Received: 22 May 2024 | Revised: 2 July 2024 | Accepted: 29 July 2024

DOI: 10.1002/aocs.12891

ORIGINAL ARTICLE

AOCS WILEY

Antioxidant and prooxidant activity of acid-hydrolyzed phenolic extracts of sugar beet leaves in oil-in-water emulsions

Peyman Ebrahimi[1] | **Ipek Bayram**[2] | **Anna Lante**[1] | **Eric A. Decker**[2]

[1]Department of Agronomy, Food, Natural Resources, Animals, and Environment—DAFNAE, University of Padova, Legnaro, Italy

[2]Department of Food Science, University of Massachusetts, Amherst, Massachusetts, USA

Correspondence
Anna Lante, Department of Agronomy, Food, Natural Resources, Animals, and Environment—DAFNAE, University of Padova, Viale dell'Università, 16, 35020 Legnaro, Italy.
Email: anna.lante@unipd.it

Funding information
Fondazione Cassa di Risparmio di Padova e Rovigo (CARIPARO)

Abstract

This study aimed to enhance the oxidative stability of soybean oil-in-water emulsions using acid-hydrolyzed and unhydrolyzed extracts obtained from sugar beet leaves. The optimum extraction process, which includes 8 min of ultrasonication followed by a 2-h acid hydrolysis, released new phenolics (e.g., catechin, myricetin, etc.) and increased the total phenolic content (TPC) from 586.24 ± 11.45 to 982.42 ± 6.61 μmol gallic acid equivalent (GAE)/L, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition from 46.63 ± 1.39 to 60.87 ± 1.12%. Acid hydrolysis increased the cupric chelating activity of the extracts while decreasing ferrous chelating activity and *trans*-ferulic acid concentration significantly ($p < 0.05$). The acid-hydrolyzed extract at a TPC of 100 μmol GAE/L prolonged the lag phase of hexanal accumulation in the emulsion from 0 to 8 days, while 400 μmol GAE/L TPC of unhydrolyzed extract increased the lag phase to 12 days. The results show that acid-hydrolyzed extracts in high concentrations may act as prooxidants.

KEYWORDS
acid hydrolysis, extraction process, glycosidic phenolic compounds, oxidative stability, polyphenols, soybean oil

INTRODUCTION

Lipid oxidation is a prevalent problem in emulsion-based foods caused by oxygen, light, and heat exposure. This process produces volatile compounds and fatty acid decomposition products, resulting in unpleasant flavors and reduced food quality (Inchingolo et al., 2021). To address this issue, developing antioxidant technologies, such as natural phenolic extracts, is crucial (Tian et al., 2022). Therefore, there is a pressing need to find plant sources abundant in natural antioxidants, particularly polyphenols, to combat lipid oxidation effectively.

The leaves of the sugar beet plant (*Beta Vulgaris* L.), often considered food by-products, are rich in polyphenols, making them a promising source for extracting these compounds (Ebrahimi et al., 2022; Nutter et al., 2020). However, not all these polyphenols can be recovered in free or aglycone form, and they may be extracted in soluble (e.g., glycosides) and insoluble bound forms (Li et al., 2020). Since the existence of polyphenols in glycoside form can limit their antioxidant activity, finding an extraction process that efficiently converts glycosides into aglycones is essential (Tu et al., 2017).

Ultrasound-assisted extraction (UAE) utilizes ultrasound waves to induce mechanical vibrations, disrupting cell walls for the effective extraction of polyphenols. However, UAE may not always liberate the phenolic compounds in their free form (Đurović et al., 2018). Therefore, it is crucial to perform a post-treatment like acid hydrolysis to release the aglycone form of phenolic compounds. Acid hydrolysis combines high temperatures (62–90°C) and high acid concentrations (HCl) at different durations (15 min to 24 h) to release the aglycones and simple sugars from the structure of glycosidic phenolic compounds (Martins et al., 2023). To date, several studies have addressed the extraction and isolation

of polyphenols from sugar beet leaves (Ebrahimi et al., 2022; Maravić et al., 2022); however, no research has investigated how acid hydrolysis affects the antioxidant characteristics of these extracts in a real food system.

This research aims to determine whether acid hydrolysis treatment enhances the antioxidant activity of the extract or induces prooxidant activity within oil-in-water emulsions. The emulsions were created using soybean oil, which is highly susceptible to lipid oxidation as it has more than 50% linoleic acid (18:2) content (Cherif & Slama, 2022). The extraction process and acid hydrolysis condition were optimized based on the total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the extracts. Then, the chlorophyll content, phenolic composition, and metal chelating activity of the optimum acid-hydrolyzed and unhydrolyzed extracts were evaluated to ensure the capacity of the extracts as a possible antioxidant. The formation of the primary (lipid hydroperoxides) and secondary (headspace hexanal) products of lipid oxidation in the emulsions was measured during the storage time.

MATERIALS AND METHODS

Chemicals and materials

The soybean oil used for preparing the emulsions contained 14.29% saturated, 21.43% monounsaturated, and 57.14% polyunsaturated fatty acids and was purchased from a retail store in Hadley, MA, USA. The leaves used in the extraction were of a 10-month-aged sugar beet plant (*Beta vulgaris* L., var. SMART DJERBA-KWS) granted by a local farm in Padova, Italy. All the chemicals used were of analytical grade. Methanol, ammonium thiocyanate, hydrochloric acid, 1-butanol, isooctane, isopropanol, and hexane were purchased from Fisher Scientific (Fair Lawn, NJ, USA), and all the other chemicals were bought from Sigma-Aldrich (St. Louis, MO, USA).

Ultrasound-assisted extraction

The extraction of polyphenols was performed according to Ebrahimi et al. (2022) with some minor modifications. An ultrasound apparatus equipped with a titanium probe (FB 505, Fisher Scientific, PA, USA) with 500 W output power, 20% amplitude, and 20 kHz frequency was utilized for UAE. The probe was immersed 2 cm into a mixture of 0.4 g freeze-dried sugar beet leaf powder and 10 mL of 75% ethanol. Extraction was carried out in different durations (2, 4, 6, 8, 10 min) to find the optimum time. During the ultrasonication, the extracting cell was positioned in a beaker containing ice to avoid elevated temperatures. The UAE was followed by a 10-min centrifugation at 4000×*g*. The extraction was performed in 2 batches, the obtained supernatants were combined, and the volume was adjusted to 20 mL using 75% ethanol. The extracts were stored at −80°C until further steps.

Acid hydrolysis post-treatment

To hydrolyze the glycosidic phenolic compounds present in the optimum phenolic extract obtained in Section 2.2, an acid hydrolysis post-treatment (Figure 1a) was carried out as described by Ebrahimi et al. (2024). The hydrolysis was performed in different durations (1, 2, and 3 h) in a reflux vessel (Figure 1b), which included a condenser attached to a round-bottom flask containing 10 mL of ethanolic extract, 2.5 mL of distilled water, and 2.5 mL of 3.6 M HCl. Mixing the extract with HCl and water resulted in final HCl and ethanol concentrations of 0.6 M and 50%, respectively. The round-bottom flask was positioned in a beaker filled with water on a magnetic stirrer hotplate for controlling the temperature at 80°C and continuous stirring. After the hydrolysis, the HCl was neutralized with 2.5 mL of 3.6 M NaOH. The final hydrolyzed extracts were filtered using Whatman No. 1 filters and then stored at −80°C until the following analyses. Considering the combined volume of HCl, NaOH, and water added to the extract in this step, a 1.75 dilution factor was applied to the results of analytical tests for the acid-hydrolyzed extract.

