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Acid-hydrolyzed phenolic extract of parsley (*Petroselinum crispum* L.) leaves inhibits lipid oxidation in soybean oil-in-water emulsions

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ABSTRACT

The antioxidant activity of the natural phenolic extracts is limited in particular food systems due to the existence of phenolic compounds in glycoside form. Acid hydrolysis post-treatment could be a tool to convert the glycosidic polyphenols in the extracts to aglycones. Therefore, this research investigated the effects of an acid hydrolysis post-treatment on the composition and antioxidant activity of parsley extracts obtained by an ultrasound-assisted extraction method to delay lipid oxidation in a real food system (i.e., soybean oil-in-water emulsion). Acid hydrolysis conditions were varied to maximize total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. When extracts were exposed to 0.6 M HCl for 2 h at 80 °C, TPC was 716.92 ± 24.43 μmol gallic acid equivalent (GAE)/L, and DPPH radical scavenging activity was 66.89 ± 1.63 %. Not only did acid hydrolysis increase the concentrations of individual polyphenols, but it also resulted in the release of new phenolics such as myricetin and gallic acid. The extract's metal chelating and ferric-reducing activity increased significantly after acid hydrolysis. In soybean oil-in-water emulsion containing a TPC of 400 μmol GAE/L, the acid-hydrolyzed extract had an 11-day lag phase for headspace hexanal compared to the 6-day lag phase of unhydrolyzed extract. The findings indicated that the conversion of glycosidic polyphenols to aglycones in phenolic extracts can help extend the shelf-life of emulsion-based foods.

1. Introduction

Many food products and beverages are O/W[1] emulsions with small amounts of lipids emulsified in a water phase (Tian et al., 2022). However, these emulsified lipids used in food applications often suffer from poor oxidative stability, leading to the deterioration of emulsion quality by affecting aroma, taste, and nutritional value (Tian et al., 2021). To enhance the quality of these food emulsions, it is crucial to inhibit lipid oxidation using antioxidant technologies. In recent years, natural antioxidant extracts have attracted significant attention for their ability to prolong the shelf-life of lipid-containing foods and beverages by delaying the oxidation process (Bayram, Laze, & Decker, 2023; Bonciolini et al., 2023; Ebrahimi & Lante, 2022). Therefore, many researchers have recently focused on finding natural antioxidants with plant sources to encounter the adverse effects of lipid oxidation on food quality (Tian et al., 2022).

Parsley (*Petroselinum crispum* L.) is a widely used herb known for its rich polyphenolic content, which makes it an attractive source for the extraction of natural antioxidants (Poureini, Mohammadi, Najafpour, & Nikzad, 2020). To date, many investigations are dedicated to determining the antioxidant properties of parsley (Janković, Mitić, Arsić, & Stankov-Jovanović, 2021). Polyphenols present in parsley are complex mixtures of various types of phytochemicals, including quercetin, myricetin, apigenin, *p*-coumaric acid, etc. (Stan, Soran, Varodi, & Lung, 2012; Yildiz, Başkan, Tütem, & Apak, 2008). Some of these compounds exist in glycoside forms (El-Zaeddi et al., 2017), meaning that during the extraction process, they are recovered in glycosidic form. The presence of the glycosides in the phenolic extracts can block the radical scavenging group of the molecule limiting their antioxidant activity. Therefore, it is crucial to find an extraction process that includes a step to convert glycosidic polyphenols into aglycones, thereby enhancing the extract's antioxidant activity (Tu et al., 2017).

Selecting a green extraction method that can reduce the amount of solvent and save time and energy, together with achieving high yields of

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[1] Oil-in-water.

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phenolic compounds is a key factor for the development of food antioxidants from natural sources (Ebrahimi & Lante, 2022). UAE[2] is a green method that utilizes ultrasonic waves to make mechanical vibrations to disrupt the plant cell structure, resulting in the release of phenolic compounds (Alshehhi, Wu, Kangsadan, Chew, & Show, 2023; Tao et al., 2019, 2022). Since most phenolic compounds extracted from natural sources are bound to carbohydrates through glycosidic bonds, a further step after UAE is needed to increase the purity and activity of phenolic compounds inside the extract (Tolosa, Rogez, Silva, & Souza, 2018; Tu et al., 2017). Acid hydrolysis can break the glycosidic bonds between phenolic compounds and sugars, releasing the aglycone form of phenolic compounds (Vargas-León et al., 2018). This study uses acid hydrolysis as an additional step conducted after obtaining the extract using the UAE method. While many studies have addressed the extraction and separation of polyphenols (El-Zaeddi et al., 2017; Poureini et al., 2020; Yildiz et al., 2008), none have investigated the impact of acid-hydrolyzed extracts on lipid oxidation in O/W emulsions. This research fills the gap in the knowledge on the effect of acid-hydrolyzed phenolic extracts on improving the oxidative stability of a food model system, compared to unhydrolyzed extracts.

Therefore, the aim of this study is to develop an efficient acid hydrolysis post-treatment to be carried out after the extraction step and to determine if the formation of aglycone can increase the ability of parsley extract to inhibit lipid oxidation in O/W emulsions. In this respect, the acid-hydrolyzed phenolic extract could be an efficient preservation strategy for food industry applications. The optimal acid hydrolysis condition was found according to TPC,[3] and DPPH[4] radical scavenging activity of the extracts. Then, the chlorophyll content, phenolic composition, metal chelating activity, and ferric-reducing activity of the extracts before and after acid hydrolysis post-treatment were evaluated to ensure the ability of the extracts as a potential antioxidant. Then, O/W emulsions were created using soybean oil since it is susceptible to oxidation due to its high content of unsaturated fatty acids (Cherif & Slama, 2022). After adding the parsley extracts, with and without acid hydrolysis, to the O/W emulsions, the primary and secondary products of lipid oxidation were monitored during storage by measuring the content of lipid hydroperoxides and headspace hexanal, respectively.

2. Materials and methods

2.1. Chemicals and materials

Soybean oil and parsley leaves were purchased from a retail store in Hadley, MA, USA. Activated charcoal, silicic acid, imidazole, sodium acetate anhydrous, Tween 20, hexanal, sodium carbonate, Folin–Ciocalteu reagent, DPPH, iron(II) sulfate heptahydrate, barium chloride dihydrate, cumene hydroperoxide, EtOH,[5] iron(II) chloride, iron(III) chloride, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt (ferrozine), acetonitrile, sulfuric acid, sodium hydroxide, sodium methoxide, potassium carbonate, acetic acid, gallic acid, 3,4-dihydroxybenzoic acid, copper(II) sulfate, pyrocatechol violet, catechin, vanillic acid, myricetin, and quercetin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonium thiocyanate, hydrochloric acid, MetOH,[6] 1-butanol, isopropanol, isooctane, and hexane were bought from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Preparation of parsley leaves

Parsley leaves were rinsed with distilled water to remove soil, and their stems were separated. Then, the leaves were freeze-dried at − 70 °C for four days using a freeze dryer (Genesis SQ EL-85, SP Scientific Virtis, PA, USA). The freeze-dried parsley leaves were then ground using a mortar and pestle. All samples were vacuum-sealed and stored at − 80 °C until the following analyses.

