SPRINGER NATURE Link

Log in

Development of a copper-based accelerated oxidation protocol for evaluating the oxidative stability of mayonnaise enriched with hydrophilic and lipophilic grape seed extracts

Original Paper Open access Published: 02 October 2026

Volume 252, article number 397 (2026) Cite this article

You have full access to this open access article



European Food Research and Technology

Aims and scope →

Submit manuscript →

Sonia Medouni-Adrar, Amina Kessaissia, Lamia Medouni-Haroune, Amel Aidli-Ouadah, Aida Mekhoukhe, Ouahiba Soufi-Maddi, Sabine Chabane, Salima Dib-Djafri, Athmane Azouaou, Samir Hadjal & Javier S. Perona

201 Accesses Explore all metrics →

Abstract

Lipid oxidation limits the shelf life of oil-in-water emulsions such as mayonnaise, while conventional accelerated tests based on high temperatures may destabilize emulsion structure and distort oxidation kinetics. This study proposes a $CuSO_4$-assisted accelerated oxidation protocol developed under mild thermal conditions (40 °C), in which the catalyst concentration was systematically screened to establish operating conditions that generated a clear induction phase followed by a quantifiable propagation phase within a 96-h experimental window while maintaining the mayonnaise matrix without visible phase separation throughout the oxidation experiment. Mayonnaise was enriched with hydrophilic grape seed extracts (HE50–150 ppm), a lipophilic grape seed oil macerate

(OM, 2.5%), or dual-phase combinations (LHE50–150). Grape seed extracts were characterized for total phenolics, tocopherols, proanthocyanidins, and antioxidant activity using the DPPH radical scavenging assay and the ferric reducing antioxidant power (FRAP) assay. Oxidative stability was assessed using peroxide value (PV) kinetics, UV indices (K232, K270), titratable acidity (TA), lag-time analysis, and kinetic modeling. The predicted time required to reach a common PV threshold (10 meq O_2/kg), extrapolated from the fitted kinetic model beyond the experimental observation period, was used as a comparative indicator of oxidative stability among formulations. Together with UV lag time, this parameter provided the greatest discrimination among formulations. Based on these model-predicted threshold times, dual-phase formulations (LHE100–150) and HE150 exhibited 2.3–2.6-fold longer times to reach the selected PV threshold than the control. Overall, the proposed protocol offers a realistic and practically applicable alternative to high-temperature accelerated tests, and dual-phase antioxidant distribution represents a promising clean-label strategy for stabilizing mayonnaise that warrants further evaluation in other emulsion-based food systems.

Explore related subjects

Discover the latest articles, books and news in related subjects, suggested using machine learning.

Fatty alcohols Fatty acids Lipid peroxides

Non-photochemical quenching Food Chemistry Proteins

Oxidative Stability in Edible Oils

Introduction

Lipid oxidation is a chain reaction that alters food structure and reactivity, producing hydroperoxides and aldehydes [1], which are major contributors to quality deterioration affecting flavor, color, texture, and nutritional value [2, 3]. This phenomenon is especially critical in oil-in-water emulsions such as mayonnaise, where the large interfacial area accelerates oxidation [4]. Mayonnaise is highly susceptible to lipid oxidation due to its

composition and interfacial dynamics [5], leading to reduced shelf-life and sensory quality [4]. Oxidative stability of emulsified systems depends on oil composition, emulsifier type, and micellar dynamics, which govern the distribution of pro- and antioxidants [6].

To limit lipid oxidation, synthetic antioxidants and metal chelators, including BHA, BHT and TBHQ, are widely used in food formulations in accordance with Codex Alimentarius provisions for food additives [7]. Although effective, their potential health risks remain controversial, and growing clean-label demand has driven interest toward natural alternatives [8]. In this regard, plant polyphenols from herbs, spices, seaweeds, and fruit by-products have shown strong antioxidant potential [9]. In particular, grape seeds, a major by-product of the juice and wine industries, are particularly rich in hydrophilic and lipophilic antioxidants [10]. Phenolic compounds are mainly concentrated in the seeds, which contain 60–70% of the total polyphenols present in the grape [11] with high concentrations of flavan-3-ols, catechins, and proanthocyanidins [12]. Grape seeds also contain 7–18% of oil [13], which is rich in tocopherols and tocotrienols [14], making them valuable natural antioxidants for foods [15]. Their dual hydrophilic–lipophilic composition favors interfacial protection in emulsions.

Traditionally, accelerated oxidation tests such as the Schaal oven test (60–70 °C), Rancimat (100–120 °C), or oxygen uptake methods have been widely used to evaluate lipid stability in food systems [3]. However, approaches relying on severe thermal conditions are poorly suited to mayonnaise-type emulsions, as the high temperatures required to accelerate oxidation may simultaneously destabilize the emulsion, causing oil phase separation and generating artefactual oxidation kinetics. Reducing the incubation temperature to ~40 °C better preserves emulsion integrity [16], but oxidation kinetics under these mild conditions remain extremely slow, making direct thermal assessment impractical within a short experimental timeframe.