Total phenolic content, chlorophyll and carotenoid content and radical scavenging activity of extracts

The Folin–Ciocalteu method was employed to determine the TPC of extracts spectrophotometrically (Azuma et al., 1999). A calibration curve ($R^2 = 0.99$) prepared with different concentrations of gallic acid (0–750 μM) was used to express the results as μmol of gallic acid equivalent per L of extract (μmol GAE/L).

Moreover, the DPPH radical scavenging activity of extracts was assessed according to Ebrahimi et al. (2022). The inhibition of DPPH radical was calculated based on Equation (1).

$$\text{Inhibition of DPPH radical}\,(\%) = \frac{(A_0 - A_1)}{A_0} \times 100 \quad (1)$$

where A_0 is the absorbance of the blank, and A_1 is the absorbance of the extracts at 517 nm.

The content of chlorophylls (i.e., chlorophyll a and b) and carotenoids in the extracts was determined spectrophotometrically according to Lichtenthaler and Buschmann (2001).

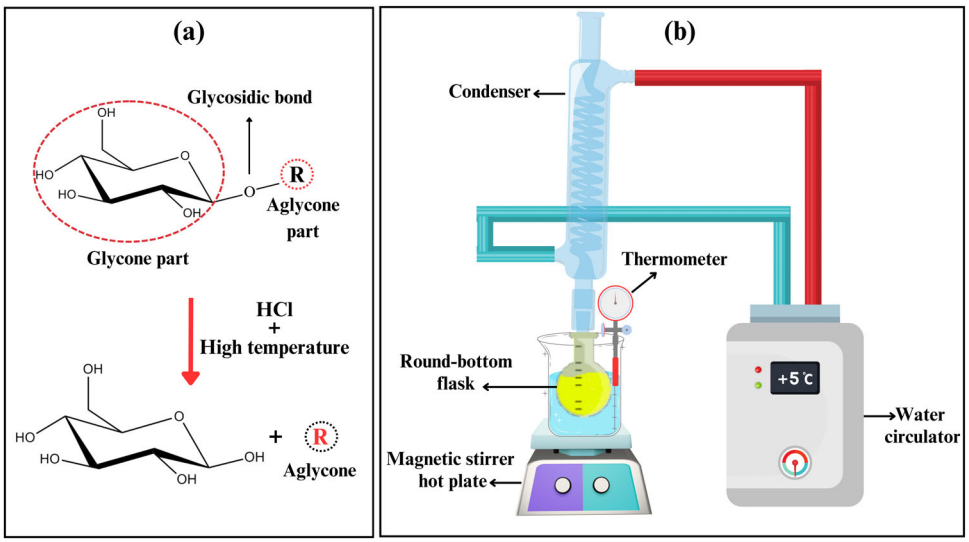

FIGURE 1 (a) The chemical reaction involved in the acid hydrolysis of glycosidic phenolic compounds, (b) Schematic of the reflux vessel used for acid hydrolysis of phenolic extracts.

Analysis of individual phenolic compounds

The aglycones in the optimum unhydrolyzed and acid-hydrolyzed extracts were identified using the HPLC method described by Ebrahimi et al. (2024). The analysis was performed using an HPLC-UV–Vis photodiode array detector apparatus (LC-2030C 3D, Shimadzu, Tokyo, Japan) equipped with an Ascentis Express 90 Å 18 reversed-phase column (5 µm i.d., 250 × 4.6 mm; Supelco, Bellefonte, PA, USA). The stock solutions of standards, including gallic acid, *p*-coumaric acid, *trans*-ferulic acid, catechin, vanillic acid, myricetin, and quercetin, were prepared in a concentration of 5 mM in 75% ethanol. For each of the standards, a calibration curve was prepared (2–100 µM) to report the results in µmol/L.

Concentrating the extracts

The optimum unhydrolyzed and acid-hydrolyzed extracts were subjected to a tenfold concentration process using a vacuum rotary evaporator (Rotavapor® R-100, Büchi, Essen, Germany) at a controlled temperature of 35°C. After rotary evaporation, the TPC of the obtained concentrated extract was measured again following the procedure outlined in Section 2.4. This measurement determined the volumes needed to achieve specific phenolic concentrations in both the emulsions and the sodium acetate-imidazole buffer for the metal chelating test. To ensure experimental accuracy, the extract volumes were standardized by evaporating them under nitrogen gas before their use in both the metal chelating activity test and the preparation of extract-added emulsions.

Metal chelating activity

For the assessment of cupric (Cu^{2+}) and ferrous (Fe^{2+}) ion chelating activity, the concentrated extracts acquired in Section 2.6 were resuspended in a sodium acetate-imidazole buffer (10 mM, pH 7.0) to achieve a final TPC of 100, 200, 400, 800 and 1000 µmol GAE/L. The ability of different phenolic concentration of extracts in chelating Cu^{2+} and Fe^{2+} ions was measured as described by Ebrahimi et al. (2024). Equation (2) was used to calculate the inhibition of Cu^{2+}-pyrocatechol violet complex formation and Fe^{2+}-ferrozine complex formation using the absorbances at 632 and 562 nm, respectively.

$$\text{Metal chelating activity}\,(\%) = \frac{(A_0 - A_1)}{A_1} \times 100 \qquad (2)$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extracts.

Preparation and characterization of oil-in-water emulsion

The minor compounds, such as tocopherols, impacting oxidation kinetics were removed from the soybean oil through a stripping method, confirmed by an HPLC method described by Bayram et al. (2023). Before stripping, the oil contained 111.0 ± 2.3 mg/kg alpha-tocopherol, 831.8 ± 17.5 mg/kg gamma-tocopherol, and 193.0 ± 4.0 mg/kg delta-tocopherol, while none of these compounds were detected after stripping. The soybean oil-in-water emulsions were created using a microfluidizer (M-110 L Microfluidics, Newton, MA, USA) by mixing 2% (w/v) stripped soybean oil, and 0.2% (w/v) Tween

20 as a surfactant in a sodium acetate-imidazole buffer (10 mM, pH 7.0) according to Bayram et al. (2023). The concentrated extracts from Section 2.6 were added to the obtained emulsions while stirring at 300 rpm for 1 h at 4°C, to yield final TPCs of 100, 200, and 400 μmol GAE/L. The blank emulsion, without the addition of extracts, served as the control group. Subsequently, 1 mL of emulsion samples was transferred into 10 mL GC headspace vials (Supelco, Bellefonte, PA, USA), sealed with aluminum caps with PTFE/silicone septa, and stored in light-resistant containers at 45°C throughout the oxidation study. Three vials from each treatment were analyzed to assess the development of primary and secondary lipid oxidation products on a daily basis. The prepared emulsions were characterized by determining droplet size distribution through light scattering and zeta potential via micro-electrophoresis using a Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, United Kingdom) following the method outlined by Bayram et al. (2023).