2.3. Proximate composition of parsley leaves

All the analyses of this part were carried out on freeze-dried parsley leaves to help plan a more optimized extraction process in the next stage. Water activity was measured using a LabMaster.aw instrument (Novasina AG, Lachen, Switzerland) at 25 °C. The soluble carbohydrates (glucose and fructose) were extracted from 0.2 g freeze-dried parsley leaves in 50 mL of a 0.1 N sulfuric acid solution and stirred for 60 min and then quantified using an HPLC[7] method (J. S. Kim, Lee, & Torget, 2001; Kupiainen, Ahola, & Tanskanen, 2010; Lopez-Hernandez et al., 1994). The crude lipid, crude protein, crude fiber, moisture, and ash content were investigated according to the methods described by Meng et al., (2022). Crude lipid content was determined using the Soxhlet technique with a Soxtec™ 2046 Extraction System (FOSS, Hillerød, Denmark). Crude protein content was evaluated using the Kjeldahl method, and the results were adjusted by multiplying by the nitrogen conversion factor (6.25). Crude fiber content was determined by boiling the leaves in 0.26 M sulfuric acid for 30 min. The insoluble residue obtained was filtered and washed, and the filtrate was subjected to boiling in 0.31 M sodium hydroxide, followed by filtration and rewashing. The final filtrate was dried at 130 °C for 120 min, with weight loss measurements taken at 350 °C. Moisture content was assessed using the oven-drying method at 105 °C for 24 h. Ash content was measured by weighing the samples before and after burning them in a muffle furnace at 550 °C for 6 h.

2.4. Ultrasound-Assisted extraction (UAE)

The phenolic extract was obtained from freeze-dried parsley leaves using the methods reported by Ebrahimi et al., (2022) with some minor modifications. The extraction parameters were set according to some initial pretests. Firstly, 0.4 g freeze-dried parsley was added to a centrifuge tube containing 10 mL of 75 % EtOH. UAE was performed using an ultrasound apparatus (FB 505, Fisher Scientific, PA, USA) equipped with a titanium probe immersed 2 cm in the ethanol-parsley mixture. The UAE conditions were as follows: 500 W output power, 20 % amplitude, 20 kHz frequency, and 8 min extraction time. During the UAE, the extracting cell was placed in a beaker containing ice to prevent increasing the temperature. After the sonication processes, the obtained mixtures were centrifuged at 4000 × g for 10 min. The extraction process was then repeated on the pellet resulting from centrifugation and the two extracts were combined, and the volume was adjusted to 20 mL using 75 % EtOH. The extracts were stored at − 80 °C until further use.

2.5. Acid hydrolysis post-treatment of extracts

Although UAE is a rapid extraction method, an acid hydrolysis post-treatment is needed to hydrolyze glycosides into aglycones to potentially increase the efficiency of extraction and to activate the antioxidants (Li et al., 2020; Tu et al., 2017; Vargas-León et al., 2018). Acid hydrolysis was carried out according to the method described by Nuutila et al., (2002) with some modifications. First, 20 mL of parsley extract in 75 % EtOH and 5 mL double distilled water were added to a round-bottom flask. Then, 5 mL HCl at concentrations of 1.8, 3.6, and 7.2 M was added to make 30-mL mixtures with final HCl concentrations of 0.3, 0.6, and 1.2 M, respectively. The final hydrolysis mixture consisted of

[2] Ultrasound-assisted extraction.
[3] Total phenolic content.
[4] 2,2-diphenyl-1-picrylhydrazyl.
[5] Ethanol.
[6] Methanol.
[7] High pressure liquid chromatography.

50 % water, which is needed for hydrolysis (Tu et al., 2017). The hydrolysis was done in a reflux vessel with a condenser attached to the round-bottom flask containing the reaction mixture. The round-bottom flask was positioned in a 400 mL beaker filled with water, equipped with a thermometer, and placed on a magnetic stirrer hotplate to regulate the temperature and maintain continuous stirring during the reaction. The mixtures were refluxed at 80 ℃ for 1, 2, and 3 h. After the acid hydrolysis, the extracts were neutralized using an equal concentration of NaOH to that of HCl in each mixture. All the extracts were filtered with Whatman No. 1 filters and stored at − 80 ℃ until the analytical measurements. The TPC and DPPH radical scavenging activity of different acid-hydrolyzed extracts were used to choose the optimum condition of acid hydrolysis.

2.6. Total phenolic content, total flavonoid content, and DPPH radical scavenging activity of extracts

The TPC was measured using the Folin-Ciocalteu method (Azuma, Ippoushi, Ito, Higashio, & Terao, 1999). The results were expressed as μmol GAE/L[8] using a calibration curve (R^2 = 0.99) prepared with different concentrations of gallic acid (0–750 μM).

The method described by Mihaylova et al. (2021) was used to measure TFC.[9] The results were expressed as μmol QE/L[10] using a calibration curve (R2 = 0.99) prepared with different concentrations of quercetin (3–330 μM).

The method described by Ebrahimi et al., (2022) with some modifications was used to measure the DPPH radical scavenging activity of extracts. Briefly, 0.1 mL of extracts was added to 0.9 mL of a DPPH solution (0.1 mM in EtOH 96 %, v/v) and left to incubate at room temperature for 30 min. The extracting solvent was used instead of the extract in the blank samples. The absorbance was recorded at 517 nm. The antioxidant activity of extracts was calculated based on Eq. (1).

$$\text{Antioxidant activity}\ (\%) = \frac{(A_0 - A_1)}{A_0} \times 100 \quad (1)$$

Where A_0 is the absorbance of the blank, and A_1 is the absorbance of the extracts.

2.7. Chlorophyll content of extracts

The concentration of chlorophylls was determined spectrophotometrically using the method described by Lichtenthaler and Buschmann, (2001). After diluting the extract using 95 % EtOH, they were filtered through 0.22 μm cellulose acetate (CA) syringe filters (Test Scientific, Perugia, Italy) to decrease turbidity. The spectrum of the extract was recorded from 400 to 700 nm and the absorbance values at 664.1, and 648.6 nm were used to calculate the concentration of chlorophyll *a* and chlorophyll *b* using Eq. (2), and 3, respectively. Since chlorophyll *a* and b do not have any absorbance at 750 nm, each value of absorbance was corrected by subtracting that of the mixture at 750 nm from the measured absorbance.

$$\text{chl}_a\left(\frac{\mu g}{mL}\right) = 13.36(A_{664.1} - A_{750}) - 5.19(A_{648.6} - A_{750}) \quad (2)$$

$$\text{chl}_b\left(\frac{\mu g}{mL}\right) = 27.43(A_{648.6} - A_{750}) - 8.12(A_{664.1} - A_{750}) \quad (3)$$

Where chl_a is chlorophyll *a*, chl_b is chlrophyll b, and A is the absorbance.