Metal-catalyzed oxidation using Cu^{2+} or Fe^{2+} is a well-established approach in lipid oxidation research [17, 18]. In the present study, $CuSO_4$ was selected as a catalytic initiator because it enables measurable and reproducible oxidation under mild thermal conditions (40 °C), thereby avoiding the elevated temperatures that would otherwise promote visible phase separation and alter the native mayonnaise matrix. Accordingly, the proposed protocol combines mild thermal conditions with controlled catalytic initiation to generate

reproducible and interpretable oxidation kinetics within a manageable experimental timeframe.

The specific contribution of this study lies in the development and establishment of a $CuSO_4$-assisted accelerated oxidation protocol for mayonnaise systems, enabling controlled and consistent oxidation kinetics suitable for the comparative evaluation of antioxidant efficacy. Unlike conventional accelerated oxidation methods, which rely primarily on elevated temperatures to accelerate lipid oxidation, the proposed protocol maintains structurally non-disruptive thermal conditions (40 °C), while $CuSO_4$ provides the controlled catalytic initiation required to accelerate oxidation within the native mayonnaise matrix.

In this context, the present study was designed to bridge this methodological gap and evaluate the performance of natural antioxidant systems within a realistic oxidation framework. Accordingly, it aimed to: (i) develop and establish a $CuSO_4$-based accelerated oxidation protocol for evaluating the oxidative stability of mayonnaise under controlled catalytic and thermal conditions; (ii) characterize the hydrophilic grape seed extract and lipophilic grape seed oil macerate in terms of total phenolics, tocopherols, and antioxidant activity; and (iii) assess the individual and combined antioxidant effects of both systems on mayonnaise oxidative stability through modeling peroxide value (PV) and titratable acidity (TA) kinetics and spectrophotometric monitoring (K232, K270).

Materials and methods

Preparation of lipophilic and hydrophilic grape seed extracts

Preparation of plant material

Grape berries (*Vitis vinifera* L., Red Globe) were sorted, washed, and halved to collect seeds. Seeds were shade-dried at ambient temperature for 7 days until constant weight was achieved, ground in electric blender (Profi-Cook, PC- KSW 1021 N, Germany) to a fine powder (≤ 500 μm), and stored in airtight glass jars at 4 °C, protected from light and humidity until use.

Extraction of hydrophilic compounds

Hydrophilic compounds were extracted according to a previously described method [19]. In brief, 10 g of grape seed powder were mixed with 100 mL of 70% ethanol (v/v) under magnetic stirring for 20 min at room temperature. After centrifugation (5000 rpm, 15 min), the residue was re-extracted two to three times. The supernatants were pooled, concentrated by rotary evaporation, freeze-dried (Christ, Alpha 1–4 LD plus, Osterode am Harz, Germany), and stored at 4 °C in the dark until use.

Extraction of lipophilic compounds

Lipophilic compounds (including tocopherols, carotenoids, and some polyphenols) were transferred to soybean oil suitable for emulsions such as mayonnaise by solid–liquid extraction (maceration) [20]. Briefly, 25 g of grape seed powder (≤ 500 μm) was mixed with 200 mL refined soybean oil without added antioxidants (Cevital industrial complex, Bejaia, Algeria) and macerated for 10 days at room temperature in a dark, dry place. No detailed particle size distribution analysis was performed. During maceration, the mixture was manually stirred twice daily for approximately 3 min to maintain homogeneous solid–liquid contact. After maceration, the oil phase was separated from the solid residue by centrifugation at 5000 rpm for 15 min at 25 °C, and the clear supernatant was collected and used as OM. The maceration duration was selected empirically based on preliminary trials to achieve effective extraction under mild conditions without heat treatment.

Characterization of grape seed extracts

Preparation of extracts for analysis

The dry hydrophilic extract obtained from ethanolic extraction was reconstituted in acidified ethanol (ethanol: water: acetic acid, 70:30 v/v) adjusted to pH = 3.5 with acetic acid prior to analysis.

Phenolic compounds were extracted from the grape seed OM according to a previously described method [21], with minor modifications. Briefly, 1 g of sample was extracted three times with 80% methanol, followed by vortexing and centrifugation to ensure exhaustive recovery of phenolics.