Oxidation study: Lipid hydroperoxides, headspace hexanal, and depletion of phenolic compounds in the emulsions

The accumulation of lipid hydroperoxides in the control and extract-added emulsions was spectrophotometrically determined during the storage period according to Bayram and Decker (2024). The results were expressed as μmol/kg oil, utilizing a standard curve generated from various concentrations of cumene hydroperoxide (0.1 to 0.5 mM).

The accumulation of secondary oxidation products, headspace hexanal, in the control and extract-added emulsions was determined during the storage period using a GC method described by Culler et al. (2022). The results were expressed as μmol/kg oil using a standard curve prepared with 0–200 μM authentic hexanal.

The phenolic compounds from the emulsions were extracted by adding 1 mL of ethyl acetate to 0.5 mL of emulsion. After vertexing the mixtures for 5 min, they were centrifuged for 2 min at 4000×*g*. The TPC in the supernatant was assessed as described in Section 2.4. The depletion of phenolic compounds during the length of the oxidation study was measured using Equation (3).

$$\text{Depletion of phenolic compounds} = \frac{TPC_x}{TPC_0} \times 100 \quad (3)$$

where TPC_0 is the total phenolic content on day 0, and TPC_x is the total phenolic content on each day of the storage period.

Statistical analysis

All the analyses and sample preparations were conducted in triplicate for statistical analysis and the results were presented as mean ± standard deviation. Data were processed using IBM SPSS Statistics (Version 20.0, SPSS Inc, Chicago, IL, USA) and subjected to one-way Analysis of Variance (ANOVA), and independent *t*-test with a significance level and confidence level of 0.05 and 95%, respectively. Tukey's test was employed for making comparisons. The lag phases of lipid hydroperoxide and headspace hexanal were determined based on one-tailed Dunnett's test. The lag phase was defined as the first data point that significantly differed ($p < 0.05$) from the initial measurement (day 0) of the experiment.

RESULTS AND DISCUSSION

Development of extraction process and acid hydrolysis post-treatment

To choose a single optimum extract for the acid hydrolysis, the TPC (Figure 2a) and DPPH radical scavenging activity (Figure 2b) of different unhydrolyzed extracts were assessed at various ultrasonication times. Results indicate that longer ultrasonication times increased the extraction yield, similar to previous studies (da Rosa et al., 2019). However, significant increases in TPC and DPPH radical scavenging activity were observed only up to 8 min of extraction, and further ultrasonication did not significantly improve these values. This finding aligns with the study conducted by Tan et al. (2024), where they observed a similar trend in polyphenol extraction from litchi peels. Beyond a certain threshold, ultrasonication may not increase phenolic yield, as the majority of the phenolic compounds may have already been extracted. This condition could be justified through Fick's Second Law of Diffusion, which asserts that after a specific duration, an equilibrium concentration is established between a solution and a solid matrix, making it unnecessary to prolong the extraction time (Kaur et al., 2022). Therefore, the extract obtained by 8 min of ultrasonication was chosen as the optimum extract for investigating the impact of acid hydrolysis post-treatment.

Acid hydrolysis, in all durations, significantly increased the TPC (Figure 2c) and DPPH radical scavenging activity (Figure 2d) of the extract obtained from 8 min of ultrasonication. This rise may be attributed to the liberation of free phenolic compounds due to the breakage of the glycosidic bond in the soluble conjugate form of polyphenols (Đurović et al., 2018; Li et al., 2020). An increase in the antioxidant activity and the content of free polyphenols in the acid-hydrolyzed phenolic extracts has been reported by several studies (Komes et al., 2011; Sani et al., 2012). The consistent increase in the phenolic content and antioxidant activity

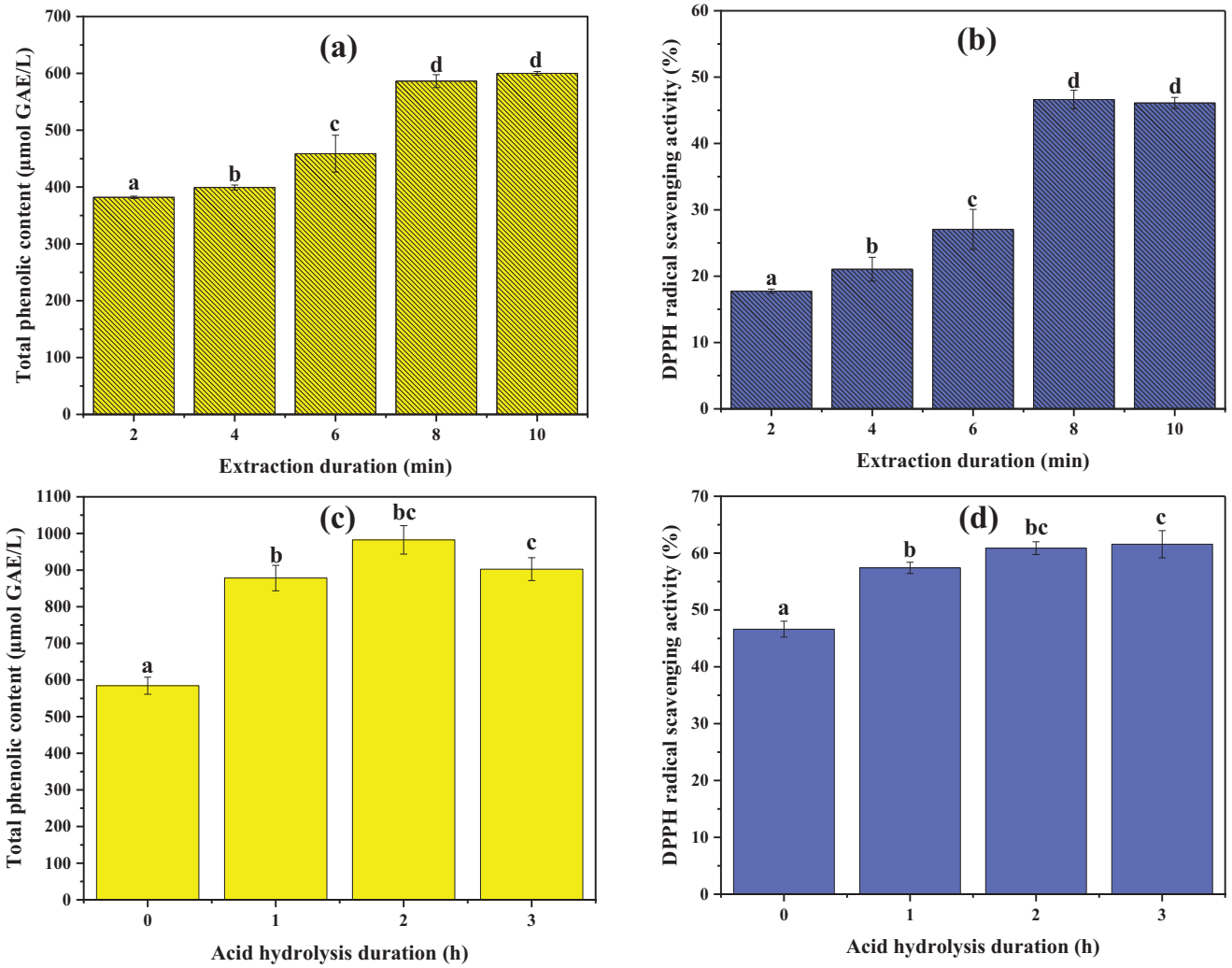

FIGURE 2 Total phenolic content (a) and DPPH radical scavenging activity (b) of unhydrolyzed extracts at different ultrasonication durations, and the total phenolic content (c) and DPPH radical scavenging activity (d) of the extract ultrasonicated for 8 min at different acid hydrolysis durations. Data are presented as mean ± SD ($n = 3$). Different letters show that there is a significant difference ($p \leq 0.05$), according to one-way ANOVA and Tukey's Honestly Significant Difference (HSD) tests.