2.8. Detection of phenolic compounds in the extracts using HPLC

Phenolic compounds in extracts were separated and analyzed using an HPLC apparatus (LC-2030C 3D, Shimadzu, Tokyo, Japan) equipped with a UV–Vis Photodiode Array Detector. The analysis was performed on an Ascentis Express 90 Å C18 reversed-phase column (5 μm i.d., 250 × 4.6 mm; Supelco, Bellefonte, PA, USA). The column and the guard column were thermostatically controlled at 40 ℃. The mobile phase for the elution of phenolic compounds was composed of ultrapure water acidified with 0.1 % acetic acid (designated as solvent A) and acetonitrile (designated as solvent B). The elution of extract and standards was performed under a flow rate of 1 mL/min. The solvent gradient in volumetric ratios of solvents A and B was as follows: 10–30 % B for 30 min and 30–10 % B for 10 min. After each analysis, the column was conditioned by running the HPLC system for 15 min with the mobile phase composition set at 10 % acetonitrile. The injection volume was 20 μL, and the wavelength spectrum was recorded from 200 to 400 nm to determine the optimum absorbance wavelength for the phenolic compounds. The best detection wavelength for monitoring the phenolic compounds was determined to be 280 nm. The stock solution of standards (5 mM), including gallic acid, catechin, vanillic acid, myricetin, and quercetin, was prepared in 75 % EtOH. To calculate the final concentration of detected phenolic compounds, calibration curves of each standard were prepared in the concentrations ranging from 2-100 μM.

2.9. Determination of ferric-reducing activity of extracts

The method described by Bayram et al., (2023) with some modifications was used to determine ferric ion reducing activity. First, different concentrations of total phenolic concentrations were prepared. This involved subjecting the extracts to rotary evaporation at 35 ℃ to decrease the volume 10 times and resuspending them in 75 % EtOH, yielding a concentrated extract. Then, different dilutions were prepared using sodium acetate-imidazole buffer (10 mM, pH 7.0) with final phenolic concentrations of 100, 200, 400, and 800 μmol GAE/L. After preparing extracts with different phenolic concentrations, 50 μL of 10 mM ferric chloride solution was added to 5 mL of each extract. Then, 130 μL of 40 mM ferrozine stock solution was added. After vortexing, the samples were incubated at room temperature in the dark for 10 min, and the absorbance was recorded at 562 nm. The results were expressed as the μmol FE/L[11] using a calibration curve (R^2 = 0.93) prepared with different concentrations of ferrous chloride (5–100 μM).

2.10. Metal chelating activity of extracts

To evaluate the cupric and ferrous ion chelating activity, different concentrations of the extract were prepared as described in section 2.9. The cupric (Cu^{2+}) chelating activity of extracts was determined according to Liu et al., (2015) with some modifications. First, 50 μL of a 5 mM copper (II) sulfate solution was added to 2.5 mL of each concentration of extract prepared in the sodium acetate-imidazole buffer (10 mM, pH 7.0). In the blank sample, the buffer was used instead of extracts. After 30 min of incubation at room temperature, 50 μL of 4 mM pyrocatechol violet was added, and absorbance was recorded at 632 nm. The percentage of inhibition of Cu^{2+}-pyrocatechol violet complex formation was calculated according to Eq. (4).

The ferrous (Fe^{2+}) chelating activity of the extract was measured using the method described by Bayram et al., (2023) with some slight modifications. Ten μL of 10 mM ferrous chloride solution and 50 μL of 0.05 M ferrozine solution were added separately into 2.5 mL of given concentrations of extract prepared in the sodium acetate-imidazole buffer (10 mM, pH 7.0). After vortexing, the samples were incubated at room temperature in the dark for 10 min, and the absorbance was

[8] Gallic acid equivalent per Liter.
[9] Total flavonoid content.
[10] Quercetin equivalent per Liter.
[11] Ferrous ion equivalent per Liter.

recorded at 562 nm. The percentage of inhibition of Fe^{2+}-ferrozine complex formation was calculated according to Eq. (4).

$$\text{Chelating activity}\,(\%) = \frac{(A_0 - A_1)}{A_0} \times 100 \quad (4)$$

Where A_0 is the absorbance of the blank, and A_1 is the absorbance of the extracts.

2.11. Preparation of soybean oil-in-water (O/W) emulsions

First, the soybean oil was stripped to remove minor compounds from soybean oil that can impact oxidation kinetics, such as tocopherols, phospholipids, and carotenoids, using the technique described by Inchingolo et al., (2021). Then, O/W emulsions were prepared by adding 2 % (w/v) stripped soybean oil and 0.2 % (w/v) Tween 20 as a surfactant to a sodium acetate-imidazole buffer (10 mM, pH 7.0). A hand-held homogenizer (M133/1281 − 0, BioSpec Products Inc., Bartlesville, OK, USA) was used for 2 min to mix the oil, surfactant, and buffer to form a coarse emulsion. The coarse emulsion was then passed through a microfluidizer (M−110L Microfluidics, Newton, MA, USA) three times at 39 psig to further reduce the particle size. The microfluidizer chamber and coil were kept cold using an ice-water mixture to minimize lipid oxidation. The final emulsion samples without adding the extracts were used as a control group.

A rotary evaporator at 35 ℃ was used to concentrate the phenolic extracts and their acid-hydrolyzed counterparts, and their TPC was determined followed by addition of extract to the emulsions to obtain 400 and 800 μmol GAE/L. The samples were stirred with a magnetic stirrer at 300 rpm for 1 h at 4 ℃ to ensure homogeneous distribution. Then, 1 mL of emulsion samples were pipetted into 10 mL GC headspace vials (Supelco, Bellefonte, PA, USA), sealed with aluminum caps with PTFE/silicone septa, and kept in light-blocking containers at 37 ℃ during the oxidation study. At regular intervals, three vials from each treatment were examined to evaluate the formation of primary and secondary lipid oxidation products.

2.12. Physical characteristics of emulsions

The physical characteristics of emulsions were studied 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 and Decker (2023a). Both the particle size distribution and ζ-potential were determined during the storage period of the emulsions. To optimize the attenuation of measurements, each emulsion was diluted 100-fold with a sodium acetate-imidazole buffer (10 mM, pH 7.0). The measurements were carried out at 25 ℃ with a 60-second equilibration time.

