenic acid; CfA: caffeic acid; pCA: *p*-coumaric acid; FA: ferulic acid; Rut: rutin; Qu: quercetin; TPC: total phenolic content.

Among the polyphenols detected, (−)-epigallocatechin (EGC), catechin, epicatechin (EC), (−)-epigallocatechin-3-gallate (EGCG), quercetin-3-*O*-rutinoside (rutin) and quercetin belong to the flavonoids group. It is reported by Smith *et al.*[40] that flavonoids are susceptible to being degraded upon exposure to UV light. The content of EGCG and EC initially increased significantly with lower radiation doses and then decreased again with longer UV-A treatments. The photolysis of EC via UV light leads to the cleavage of the ether bond within the heterocyclic ring of flavan-3-ols, facilitating the conversion of catechin to its epimerized form[41]. Moreover, since EGCG is a galloylated catechin, the observed decrease in its content under the highest dosage of UV-A exposure could be attributed to the cleavage of the bond between catechin and gallic acid. This cleavage likely results in a significant increase in the content of these individual compounds in the extracts.[42] Therefore, the observed increase in the content of catechin and gallic acid after UV-A treatment can be justified.

Among the detected flavonoids in the present research, rutin content decreased significantly ($P < 0.05$) in high radiation dosages of UV-A treatment. Scheepers[6] reported that 98.6% of rutin remains in extracts after UV exposure. This is consistent with the results obtained in the present research. The extent of photoreactivity of flavonoids depends on the positions of the hydroxyl group substitution on the aromatic nucleus (B-ring). The presence of the 3-OH group on the B-ring can decrease the photostability of phenolic compounds significantly.[40] Thus, the presence of a 3-OH group in the structure of rutin likely accounts for the significant decrease in its content.

The extract treated at 10 V with 30 UV-A LEDs had the highest total phenolic content. However, the ferrous chelating activity of the extracts did not change significantly, and the DPPH radical scavenging activity decreased after UV exposure. The reason for the decrease in the antioxidant activity of extracts could be the degradation of chlorophylls due to UV-A treatment. Various studies have shown that chlorophylls have antioxidant effects and are capable of inactivating the oxidant compounds.[43] While the exposure of chlorophylls to light can indeed result in the generation of singlet oxygen,[4] it is important to note that the lifetimes of singlet states are extremely brief, and the likelihood of bimolecular encounters with oxygen in homogeneous solutions, such as ethanolic extracts, is low.[40]

### Correlation among different parameters

Figure 4 presents a heatmap illustrating the correlations among various extract properties, as determined by Pearson correlation analysis. The heatmap reveals a significant positive correlation between glucose and fructose content and several phenolic compounds, including gallic acid, catechin, EGC, hydroxybenzoic acid, *m*-coumaric acid, chlorogenic acid, caffeic acid and ferulic acid. The phenolic compounds which significantly affected the total phenolic content, after the UV treatment, were vanillic acid, syringic acid, EC and EGCG. Additionally, a significant positive correlation is observed between the depletion of chlorophyll content and DPPH radical scavenging activity, indicating that decreased chlorophyll levels correspond to lower radical scavenging activity.

As depicted in the heatmap, there is a significant negative correlation between chlorophyll content and all phenolic compounds with increased concentrations, which means that the degradation of chlorophylls in the extract may indirectly lead to the generation of more phenolic compounds. As mentioned previously, the exposure of chlorophylls to UV-A may induce the generation of singlet oxygen, a reactive oxygen species (ROS).[44] The generated ROS could readily oxidize molecules with which it comes into contact. The oxidative stress induced by singlet oxygen exposure initiates a cascade of biochemical events within the extract, ultimately leading to the synthesis and accumulation of secondary metabolites such as phenolic compounds. One of




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the primary consequences of oxidative stress induced by UV exposure is the activation of defence mechanisms within the extract to balance the excess ROS produced. In response to the presence of ROS, the extract may initiate the activity of various enzyme systems to counteract the oxidative damage. These enzymes could be responsible for the production of phenolic precursors or enhance the activity of enzymes involved in the conversion of these precursors into active phenolic compounds.[45,46]

### Oxidative stability of extract-added corn oil

The integration of external antioxidants into food systems is often essential, particularly when food producers aim to create healthier products rich in vulnerable unsaturated fatty acids.[47] In this study, the oxidative stability of extract-added corn oil was measured based on the formation of volatile secondary oxidation compounds. The volatile oxidation products, generated due to high temperature and airflow in the Rancimat method, cause a breakpoint in the plotted curve of conductivity. The time in which this breakpoint happens is called the induction period. The shorter the induction period, the faster the lipid oxidation.[48]