is not unexpected, given the strong association between antioxidant activity and phenolic content (Ebrahimi & Lante, 2021; Sinlapapanya et al., 2024). However, the increase in the phenolic content due to acid hydrolysis happened only up to 2 h, and it decreased significantly after that point. This decline may be attributed to the degradation of certain phenolic compounds, such as phenolic acids, at high temperatures during acid hydrolysis, as reported by Nuutila et al. (2002). Thus, the 2-h-acid-hydrolyzed extract was chosen to be evaluated for the next steps of the analysis.

Chemical properties of the unhydrolyzed and acid-hydrolyzed extracts

Optimization of UAE and acid hydrolysis durations in Section 3.1 yielded two optimal extracts for analysis: the unhydrolyzed extract, obtained via 8 min of ultrasonication without acid hydrolysis, and the acid-hydrolyzed extract, obtained via 8 min of ultrasonication followed by a 2-h acid hydrolysis. Table 1 shows detected phenolic compounds, total phenolic content, DPPH radical scavenging activity, and chlorophylls and carotenoid content of these extracts. The phenolic compounds were selected based on their prevalence in sugar beet leaves (Maravić et al., 2022).

The results show that the free forms of catechin, vanillic acid, *p*-coumaric acid, and myricetin were not detected in the unhydrolyzed extract but only after acid hydrolysis. Moreover, quercetin and gallic acid content significantly increased with acid hydrolysis. The release of catechin and the significant increase in gallic acid content could be linked, as acid hydrolysis may break the bond in galloylated catechins and lead to an increase in the content of catechin and gallic acid.

TABLE 1 Phenolic profile, total phenolic content, DPPH radical scavenging activity, and chlorophyll and carotenoid content of the optimized unhydrolyzed and acid-hydrolysed extracts.

Components	λ_{max} (nm)	Retention time (min)	Concentration	
			Unhydrolyzed extract	Acid-hydrolysed extract
Gallic acid (μmol/L)	272	2.82	1.87 ± 0.04[a]	77.82 ± 5.51[b]
Catechin (μmol/L)	280	5.13	ND	124.88 ± 7.93
Vanillic acid (μmol/L)	261	6.74	ND	7.69 ± 0.20
p-coumaric acid (μmol/L)	307	9.64	ND	3.14 ± 0.15
trans-ferulic acid (μmol/L)	322	11.73	70.50 ± 15.85[b]	28.28 ± 3.91[a]
Myricetin (μmol/L)	371	16.16	ND	5.66 ± 0.28
Quercetin (μmol/L)	370	23.24	1.98 ± 0.22[a]	3.25 ± 0.38[b]
Total phenolic content (μmol GAE/L)	—	—	586.24 ± 11.45[a]	982.42 ± 6.61[b]
DPPH radical scavenging activity (%)	—	—	46.63 ± 1.39[a]	60.87 ± 1.12[b]
Chlorophyll a (μg/mL)	—	—	25.54 ± 1.80[a]	9.36 ± 0.20[b]
Chlorophyll b (μg/mL)	—	—	16.60 ± 0.45[a]	10.50 ± 0.43[b]
Carotenoids (μg/mL)	—	—	9.60 ± 0.54	ND

Note: Data is reported as mean ± SD of triplicate samples ($n = 3$). Different letters show that there is a significant difference ($p \leq 0.05$) according to an independent *t*-test. ND: Not detected.

Additionally, the rise in other phenolic compounds likely results from the cleavage of glycosidic linkages (Ebrahimi et al., 2024; Nuutila et al., 2002; Tolosa et al., 2018). These changes can contribute to the significant increase in TPC and DPPH radical scavenging activity observed in the acid-hydrolyzed extract. Similar to the present study, Komes et al. (2011) reported that acid hydrolysis with 2 M HCl for 2 h at 95°C significantly released *p*-coumaric acid in the phenolic extracts obtained from olive leaves, nettle, lavender, and blackberry leaves. Begum et al. (2015) reported that in some species of rice bran, *p*-coumaric acid, and vanillic acid appeared only when the extraction was performed with acid hydrolysis. Sani et al. (2012) also reported the release of gallic acid and vanillic acid after acid hydrolysis in germinated brown rice extracts.

The increase in the recovery of quercetin and catechin during the acid hydrolysis of red onion samples with 1.2 M HCl at 80°C for 2 h was reported by Nuutila et al. (2002). Additionally, myricetin release and concentration increase due to acid hydrolysis have been reported by several authors (Mehrdad et al., 2009; Nuutila et al., 2002; Tolosa et al., 2018). According to Tolosa et al. (2018), using 2.5 M HCl at 75°C for 60 min effectively cleaves the glycosidic bond of myricetin-3-O-rhamnoside, a glycoside of myricetin. The release of these compounds after acid hydrolysis could significantly contribute to the increase in the TPC and DPPH radical scavenging activity of the extract.

The *trans* isomer of ferulic acid exists abundantly in many plants, notably in sugar beet leaves (Maravić et al., 2022; Truong et al., 2020). In the present research, *trans*-ferulic acid had the highest concentration in the unhydrolyzed extract, and it was the only phenolic compound, among those detected, reducing after the acid hydrolysis. Sensitivity to hydrolysis, involving high temperature and acidity, can degrade the content of certain phenolic compounds (Vargas-León et al., 2018). Nuutila et al. (2002) reported that the degradation of phenolic acids, such as ferulic acid, occurs due to high temperatures during acid hydrolysis.

In addition to the phenolic profile, the antioxidant activity of phenolic extracts may be affected by the presence of carotenoids and chlorophylls (Ebrahimi et al., 2023). Table 1 indicates a significant reduction in chlorophyll a and b, and removal of carotenoids after acid-hydrolysis. Arvola (1981) reported a shift in the maximum absorbance of the chlorophyll extracts to 663 nm under acidic conditions (0.7 M HCl), showing the conversion of chlorophylls to other compounds. Generally, high temperatures (>60°C) and acidity trigger chlorophyll conversion to other compounds, reducing total chlorophyll via the replacement or removal of magnesium in the structure of chlorophylls (Pareek et al., 2017). For example, it is reported that pH 3.0 and 80°C for 30 min can change chlorophylls to pheophytin (Kang et al., 2018). This condition is very similar to the condition of acid hydrolysis in the present research. Therefore, it is expected that the same compound was generated in the acid-hydrolyzed extracts, which may present a different antioxidant activity.