2.13. Measurement of primary oxidation products (lipid hydroperoxides)

To track the formation of primary oxidation products in the prepared emulsions, lipid hydroperoxides were measured using the method developed by Shantha & Decker, (1994) with some modifications. Hydroperoxide measurements were conducted daily. Briefly, 300 μL of emulsions from the GC vials was added to 1.5 mL of isooctane:2-propanol solution (3:1, v/v) in test tubes. The mixture was vortexed for 30 s and then centrifuged for 3 min at 1785 × g (CL10 centrifuge, Thermo Fisher Scientific Inc., Waltham, MA, USA). Then, 200 μL of supernatant was added to 2.8 mL of a MetOH:1-butanol solution (2:1, v/v), followed by the addition of 30 μL of a 1:1 mix of 3.94 M ammonium thiocyanate and ferrous iron. The ferrous ion was prepared from a mixture of 0.144 M iron (II) sulfate solution with 0.132 M barium chloride in 0.4 M HCl at a 1:1 ratio, followed by centrifuging for 5 min at 1785 × g and collecting the supernatant. The samples were incubated for 20 min in the dark at room temperature. The absorbance of the samples was measured at 510 nm. Samples with high absorbances (>1.2) were diluted 10 times with MetOH/1-butanol (2:1, v/v) before the measurements. The results were expressed as μmol/kg oil using a standard curve prepared with different concentrations of cumene hydroperoxide (0.1–0.5 mM).

2.14. Measurement of secondary oxidation products (headspace hexanal)

To track the formation of secondary oxidation products in the prepared emulsions, headspace hexanal was measured as described by Culler et al., (2022). The analysis was performed via solid-phase microextraction coupled to a gas chromatograph with a flame ionization detector (SPME-GC-FID) using a Shimadzu GC-2010 with a Shimadzu AOC-6000 autosampler (Shimadzu, Kyoto, Japan). GC vials were heated in an autosampler at 55 ℃ for 10 min. After heating, the volatiles were absorbed on the surface of a 50/30 mm divinylbenzene/carboxen/polydimethylsiloxane SPME fiber needle (Supelco, Bellefonte, PA, USA), which was inserted for 2 min into the GC vials at the end of the heating process. The fiber needle carrying the volatile compounds was placed into the injector port of the GC where they are desorbed at 250 ℃ for 3 min and separated on a 30 m × 0.32 mm i.d. × 1 μm fused silica capillary Equity-1 column for 10 min using helium as the carrier gas. The oven temperature was 65 ℃, while the FID was at 250 ℃ and a split ratio of 1:7 was used. The results were expressed as μmol/kg oil using a standard curve prepared with 0–200 μM authentic hexanal.

2.15. Statistical analysis

All the analyses were carried out in triplicate (n = 3) for the statistical analysis, and the results were expressed as mean ± standard deviation. The data collected from the assays were processed using IBM SPSS Statistics (Version 20.0, SPSS Inc, Chicago, IL, USA) and subjected to a one-way Analysis of Variance (ANOVA) with a significance level and confidence level of 0.05 and 95 %, respectively. The comparisons were made using Tukey's test. Lipid hydroperoxide and headspace hexanal lag phases were determined based on Dunnett's test. The lag phase was defined as the first data point that differed statistically from the day zero reading of the experiment ($p \leq 0.05$). In metal chelating activity tests, an independent *t*-test was utilized to compare the data between the samples before and after acid hydrolysis. Origin Pro 2022 (Northampton, MA, USA) was used for graphing the data.

3. Results and discussion

3.1. Chemical properties of parsley leaves

Plant-derived bioactive compounds are susceptible to degradation during processing and storage, influenced by various factors such as enzymes, water activity, sugars, lipids etc. (Maldonado-Astudillo et al., 2019). Therefore, it is crucial to know the chemical composition of the

Table 1
Proximate composition and soluble sugars of freeze-dried parsley leaves.

Component	Content
Water activity	0.49 ± 0.02
Moisture (%)	9.37 ± 0.68
Dry matter (%)	90.63 ± 0.68
Ash (g/100 g DM)	13.74 ± 0.25
Crude protein (g/100 g DM)	30.32 ± 0.34
Crude fiber (g/100 g DM)	27.53 ± 0.53
Crude lipid (g/100 g DM)	6.14 ± 0.20
Glucose (g/100 g DM)	4.54 ± 0.06
Fructose (g/100 g DM)	1.54 ± 0.01

Data is reported as mean ± SD (n = 3).

plant materials before using them for the recovery of bioactive compounds. Table 1 shows the proximate composition, and soluble sugar content of freeze-dried parsley leaves.

Since water content highly impacts the degradation of sensitive compounds, knowing the moisture content and water activity (a_w) of raw material is essential (Ebrahimi et al., 2022). Despite the low moisture content of the freeze-dried parsley leaves, a_w was higher than expected. Maldonado-Astudillo et al., (2019) reported that polyphenols are more extractable in higher water activities (aw $\geq$ 0.5) due to higher content of free phenolic contents. A high water activity also enhances the likelihood of extracting polyphenols in their soluble glycosidic form, resulting in an extract with the potential to yield a substantial quantity of free phenolic compounds when subjected to acid hydrolysis. Furthermore, a high a_w positively influences the activity of the phenylalanine ammonia-lyase enzyme, which in turn can increase the content of polyphenols, since it is a key enzyme in phenolic biosynthesis during the storage of fruits and vegetables (Maldonado-Astudillo et al., 2019; Oufedjikh, Mahrouz, Amiot, & Lacroix, 2000). Therefore, the a_w of the parsley leaves in the present research, which is close to 0.5, could help increase the efficiency of the extraction process.

In case of a high lipid content, removing lipids can improve the overall efficiency of the extraction process. Thus, estimating the lipid content in the plant material is an essential step in the extraction process (Ebrahimi et al., 2022). Defatting is normally done before the extraction of phenolic compounds if the lipid content of plant tissue is high (Đurović et al., 2018; Teh & Birch, 2014). In the present paper, the lipid content of parsley leaves is negligible (6.14 $\pm$ 0.20 g/100 g DM), making it unnecessary to remove the lipids from the extracts.

The high protein and fiber content of parsley leaves makes it difficult to extract all the phenolic compounds present in the plant tissue since a considerable quantity of polyphenols may remain unextractable due to the interaction of proteins and polysaccharides with polyphenols by forming ionic, hydrogen, or hydrophobic bonds. This may affect the stabilization and bioactivity of polyphenols and potentially influence the efficiency and effectiveness of the extraction process. Therefore, it is prominent to consider that the usage of conventional extraction methods is not suitable for this kind of plant matrix, and there is a need to employ a novel extraction method like UAE with the capability of releasing the unextractable phenolic compounds (Ding, Morozova, Scampicchio, & Ferrentino, 2020; Wu et al., 2020). Đurović et al., (2018) reported that when UAE is used as the extraction technique, some phenolic compounds can be released even without alkaline hydrolysis, which is normally needed for the release of insoluble-bound phenolic acids. Therefore, UAE was chosen as the preferred extraction method for this study.