Figure 5 shows the induction period of oxidation in the corn oils and the AAI of different extracts. The induction period in all the samples enriched with the treated/untreated extracts increased significantly ($P < 0.05$). This increase could be because of the rich phenolic content of the added extracts. Polyphenols exhibit antioxidant activity through multiple pathways, including scavenging free radicals, chelating metal ions (e.g. $Fe^{2+}$, $Cu^{2+}$) and inhibiting oxidative enzymes.[49,50] A similar increasing trend in the oxidative stability of corn oil using other plant phenolic extracts has been reported in the literature.[51-53]

Although the DPPH radical scavenging activity of the extracts decreased after UV treatment, there were no significant differences among the AAI of untreated and UV-treated extracts in the corn oil. This inconsistency might be attributed to the distinct environments in which the antioxidant scavenges the free radicals, with the DPPH test conducted in ethanol and the Rancimat test in the oil system.[17,54] Moreover, the increased solubility of polyphenols in the oil could be a possible reason for their maintained antioxidant activity in the UV-treated samples, despite the chlorophyll-removal process eliminating the antioxidant effect of chlorophylls. According to the phenolic profile (Table 3) and soluble sugar content (Fig. 3) of the extracts, UV-A treatment increased the content of free polyphenols by converting them from glycoside forms (water-soluble) to aglycone forms (lipid-soluble). Enhanced solubility in lipid environments, such as oil systems, can improve the performance of antioxidants.[55]

The Rancimat test results confirmed that the antioxidant activity of the extracts did not diminish after colour removal using UV-A. The stable antioxidant activity index of the extracts after colour removal implies that the UV-A treatment not only effectively reduces the chlorophyll content but also preserves the antioxidant properties. This is important because the addition of chlorophylls to edible oils can be considered fraudulent and adulteration,[56,57] as a green-coloured phenolic extract can alter the visual characteristics of the oil.

Moreover, the results show that both UV-treated and untreated extracts are comparable to 0.02% (w/v) of BHT, which is the maximum permissible level established by Codex Alimentarius.[58] This highlights that the decoloured extract obtained from sugar beet leaves could be a potential substitute for synthetic antioxidants, which are proven to have many adverse effects on consumer health.

## CONCLUSION

Utilizing UV-A technology as a post-treatment to eliminate the green colour from chlorophyll-containing phenolic extracts opens a new window for improving the quality of extracts for use in real food systems. This study demonstrates that UV-A treatment can effectively eliminate the colour of chlorophylls from plant extracts without adversely impacting their antioxidant activity in food systems. The ability of the decoloured extract to enhance the oxidative stability of food systems suggests a promising potential for substituting synthetic antioxidants with natural extracts, thereby supporting the development of clean-label food products.

However, finding the optimal UV-A dosage and applicability of the proposed method to other plant extracts with different profiles of bioactive compounds requires further study. Additionally, excessive UV-A treatment to remove all chlorophyll content may negatively impact the phenolic content of extracts. The findings

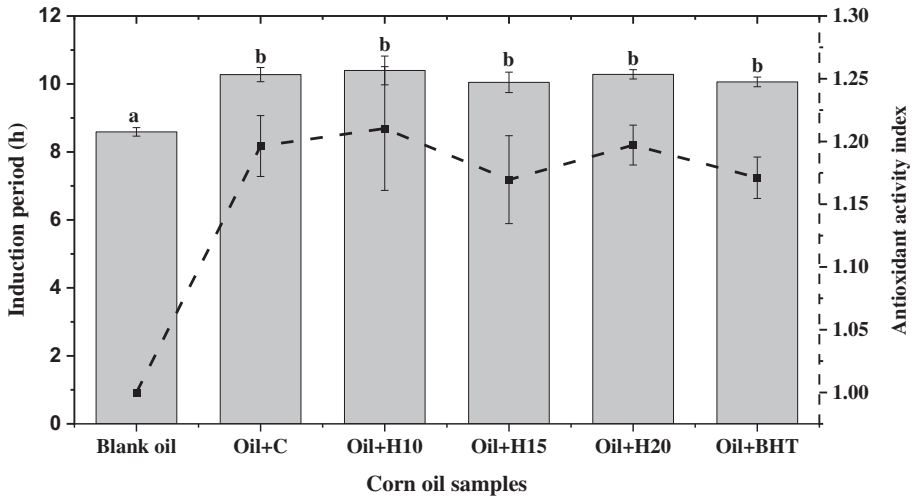


**Figure 5.** Induction period and antioxidant activity index of corn oil with and without extracts (10% v/v) or BHT (0.02% w/v). Bars show the induction period and the dashed line shows the antioxidant activity index. Results are indicated as mean ± SD ($n = 3$). Different characters show a significant difference ($P \leq 0.05$) among the samples, as assessed by one-way ANOVA and Tukey's honestly significant difference tests.






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of this study could serve as a foundation for further investigation into the mechanisms underlying the decolourization of phenolic extracts under light.

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## CONFLICT OF INTEREST

The authors assert that they have no conflicts of interest that could potentially influence the work reported in this article.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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