Metal chelating activity

Free ions of iron and copper (Fe^{2+}, Fe^{3+}, and Cu^{2+}) can function as prooxidants, accelerating lipid oxidation in foods by catalyzing the activity of free radicals and prompting reactive oxygen species generation. Polyphenols can counteract these issues by chelating

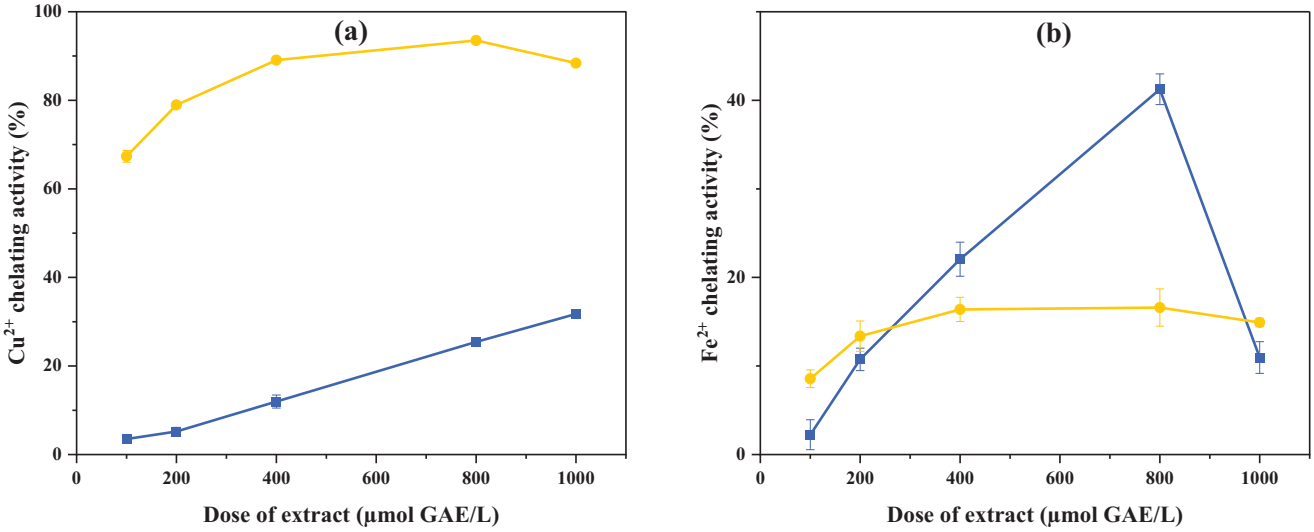

FIGURE 3 Cupric chelating activity (a) and ferrous chelating activity (b) of the optimized unhydrolyzed and acid-hydrolyzed extracts in dosages of extracts based on phenolic concentration. Data are presented as mean ± SD ($n = 3$). Some error bar are beneath the data points. ■: Unhydrolyzed extract, ●: Acid-hydrolyzed extract.

the metal ions, inhibiting lipid oxidation (Bayram et al., 2023). Thus, the optimum unhydrolyzed and acid-hydrolyzed extracts in the present study were evaluated as natural metal chelators to determine their most effective concentrations for oxidation study in the next step.

Figure 3a shows a significant increase in cupric chelating activity of acid-hydrolyzed extracts at all concentrations, possibly due to enhanced phenolic availability or additional chelating groups formed with aglycone formation, compared to unhydrolyzed extract. A concentration-dependent increase in cupric ion chelation was observed with the unhydrolyzed extract, similar to the findings of Arjeh et al. (2022).

Surprisingly, ferrous chelating activity of acid-hydrolyzed extracts was lower than unhydrolyzed extracts in TPCs higher than 100 μmol GAE/L (Figure 3b). This disparity may be attributed to higher concentration of ferulic acid (see Table 1) in the unhydrolyzed extract compared to its acid-hydrolyzed counterpart, which is known to have high ferrous chelating activity (Truong et al., 2020). Both acid-hydrolyzed and unhydrolyzed extract showed an increasing ferrous chelating activity up to a TPC of 800 μmol GAE/L, beyond which it declined significantly. This suggests a dose-dependent ferrous chelating activity in both extracts. This happens when more than one phenolic compound chelates the metals at higher phenolic to metal ratios (Tian et al., 2022).

Since most of flavonoids, such as myricetin, are efficient antioxidants at very low concentrations but rather prooxidants at higher concentrations (Agraharam et al., 2022; Truong et al., 2020), only the low concentrations of the extracts (100, 200, 400 μmol GAE/L) were chosen to be added to emulsions for the oxidation study purpose.

Oxidation stability of soybean oil-in-water emulsions

To assess the impact of unhydrolyzed and acid-hydrolyzed sugar beet leaf extracts on the oxidative stability of soybean oil-in-water emulsions, the formation of lipid hydroperoxides (Figure 4a) and hexanal headspace (Figure 4b) was monitored during the storage period at 45°C. During these analyses, no creaming was observed in any of the emulsions, and they maintained their physical stability as supported by emulsion particle diameter and ζ-potential results. The average emulsion particle diameter ranged from 197.07 ± 5.59 nm to 215.37 ± 14.61 nm, and the average ζ-potential ranged between −20.57 ± 1.16 mV and −17.00 ± 1.74 mV, with no significant differences.

Together with monitoring the hydroperoxide and hexanal, the depletion of phenolic content in the extract-added emulsions was monitored (Figure 5). The results show that phenolic compounds depleted, consistent with the progress of lipid oxidation. Phenolic content within the emulsions significantly declined until the last day of the lipid oxidation lag phase in each sample.

The hydroperoxide content and headspace hexanal of the blank emulsion increased immediately at the start of the storage period. Emulsions containing 100 μmol GAE/L TPC of unhydrolyzed extract exhibited lag phases of 10 days for hydroperoxide and 9 days for hexanal accumulation. Both these values extended to 11 days when the TPC was increased to 200 μmol GAE/L. The emulsion with 400 μmol GAE/L TPC of unhydrolyzed extract demonstrated the highest stability, with lag phases of 11 days for hydroperoxide and 12 days for headspace hexanal. However, the results for the acid-hydrolyzed extracts are completely different, showing a lower stability in the emulsions, compared to