3.2. Optimization of the acid hydrolysis of parsley phenolic extract

TPC and DDPH radical scavenging activity of extracts were monitored to obtain the best condition of acid hydrolysis. Fig. 1 shows that TPC (a) and DPPH radical scavenging activity (b) of extracts increased due to acid hydrolysis for up to 2 h, after which the latter decreased when the concentration of HCl was 0.6 and 1.2 M. While the decrease in DPPH radical scavenging activity of the sample hydrolyzed with 0.6 M HCl after 2 h was not significant ($p > 0.05$), a significant decrease ($p < 0.05$) was observed with 1.2 M HCl concentration. This decline may be attributed to prolonged exposure to high temperature and harsh acidic conditions, leading to the degradation of some phenolic compounds and other antioxidant compounds (e.g., chlorophylls and carotenoids) (Nuutila et al., 2002; Pareek et al., 2017; Vargas-León et al., 2018). For instance, Yildiz et al., (2008) conducted acid hydrolysis on parsley extracts and reported a nearly halved concentration of myricetin after a 4-hour hydrolysis compared to 2 h. Given that some phenolic compounds act as potent antioxidants, synergizing with other antioxidant compounds (Bayram et al., 2023), their reduced concentration may lead to diminished antioxidant activity despite a higher TPC in the extracts hydrolysed for a longer time (Yildiz et al., 2008).

Total phenolic concentration and DPPH radical scavenging activity were greater at 0.6 and 1.2 M HCl at 2 h of hydrolysis compared to 0.3 M HCl, but there were no significant differences between samples hydrolyzed with 0.6 and 1.2 M HCl. It is reported that increasing the acid concentration would decrease the time for the hydrolysis of flavonoid glycosides (Tu et al., 2017). However, due to the insignificant increase in the TPC of extract exposed to 1.2 M HCl compared to 0.6 M HCl, the extract hydrolyzed with 0.6 M HCl was preferred as the best acidic condition for the reaction. Moreover, the antioxidant activity of the extracts reached a peak when the acid hydrolysis was done for 2 h. Therefore, the acid-hydrolyzed extract using 0.6 M HCl for 2 h was chosen to be evaluated for the next steps of the analysis.

The increase in both total phenolic concentration and DPPH radical scavenging activity in the acid-hydrolyzed extracts is not unexpected as antioxidant activity would be expected to be greater at greater phenolic concentrations (Cao et al., 2020; Ebrahimi & Lante, 2021). This data is consistent with those obtained by Shofian et al. (2011), who evaluated the correlation between the total phenolic concentration and antioxidant activity of different fruit extracts. The higher TPC and DPPH radical scavenging activity after the acid hydrolysis could be related to the increase of free phenolic compounds due to the break of the glycosidic bond of their soluble conjugate form, which results in the release of the free form of polyphenols and simple sugars (Đurović et al., 2018; Li et al., 2020). An increase in the antioxidant activity and the content of

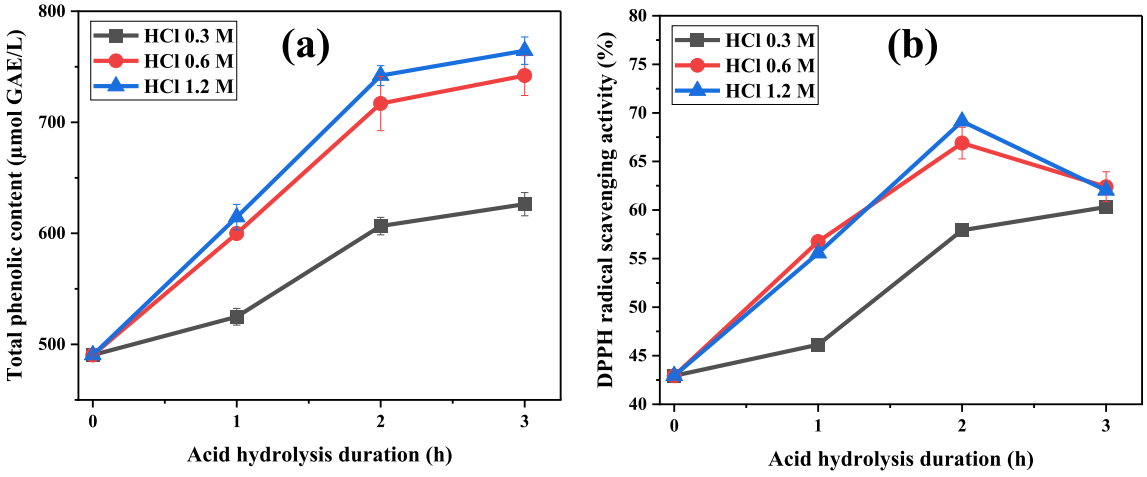

Fig. 1. Total phenolic content (a) and DPPH radical scavenging activity (b) of extracts during different conditions of acid hydrolysis Data points show mean $\pm$ SD (n = 3). Some error bars are within the data points.

free polyphenols in the acid-hydrolyzed phenolic extracts has been reported by several studies (Komes et al., 2011; Sani, Iqbal, Chan, & Ismail, 2012).

3.3. Phenolic composition, DPPH radical scavenging activity, and chlorophyll content of the extracts

Table 2 shows the phenolic content and DPPH radical scavenging activity of the extracts before and after a 2-h acid hydrolysis with 0.6 M HCL at 80 ℃. The concentrations of catechin, vanillic acid, and quercetin increased significantly ($p \leq 0.05$) after acid hydrolysis. Moreover, myricetin and gallic acid appeared only in acid-hydrolyzed extracts. The release of free phenolics after breaking their glycosylated form by acid hydrolysis can contribute to an overall increase in TPC, TFC, and DPPH radical activity of the acid-hydrolyzed extracts, which subsequently leads to an increased antioxidant potential. Fig. 2 (a) shows the mechanism of acid hydrolysis in the release of aglycones, and the chemical structure of the aglycones released after acid hydrolysis are shown in Fig. 2 (b).

Myricetin is a polyhydroxy flavonol compound that has a high antioxidant activity (Song et al., 2021). Given the reported insolubility of myricetin in water (Adeib Idris, Markom, Abd Rahman, & Mohd Ali, 2019) and solubility of glycosides in water, it is reasonable to anticipate that myricetin exists in glycosidic form in the parsley leaves. This could be a reason why myricetin was not detected in the extracts before acid hydrolysis. Tolosa et al., (2018) reported that acid hydrolysis with 2.5 M HCl at 75 ℃ for 60 min can effectively break the glycosidic bound of myricetin-3-O-rhamnoside, which is a glycoside of myricetin. The release of myricetin after acid hydrolysis in the present research can significantly contribute to the higher DPPH radical scavenging activity in the acid-hydrolyzed extract. Mehrdad et al., (2009) reported that acid hydrolysis could be used for rapid extraction of myricetin, quercetin, and kaempferol in *Rhus coriaria* L, through the conversion of glycoside form of flavonoids to aglycones.