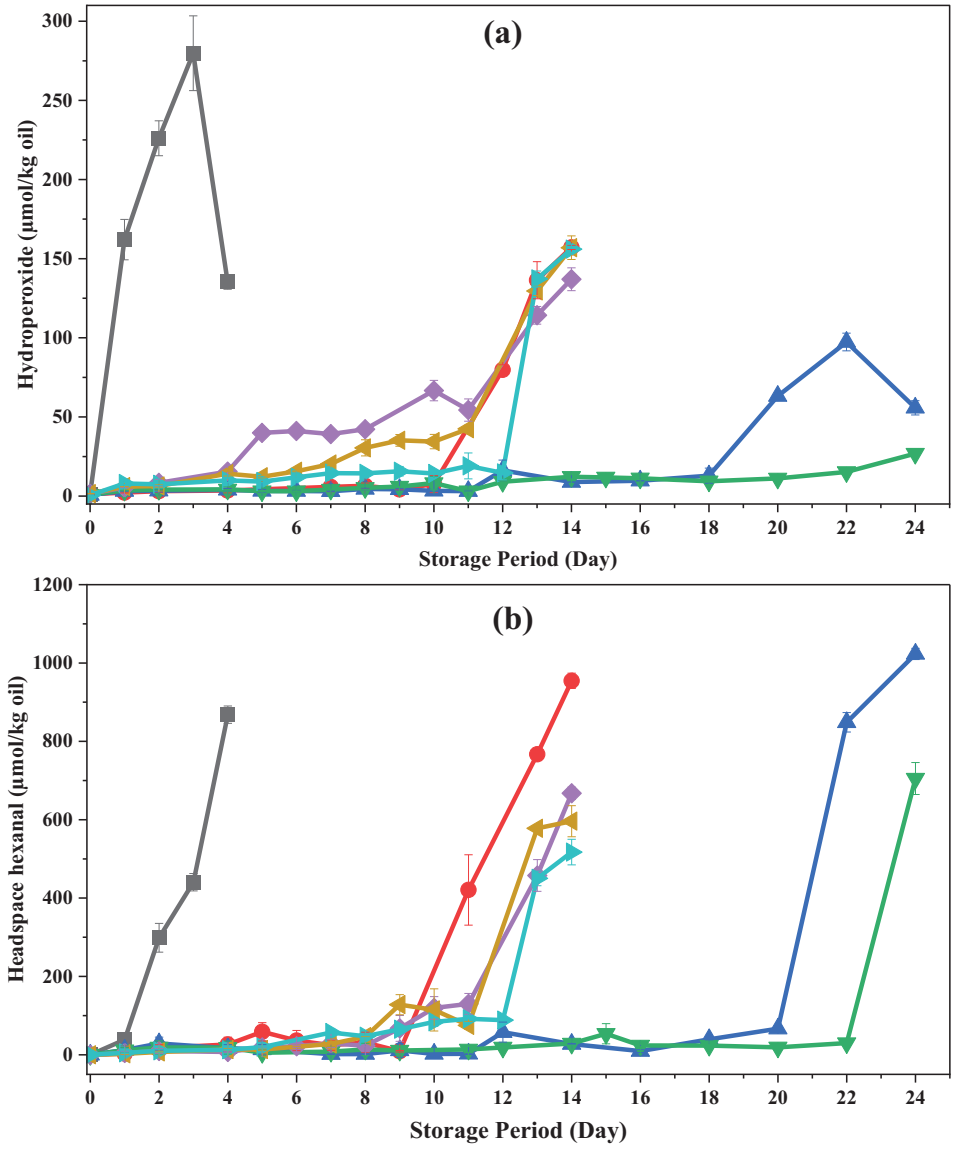

FIGURE 4 Cumulative generation of lipid hydroperoxides (a) and headspace hexanal (b) in soybean oil-in-water emulsions during the storage period at 45°C. Data points show mean ± SD ($n = 3$). Some error bar are beneath the data points. The lines depict the emulsions without extract (■), with unhydrolyzed extract at phenolic concentrations of 100 (●), 200 (▲), and 400 (▼) μmol GAE/L, and with acid-hydrolyzed extract at phenolic concentrations of 100 (♦), 200 (◀), and 400 (▶) μmol GAE/L.

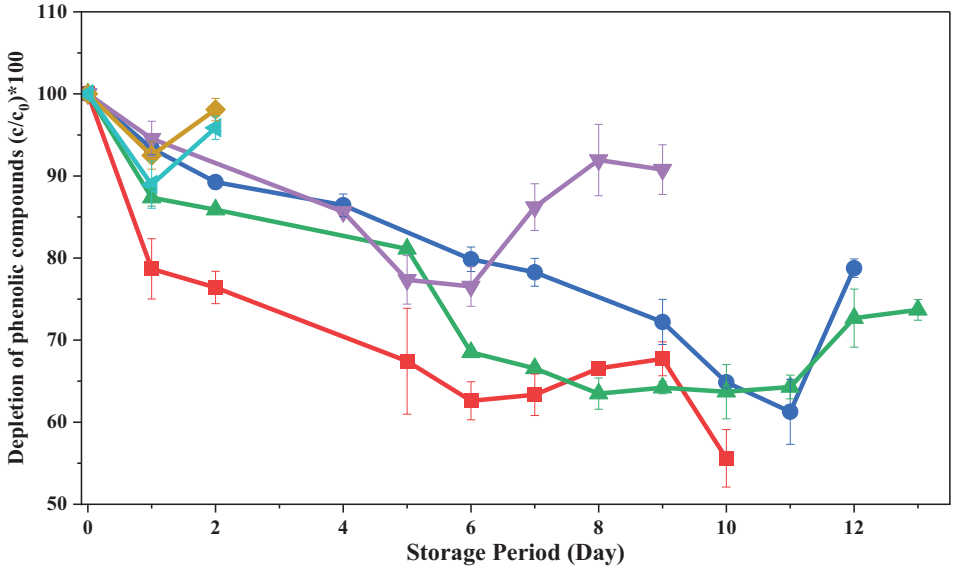

FIGURE 5 Depletion of phenolic compounds in the extract-added soybean oil-in-water emulsions during the storage period at 45°C. Data points show mean ± SD ($n = 3$). Some error bar are beneath the data points. The lines depict the emulsions containing unhydrolyzed extract at phenolic concentrations of 100 (●), 200 (▲), 400 (▼) μmol GAE/L, and emulsions containing acid-hydrolyzed extract at phenolic concentrations of 100 (♦), 200 (◀), and 400 (▶) μmol GAE/L.

unhydrolyzed extracts. A possible reason for the more antioxidant activity of unhydrolyzed extract could be the higher concentration of *trans*-ferulic acid (Truong et al., 2020). Additionally, the higher chlorophyll concentration in the unhydrolyzed extract might enhance antioxidant activity if the extract-added emulsion is stored in the dark (Ebrahimi et al., 2023; Endo et al., 1985).