The increase in the recovery of quercetin and catechin during the acid hydrolysis of onion leaves and spinach with 1.2 M HCl at 80 ℃ for 2 h was reported by Nuutila et al., (2002). The conversion of quercetin glycosides to aglycones by acid hydrolysis has also been confirmed by Tu et al., (2017). These are consistent with the results obtained in the present study. Notably, the rise in the concentration of catechin was substantially higher than other compounds. The release of gallic acid and the significant increase in the content of catechin could be related to each other. Acid hydrolysis may break the bond between catechin and gallic acid in the galloylated catechins and lead to a significant increase in the content of catechin, and the appearance of gallic acid. The bioavailability of galloylated catechins is very poor due to their large molecular size and large number of hydrogen bonds (Lante & Friso, 2013). Therefore, the possible conversion of galloylated catechins into gallic acid and catechin by acid hydrolysis could lead to an increase in their bioavailability.

Table 2
Phenolic content and DPPH radical scavenging activity of the extracts before and after a 2-h acid hydrolysis with 0.6 M HCL at 80 ℃.

Compounds	Retention time (min)	Concentration (μmol/L)	
		Before acid hydrolysis	After acid hydrolysis
Gallic acid	2.82	ND	20.87 ± 0.85
Catechin	5.13	45.43 ± 4.63[a]	307.83 ± 16.55[b]
Vanillic acid	6.74	5.04 ± 3.17[a]	20.30 ± 1.64[b]
Myricetin	16.16	ND	14.08 ± 0.06
Quercetin	23.24	2.10 ± 0.04[a]	3.82 ± 0.20[b]
TPC (μmol GAE/L)	–	490.44 + 0.89[a]	716.92 ± 24.43[b]
TFC (μmol QE/L)	–	356.83 ± 3.55[a]	560.45 ± 15.88[b]
DPPH radical scavenging activity (%)	–	42.93 + 0.36[a]	66.89 ± 1.63[b]

Data is reported as mean ± SD (n = 3).
Different letters show that there is a significant difference ($p \leq 0.05$) in each row according to an independent *t*-test.

Another vital factor controlling the antioxidant activity of phenolic extracts is chlorophyll content. The presence or absence of chlorophylls in the phenolic extracts can be correlated to their ability to inhibit lipid oxidation. When the extraction of phenolic compounds is conducted using organic solvents such as EtOH, chlorophylls are normally co-extracted. Since chlorophylls have both prooxidant and antioxidant activity, understanding their content in the extracts is important (Ebrahimi, Shokramraji, Tavakkoli, Mihaylova, & Lante, 2023). Fig. 2 (c) shows the spectrum of the optimized extracts before and after acid hydrolysis (0.6 M HCl, 2 h) in wavelengths ranging from 400 to 700 nm. The three distinct peaks at 436, 470, and 665 nm are the major absorbance peaks of chlorophyll *a*. The chlorophyll *a* and b concentrations in the extract before acid hydrolysis were 56.38 ± 2.06 and 33.93 ± 3.39 μg/g parsley, respectively. However, the extract after acid hydrolysis did not contain considerable amounts of chlorophyll *a* and b, but instead had pheophorbide, which has absorbance peaks at 408, 506, 537, 608, and 666 nm (Ge et al., 2023; Taniguchi & Lindsey, 2018). Pheophorbide forms due to the loss of magnesium from chlorophyll (Matsumoto et al., 2021), and it is known to be produced in high temperatures and acidic environments (Ebrahimi et al., 2023). It is reported that chlorophylls under exposure to light can act as prooxidants in oil-in-water emulsions (T. S. Kim, Decker, & Lee, 2012). There is no research investigating the possible effect of pheophorbide a on lipid oxidation in emulsions. However, the high antioxidant activity of pheophorbide a has been proven (Marcheafave et al., 2019).

3.4 Metal chelating activity and ferric-reducing activity of extracts

Transition metals are among the most ubiquitous prooxidants in food matrices owing to their power to form alkyl radicals and decompose lipid hydroperoxides into peroxyl and alkoxyl radicals (Bayram et al., 2023). Metal chelators impede lipid oxidation by controlling the redox cycling of metal ions, sterically preventing the contact between metal ions and hydroperoxides, reducing the solubility of iron, and blocking all metal-binding sites (Bayram et al., 2023). Lipid hydroperoxides accumulate at the O/W interface in emulsions due to their surface activities and are then decomposed into free radicals by prooxidant metals in the surrounding aqueous phase, thereby leading to further oxidative reactions (Tian et al., 2021). Polyphenols could act as metal chelators by forming complexes with residual free ions of metals (Fe^{2+}, Fe^{3+}, and Cu^{2+}) and decreasing the content of free ions (Kolev, 2022).

Metal chelators are commonly utilized in the food industry to reduce the prooxidant effect of transition metals (Bayram et al., 2023). Therefore, the natural extracts obtained from parsley leaves in the present work were tested to determine their ability to chelate transition metals and to reduce transition metals, which can increase their prooxidant activity (Table 3).

Cupric (Cu^{2+}) and ferrous (Fe^{2+}) chelating activity of extracts at all concentrations increased significantly after acid hydrolysis which could be due to more phenolics in the extracts or the release of chelating groups upon formation of the aglycones. However, the ferrous chelating activity in both extracts was dose-dependent and decreased significantly at 800 μmol GAE/L compared to lower concentrations. This could occur if more than one phenolic compound was chelating metals at higher phenolic-to-metal ratios. Multiple chelators have been shown to bind iron when chelator concentrations are high (e.g. EDTA) (Tian et al., 2022).

Ferric-reducing activity shows the concentration of ferrous ions (Fe^{2+}) formed by reducing ferric ions (Fe^{3+}). In the acid-hydrolyzed extract, the ferric-reducing activity increased significantly, which

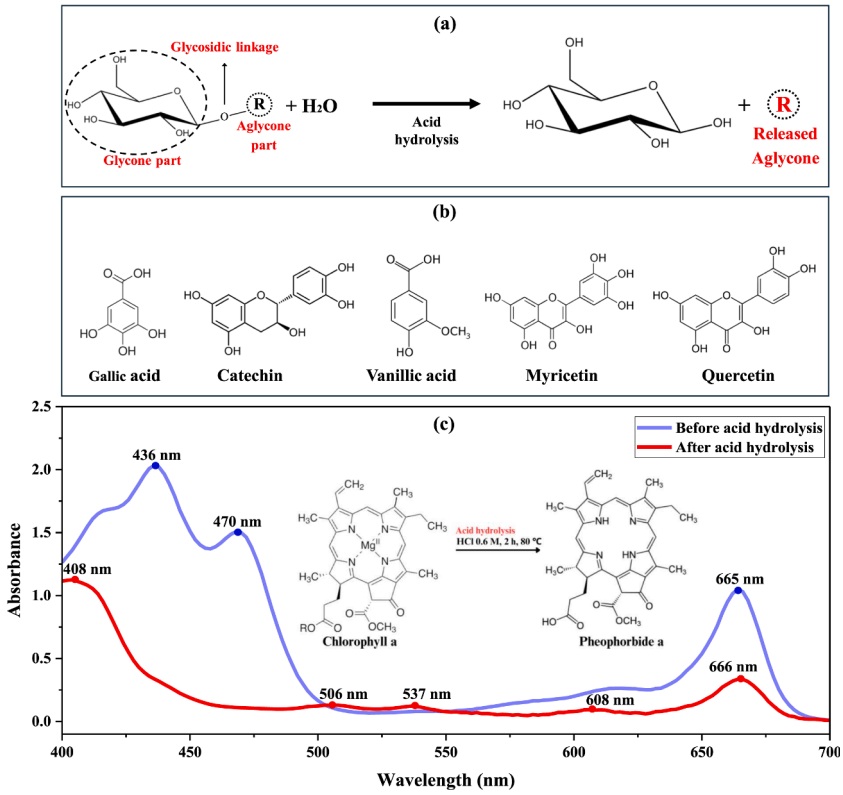

Fig. 2. (a) Mechanism of acid hydrolysis in the release of phenolic compounds, (b) Chemical structure of the aglycones detected in the parsley extracts, and (c) Spectrum of the optimized extracts before and after acid hydrolysis (0.6 M HCl, 2 h) in the wavelengths ranging from 400 to 700 nm.

Table 3
Metal chelating activity of extracts in different concentrations, before and after a 2-h acid hydrolysis with 0.6 M HCL at 80 ℃.

TPC (μmol GAE/L)	Cu^{2+} chelating activity (%)		Fe^{2+} chelation activity (%)		Ferric reducing activity(μmol FE/L)	
	Before acid hydrolysis	After acid hydrolysis	Before acid hydrolysis	After acid hydrolysis	Before acid hydrolysis	After acid hydrolysis
100	54.27 ± 2.01^{Aa}	72.94 ± 0.55^{Ab}	14.08 ± 0.21^{Aa}	16.12 ± 1.90^{Ab}	12.40 ± 0.63^{Aa}	13.91 ± 0.01^{Aa}
200	66.69 ± 1.41^{Ba}	79.33 ± 0.45^{Bb}	22.37 ± 2.79^{Ba}	24.03 ± 0.53^{Ba}	18.24 ± 1.91^{Ba}	42.12 ± 0.02^{Bb}
400	76.41 ± 1.07^{Ca}	90.89 ± 0.57^{Cb}	22.13 ± 1.63^{Ba}	36.04 ± 1.53^{Db}	38.14 ± 1.46^{Ca}	67.06 ± 0.03^{Cb}
800	86.75 ± 1.05^{Da}	91.60 ± 0.20^{Cb}	10.26 ± 2.61^{Aa}	28.62 ± 0.80^{Cb}	41.97 ± 1.67^{Ca}	42.65 ± 0.02^{Ba}

Data are presented as mean ± SD (n = 3). Different capital letters show that there is a significant difference ($p \leq 0.05$) among different concentrations, according to one-way ANOVA and Tukey's honestly significant difference (HSD) tests. Different small letters show that there is a significant difference ($p \leq 0.05$) between the samples before and after acid hydrolysis according to an independent *t*-test.

could be due to an increase in the reducing groups in the aglycones compared to their glycosylated counterparts. However, the ferric-reducing activity of the acid-hydrolyzed extract decreased significantly at 800 μmol GAE/L, but still, it was higher than the unhydrolyzed extracts. As previously discussed, acid hydrolysis released strong antioxidants, such as myricetin, in the extract. It was reported that myricetin can reduce the ferric ion (Bayram et al., 2023). Therefore, the higher reducing activity of the acid-hydrolyzed extracts could be due to the release of such aglycones. Nevertheless, just because a molecule binds to iron and copper does not mean it has antioxidant activity since reduced metals may have much stronger prooxidant activity (Eghbaliferiz & Iranshahi, 2016). Depending on the type and concentration of flavonoids used, flavonoid-iron complexes may make iron more soluble, facilitating iron access to the emulsion droplets (Bayram et al., 2023). If the metals are still able to undergo redox cycling in the flavonoid chelates, they may increase rather than decrease lipid oxidation reactions. For this reason, antioxidant activity should also be tested in the presence of oxidizing fatty acids.

3.5. Oxidative stability and physical properties of emulsions

The oxidative degradation of lipids in food products is closely related to their shelf-life and acceptability (Hu et al., 2022). In this study, the effect of parsley extracts on the oxidation stability of 2 % stripped soybean O/W emulsions was investigated by monitoring the formation of primary (lipid hydroperoxides; Fig. 3. a) and secondary (hexanal headspace; Fig. 3. b) oxidative products during the storage period. In these experiments, the particle diameter and ζ-potential did not change significantly until the lag phase of lipid oxidation (Fig. 4). No visible creaming was detected in any of the emulsions during the length of the storage studies. The physical stability of such an emulsion system for oxidation studies has been already reported by several papers (Bayram et al., 2023; Kaur, Bhatia, Bayram, Decker, & Phutela, 2023). Lv et al., (2021) added the extracts of Chinese quince into sunflower oil-in-water emulsions, and their results were consistent with the changes in zeta potential and particle size of emulsions in the present paper.

The lipid hydroperoxides and hexanal lag phases were used to determine oxidative stability since the lag phase is related to shelf-life. Control emulsions had a lag phase of 0 days for both hydroperoxides

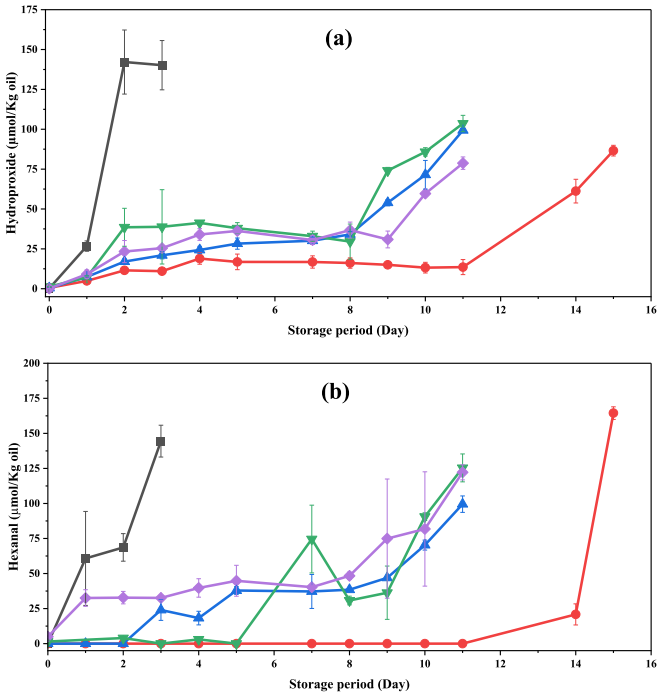

Fig. 3. Formation of lipid hydroperoxides (a), and headspace hexanal (b) in 2 % stripped soybean O/W emulsions (at pH 7.0) stabilized by Tween 20 during storage at 37 °C. Data points show mean ± SD (n = 3). Some error bars are within the data points. ■: Control emulsion, ●: Emulsion with 400 μmol GAE/L TPC of acid-hydrolyzed extract, ▲: Emulsion with 800 μmol GAE/L TPC of acid-hydrolyzed extract, ▼: Emulsion with 400 μmol GAE/L TPC of unhydrolyzed extract, ◆: Emulsion with 800 μmol GAE/L TPC of unhydrolyzed extract.