The generation of lipid hydroperoxides in all the emulsions containing acid-hydrolyzed extract started from the first day, leading to subsequent formation of hexanal headspace in the emulsions. The emulsions containing 100 μmol GAE/L TPC of acid-hydrolyzed extract had an 8-day headspace hexanal lag phase, surpassing other concentrations of acid-hydrolyzed extract. This means that the acid-hydrolyzed extract works better in lower concentrations. Despite the increase in the TPC, DPPH radical scavenging activity, and content of most individual phenolic compounds in the acid-hydrolyzed extract, the free phenolic compounds released after the acid hydrolysis increased the prooxidant activity of the extract in the emulsions. Similarly, Kikuzaki et al. (2002) reported that the antioxidant activity of hydroxycinnamic acids in a buffer-based system is not always correlated with their radical scavenging activity. The prooxidant activities of polyphenols depend on the fact that the compounds can reduce Fe(III) and O_2 to Fe(II) and $HO^{\bullet}_2$, respectively (Pan et al., 2022). Although the antioxidant activity is highly connected to the quantity of polyphenols, it should be noted that the type and quality of phenolic compounds are more responsible for the antioxidant activity (Ebrahimi et al., 2022). For example, myricetin, which was released after acid hydrolysis, was observed to display prooxidant behavior when in the presence of metal salts, and the specific type of metal salt plays a crucial role in determining whether it will exhibit prooxidant or antioxidant properties (Agraharam et al., 2022). Moreover, since acid hydrolysis released high quantity of hydrophilic phenolic compounds, including gallic acid and catechin, it can limit the efficiency of the extract in a hydrophilic system (i.e., oil-in-water emulsion), according to the polar paradox hypothesis (Frankel et al., 1994). Therefore, the reason for better function of unhydrolyzed extract in delaying the oxidation could be connected to its phenolic profile.

CONCLUSION

This study highlights the promising use of phenolic extracts derived from food by-products (e.g., sugar beet leaves) in improving the shelf-life of emulsion-based foods. It was found that acid hydrolysis can successfully convert the glycosidic phenolic compounds to aglycones resulting in increased TPC and DPPH radical scavenging activity, and copper chelating activity. However, the impact of acid hydrolysis on the phenolic extracts can either enhance antioxidant activity or induce prooxidant activity, depending on the dosage added to the food, and the phenolic profile of the extract. Therefore, it is crucial to acknowledge that predicting the behavior of an antioxidant in a given system is still a complex challenge. The effectiveness of a chosen antioxidant is not solely determined by its chemical reactivity but is also influenced by various physical–chemical phenomena within the specific systems under consideration.

AUTHOR CONTRIBUTIONS

Peyman Ebrahimi designed the study, carried out the research, analyzed the data, prepared the graphs, and wrote and edited the original draft. Ipek Bayram contributed to methodology, formal analysis, investigation, data curation, and writing-review and editing. Anna Lante supervised the study, contributed to conceptualization, methodology, writing-review and editing, Eric A. Decker supervised the study, contributed to research design, conceptualization, methodology, and writing-review and editing. All authors contributed to and approved the final draft of the manuscript.

ACKNOWLEDGMENTS

The authors acknowledge the PhD scholarship from Fondazione Cassa di Risparmio di Padova e Rovigo (CARIPARO) granted to Peyman Ebrahimi, and they appreciate the help of Carolina Cantele in some experiments.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

No humans or animal were used in this research.

ORCID

Ipek Bayram https://orcid.org/0000-0002-6604-6525
Anna Lante https://orcid.org/0000-0003-4709-9311

REFERENCES

Agraharam G, Girigoswami A, Girigoswami K. Myricetin: a multifunctional flavonol in biomedicine. Curr Pharmacol Rep. 2022;8:48–61. https://doi.org/10.1007/s40495-021-00269-2

Arjeh E, Khodaei SM, Barzegar M, Pirsa S, Karimi Sani I, Rahati S, et al. Phenolic compounds of sugar beet (*Beta vulgaris* L.): separation method, chemical characterization, and biological properties. Food Sci Nutr. 2022;10(12):4238–46. https://doi.org/10.1002/fsn3.3017

Arvola L. Spectrophotometric determination of chlorophyll a and phaeopigments in ethanol extractions. Ann Bot Fenn. 1981;18:221–7.

Azuma K, Ippoushi K, Ito H, Higashio H, Terao J. Evaluation of antioxidative activity of vegetable extracts in linoleic acid emulsion and phospholipid bilayers. J Sci Food Agric. 1999;79:2010–6. https://doi.org/10.1002/(SICI)1097-0010(199911)79:14<2010::AID-JSFA471>3.0.CO;2-U

Bayram I, Decker EA. Analysis of the mechanism of antioxidant synergism between α-tocopherol and myricetin in bulk oil. J Am Oil Chem Soc. 2024;101:477–92. https://doi.org/10.1002/aocs.12792

Bayram I, Laze A, Decker EA. Synergistic mechanisms of interactions between Myricetin or Taxifolin with α-tocopherol in oil-in-water emulsions. J Agric Food Chem. 2023;71:9490–500. https://doi.org/10.1021/acs.jafc.3c01226

Begum A, Goswami A, Chowdhury P. A comparative study on free and bound phenolic acid content and their antioxidant activity in bran of rice (*Oryza sativa* L.) cultivars of eastern Himalayan range. Int J Food Sci Technol. 2015;50:2529–36. https://doi.org/10.1111/ijfs.12920

Cherif A, Slama A. Stability and change in fatty acids composition of soybean, corn, and sunflower oils during the heating process. J Food Qual. 2022;2022:1–17. https://doi.org/10.1155/2022/6761029

Culler MD, Bayram I, Decker EA. Enzymatic modification of lecithin for improved antioxidant activity in combination with tocopherol in emulsions and bulk oil. J Agric Food Chem. 2022;70:13404–12. https://doi.org/10.1021/acs.jafc.2c05182

da Rosa GS, Vanga SK, Gariepy Y, Raghavan V. Comparison of microwave, ultrasonic and conventional techniques for extraction of bioactive compounds from olive leaves (*Olea europaea* L.). Innov Food Sci Emerg Technol. 2019;58:102234. https://doi.org/10.1016/j.ifset.2019.102234

Đurović S, Nikolić B, Luković N, Jovanović J, Stefanović A, Šekuljica N, et al. The impact of high-power ultrasound and microwave on the phenolic acid profile and antioxidant activity of the extract from yellow soybean seeds. Ind Crop Prod. 2018;122:223–31. https://doi.org/10.1016/j.indcrop.2018.05.078

Ebrahimi P, Bayram I, Lante A, Decker EA. Acid-hydrolyzed phenolic extract of parsley (*Petroselinum crispum* L.) leaves inhibits lipid oxidation in soybean oil-in-water emulsions. Food Res Int. 2024;187:114452. https://doi.org/10.1016/j.foodres.2024.114452

Ebrahimi P, Lante A. Polyphenols: a comprehensive review of their nutritional properties. Open Biotechnol J. 2021;15:164–72. https://doi.org/10.2174/1874070702115010164

Ebrahimi P, Mihaylova D, Marangon CM, Grigoletto L, Lante A. Impact of sample pretreatment and extraction methods on the bioactive compounds of sugar beet (*Beta vulgaris* L.) leaves. Molecules. 2022;27:8110. https://doi.org/10.3390/molecules27228110

Ebrahimi P, Shokramraji Z, Tavakkoli S, Mihaylova D, Lante A. Chlorophylls as natural bioactive compounds existing in food by-products: a critical review. Plan Theory. 2023;12:1533. https://doi.org/10.3390/plants12071533

Endo Y, Usuki R, Kaneda T. Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. I. Comparison of the inhibitory effects. J Am Oil Chem Soc. 1985;62:1375–8. https://doi.org/10.1007/BF02545962