and hexanal. The emulsion containing 800 μmol GAE/L of the unhydrolyzed extract did not result in a major increase in both lipid hydroperoxide and hexanal compared to the control. However, the emulsion containing 400 μmol GAE/L TPC of acid-hydrolyzed extract had an 11-day lag phase for hexanal formation, showing the highest oxidative stability after the emulsion containing 400 μmol GAE/L TPC of unhydrolyzed extract, which had a 6-day hexanal lag phase. This supports the data showing that acid hydrolysis increases both DPPH radical scavenging and metal chelation. One of the reasons for the better functionality of the acid-hydrolyzed extract in inhibiting oxidation could be the presence of myricetin. It is reported that myricetin has a synergistic effect with other antioxidants (Bayram & Decker, 2023b; Bayram et al., 2023). Therefore, the generation of myricetin in the acid hydrolysis process can help regenerate oxidized antioxidants in the system, resulting in a higher antioxidative functionality. Moreover, the significant increase in the catechin content of acid-hydrolyzed extract is another effective factor. The phenolic hydroxyl groups on the B-ring of ungalloylated catechins and B- and D-rings galloylated catechins are essential for the antioxidant activity of phenolic extracts. These active groups can neutralize free radicals by contributing hydrogen atoms, resulting in the creation of less reactive phenoxyl radicals due to the delocalization of unpaired electrons around the phenyl ring (Tian et al., 2021).

The emulsion containing 800 μmol GAE/L of the acid-hydrolyzed extract showed lower hydroperoxide (0 days) and hexanal (2 days) lag phases compared to when its concentration was 400 μmol GAE/L. This happened while the acid-hydrolyzed extract at 800 μmol GAE/L had lower ferric-reducing activity. This discrepancy might be attributed to the different environments of the ferric-reducing test, which only included the water phase, whereas oxidation in the emulsions occurred within the lipid–water interface (Lv et al., 2021; Pinchuk, Shoval, Dotan, & Lichtenberg, 2012). It has been previously reported that higher polyphenol levels lead to more significant changes in surface chemistry (Tian et al., 2022). Oxidation products, such as radicals and hydroperoxides, can accumulate at the interface, accelerating lipid oxidation. Certain phenolic compounds possess surface activity or can attach to emulsifiers (e.g., Tween 20) via hydrophobic interactions, facilitating their absorption at the oil–water interface and potentially catalyzing further oxidation (Lv et al., 2021). Therefore, it is possible that the phenolic compounds released after acid hydrolysis may make the extract a prooxidant at high concentrations due to their increased ability to reduce ferric ions into ferrous ions. The factors transforming an antioxidant to a prooxidant could be the presence of prooxidant metal ions, the concentration and redox potential of antioxidants, and the location of oxidation inducers and phenolic compounds between the lipid and water phases (Pinchuk et al., 2012; Sotler et al., 2019). This suggests that a phenolic extract may demonstrate either prooxidant or antioxidant activity in different food systems, and further research is needed to elucidate these mechanisms.

4. Conclusion

Acid hydrolysis of phenolic extracts obtained from plant resources opens a new window on the recovery of specific antioxidant bioactive compounds. The findings of the present research provide valuable insights into the potential applications of acid hydrolysis of plant extract in the food industry, particularly for extending the shelf-life of food products and beverages with an oil-in-water emulsion system. Key findings include the successful conversion of glycosidic phenolic

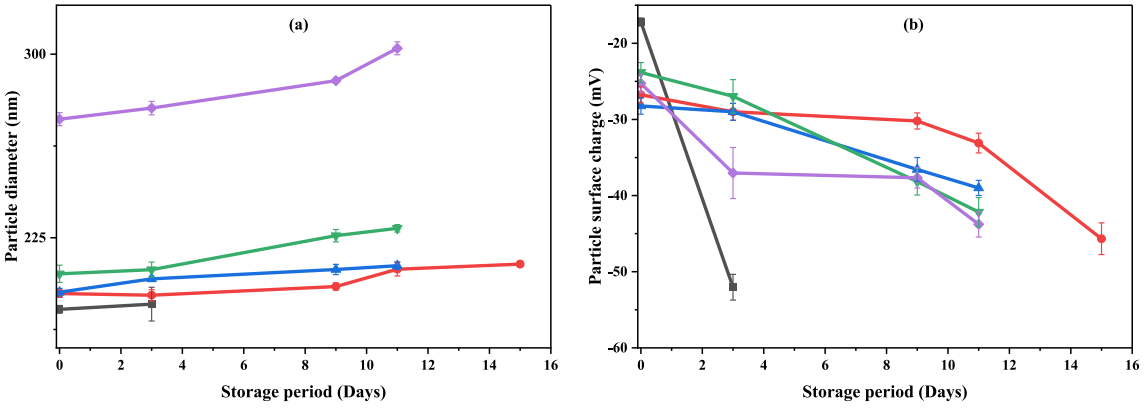

Fig. 4. Particle size (a), and particle surface charge (b) of emulsions. Data points show mean ± SD (n = 3). Some error bars are within the data points. ■: Control emulsion, ●: Emulsion with 400 μmol GAE/L TPC of acid-hydrolyzed extract, ▲: Emulsion with 800 μmol GAE/L TPC of acid-hydrolyzed extract, ▼: Emulsion with 400 μmol GAE/L TPC of unhydrolyzed extract, ◆: Emulsion with 800 μmol GAE/L TPC of unhydrolyzed extract.

compounds to aglycones by acid hydrolysis, which resulted in increased DPPH radical scavenging activity, iron and copper chelation, and inhibition of lipid hydroperoxide and hexanal formation in oil-in-water emulsions.

However, acid hydrolysis also increased the iron-reducing capacity of the parsley extracts, which could explain why higher concentrations of the extracts had less antioxidant activity in the oil-in-water emulsions. This may limit the application of acid-hydrolyzed extracts in high concentrations in food systems where metals are the major prooxidants (e.g., high moisture foods). Therefore, it is crucial to find the balance between free radical scavenging and iron reducing activity based on the nature of food system, as well as the phenolic profile of the natural extract to reach maximum antioxidant activity. To gain deeper insights into the impact of acid hydrolysis on the inhibition of lipid oxidation, it is recommended to broaden the scope in the future research by evaluating the effects of this post-treatment on extracts derived from various plants with differing phenolic compositions.

CRediT authorship contribution statement

Peyman Ebrahimi: Conceptualization, Writing – original draft, Methodology, Investigation, Data curation, Formal analysis, Writing – review & editing. **Ipek Bayram:** Methodology, Data curation, Writing – review & editing, Validation. **Anna Lante:** Conceptualization, Writing – review & editing, Supervision. **Eric A. Decker:** Conceptualization, Data curation, Writing – review & editing, Supervision.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.114452.

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