Frankel EN, Huang SW, Kanner J, German JB. Interfacial phenomena in the evaluation of antioxidants: bulk oils vs emulsions. J Agric Food Chem. 1994;42:1054–9. https://doi.org/10.1021/jf00041a001

Inchingolo R, Bayram I, Uluata S, Kiralan SS, Rodriguez-Estrada MT, McClements DJ, et al. Ability of sodium dodecyl sulfate (SDS) micelles to increase the antioxidant activity of α-tocopherol. J Agric Food Chem. 2021;69:5702–8. https://doi.org/10.1021/acs.jafc.1c01199

Kang YR, Park J, Jung SK, Chang YH. Synthesis, characterization, and functional properties of chlorophylls, pheophytins, and Zn-pheophytins. Food Chem. 2018;245:943–50. https://doi.org/10.1016/j.foodchem.2017.11.079

Kaur B, Panesar PS, Anal AK. Standardization of ultrasound assisted extraction for the recovery of phenolic compounds from mango peels. J Food Sci Technol. 2022;59:2813–20. https://doi.org/10.1007/s13197-021-05304-0

Kikuzaki H, Hisamoto M, Hirose K, Akiyama K, Taniguchi H. Antioxidant properties of ferulic acid and its related compounds. J Agric Food Chem. 2002;50:2161–8. https://doi.org/10.1021/jf011348w

Komes D, Belščak-Cvitanović A, Horžić D, Rusak G, Likić S, Berendika M. Phenolic composition and antioxidant properties of some traditionally used medicinal plants affected by the extraction time and hydrolysis. Phytochem Anal. 2011;22:172–80. https://doi.org/10.1002/pca.1264

Li W, Yang R, Ying D, Yu J, Sanguansri L, Augustin MA. Analysis of polyphenols in apple pomace: a comparative study of different extraction and hydrolysis procedures. Ind Crop Prod. 2020;147:112250. https://doi.org/10.1016/j.indcrop.2020.112250

Lichtenthaler HK, Buschmann C. Chlorophylls and carotenoids: measurement and characterization by spectroscopy. Curr Protocol Food Anal Chem. 2001;1:F4.3.1–8. https://doi.org/10.1002/0471142913.faf0403s01

Maravić N, Teslić N, Nikolić D, Dimić I, Šereš Z, Pavlić B. From agricultural waste to antioxidant-rich extracts: green techniques in extraction of polyphenols from sugar beet leaves. Sustain Chem Pharm. 2022;28:1–12. https://doi.org/10.1016/j.scp.2022.100728

Martins CC, Kahmann A, Anzanello MJ, Rodrigues RC, Rodrigues E, Mercali GD. Acid hydrolysis conditions do affect the non-extractable phenolic compounds composition from grape peel and seed. Food Res Int. 2023;174:113636. https://doi.org/10.1016/j.foodres.2023.113636

Mehrdad M, Zebardast M, Abedi G, Koupaei MN, Rasouli H, Talebi M. Validated high-throughput HPLC method for the analysis of flavonol aglycones myricetin, quercetin, and kaempferol in *Rhus coriaria* L. using a monolithic column. J AOAC Int. 2009;92:1035–43. https://doi.org/10.1093/jaoac/92.4.1035

Nutter J, Fernandez MV, Jagus RJ, Agüero MV. Development of an aqueous ultrasound-assisted extraction process of bioactive compounds from beet leaves: a proposal for reducing losses and increasing biomass utilization. J Sci Food Agric. 2020;101:1989–97. https://doi.org/10.1002/jsfa.10815

Nuutila AM, Kammiovirta K, Oksman-Caldentey KM. Comparison of methods for the hydrolysis of flavonoids and phenolic acids from onion and spinach for HPLC analysis. Food Chem. 2002;76:519–25. https://doi.org/10.1016/S0308-8146(01)00305-3

Pan Y, Qin R, Hou M, Xue J, Zhou M, Xu L, et al. The interactions of polyphenols with Fe and their application in Fenton/Fenton-like reactions. Sep Purif Technol. 2022;300:121831. https://doi.org/10.1016/j.seppur.2022.121831

Pareek S, Sagar NA, Sharma S, Kumar V, Agarwal T, González-Aguilar GA, et al. Chlorophylls: chemistry and biological functions. Fruit and vegetable phytochemicals. Chichester, UK: John Wiley & Sons, Ltd; 2017. p. 269–84.

Sani IM, Iqbal S, Chan KW, Ismail M. Effect of acid and base catalyzed hydrolysis on the yield of phenolics and antioxidant activity of extracts from germinated brown rice (GBR). Molecules. 2012;17:7584–94. https://doi.org/10.3390/molecules17067584

Sinlapapanya P, Sumpavapol P, Buatong J, Benjakul S. Ethanolic cashew leaf extract: antioxidant potential and impact on quality changes of dried fermented catfish during storage. Futur Foods. 2024;9:100296. https://doi.org/10.1016/j.fufo.2024.100296

Tan MJ, Li Y, Zhao SQ, Yue FH, Cai DJ, Wu JT, et al. Synergistic ultrasound pulsed electric field extraction of litchi peel polyphenols and determination of their properties. Int J Biol Macromol. 2024;260:129613. https://doi.org/10.1016/j.ijbiomac.2024.129613

Tian L, Zhang S, Yi J, Zhu Z, Cui L, Decker EA, et al. Factors impacting the antioxidant/prooxidant activity of tea polyphenols on lipids and proteins in oil-in-water emulsions. LWT. 2022;156:113024. https://doi.org/10.1016/j.lwt.2021.113024

Tolosa T, Rogez H, Silva E, Souza J. Optimization of acid hydrolysis of Myricetin-3-O-rhamnoside using response surface methodology. J Braz Chem Soc. 2018;9:91–7. https://doi.org/10.21577/0103-5053.20180125

Truong DH, Nhung NTA, Dao DQ. Iron ions chelation-based antioxidant potential vs. pro-oxidant risk of ferulic acid: a DFT study in aqueous phase. Comput Theor Chem. 2020;1185:112905. https://doi.org/10.1016/j.comptc.2020.112905

Tu X, Ma S, Gao Z, Wang J, Huang S, Chen W. One-step extraction and hydrolysis of flavonoid glycosides in rape bee pollen based on Soxhlet-assisted matrix solid phase dispersion. Phytochem Anal. 2017;28:505–11. https://doi.org/10.1002/pca.2699

Vargas-León EA, Díaz-Batalla L, González-Cruz L, Bernardino-Nicanor A, Castro-Rosas J, Reynoso-Camacho R, et al. Effects of acid hydrolysis on the free radical scavenging capacity and inhibitory activity of the angiotensin converting enzyme of phenolic compounds of two varieties of Jamaica (*Hibiscus sabdariffa*). Ind Crop Prod. 2018;116:201–8. https://doi.org/10.1016/j.indcrop.2018.02.044

How to cite this article: Ebrahimi P, Bayram I, Lante A, Decker EA. Antioxidant and prooxidant activity of acid-hydrolyzed phenolic extracts of sugar beet leaves in oil-in-water emulsions. J Am Oil Chem Soc. 2024. https://doi.org/10.1002/aocs.12891