Determination of total phenolic content

Total phenolic content (TPC) was measured by the Folin–Ciocalteu method [22] with minor modifications. 200 µL extract was mixed with 1.5 mL Folin–Ciocalteu reagent (1:10), then 1.5 mL 6% Na_2CO_3 added. After 60 min at room temperature, absorbance was read at 760 nm. Results expressed as mg gallic acid equivalents per 100 g dry weight of grape seed powder (mg GAE/100 g DW) for hydrophilic extract and per kg OM (mg GAE/kg OM).

Determination of condensed tannins (proanthocyanidins)

Proanthocyanidins (PA) were quantified using the acid butanol method adapted from a previously described procedure [23]. 0.25 mL of extract was mixed with 2 mL ferric sulfate–butanol–HCl reagent, heated at 95 °C for 50 min, cooled, and absorbance recorded at 530 nm. Results expressed as mg cyanidin-3-glucoside equivalents per 100 g dry weight of grape seed powder (mg C3GE/100 g DW).

Quantification of total tocopherols

Total tocopherols (TTP) in grape seed extracts were measured via a redox colorimetric method [24]. 100 µL OM was diluted in 10 mL acetone; 1 mL of this solution was mixed with 4 mL 0.01% potassium ferricyanide and 2 mL 0.01% ammonium iron (III) sulfate. pH was adjusted to 4 with 0.1 M HCl, volume completed to 10 mL with methanol. After 10 min at room temperature, absorbance at 743 nm was measured. Results expressed as mg α-tocopherol equivalents per kg OM (mg α-TE/kg OM).

Evaluation of antioxidant activity

DPPH* radical scavenging assay

The antioxidant activity of grape seed extracts was evaluated using the DPPH· radical scavenging assay following a previously described procedure [25]. 100 µL of each extract was added to 2.9 mL of a freshly prepared DPPH· solution (6×10^{-5} mM in methanol). After 30 min of incubation in the dark at room temperature, the decrease in absorbance was measured at 515 nm. Antioxidant capacity was determined from a calibration curve and expressed as micromoles of Trolox equivalents per 100 g of dry weight of grape seed powder for the hydrophilic extract and per kilogram of OM (µmol TE/100 g DW and µmol TE/kg OM, respectively).

Ferric reducing antioxidant power (FRAP) assay

The reducing power of the extracts was evaluated using the FRAP assay following a previously described procedure [26]. In this procedure, 1 mL of extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide solution (1%). The mixture was incubated at 50 °C for 20 min. After incubation, 2.5 mL of trichloroacetic acid (10%) was added, and the solution was centrifuged at 5000×g for 10 min. An aliquot of 2.5 mL of the resulting supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%). The absorbance of the green-colored complex formed was measured at 700 nm. The reducing antioxidant capacity was determined from a calibration curve and expressed as milligrams of Trolox equivalents per 100 g of dry weight of grape seed powder for the hydrophilic extract and per kilogram of OM (mg TE/100 g DW and mg TE/kg OM, respectively).

Preparation of the reference mayonnaise formulation

Mayonnaise ingredients were obtained from commercial and industrial suppliers. All mayonnaise formulations were prepared using refined soybean oil free of added antioxidants (Cevital industrial complex, Bejaia, Algeria; see Sect. 2.1.3), ensuring that the observed oxidative stability differences were attributable solely to the grape seed enrichments tested. Fresh eggs, vinegar, salt, sugar, and mustard were purchased from the local market. Potable water was boiled for microbial safety and cooled before use.

Emulsification was performed using a domestic hand-held blender (SEB, Type HB4061, 450 W, France) operating at a single fixed speed. Egg yolk was first placed in the mixing bowl, followed by gradual incorporation of the aqueous phase. Soybean oil was then added slowly under continuous mixing for approximately 3 min until formation of a stable oil-in-water emulsion.

A preliminary mixture design study (Extreme Vertices design) was conducted to optimize the proportions of the three main components (soybean oil, egg yolk, and aqueous phase); the detailed methodology and results are provided in the Supplementary data. The selected reference formulation comprised 72.8% soybean oil, 9.2% egg yolk, and 18% aqueous phase (w/w). The aqueous phase consisted of potable water (14.5%), white vinegar (2.0%), salt (1.0%), sugar (1.5%), and mustard (0.5%), adapted from [27] with modifications.

Preparation of mayonnaise samples for the study

The reference formulation described in Sect. 2.3 was used to prepare eight mayonnaise samples for evaluating the effect of grape seed enrichment on oxidative stability. The control (CTRL) contained no extract. The lipophilic sample (LE) included 2.5% OM, selected as the maximum incorporation level compatible with acceptable organoleptic properties of the emulsion based on preliminary trials. Three hydrophilic samples (HE50, HE100, and HE150) were prepared by incorporating dry hydrophilic extract at 50, 100, and 150 ppm (mg/kg mayonnaise), respectively, into the aqueous phase. These concentration levels were selected based on preliminary experiments and on ranges commonly reported for polyphenol enrichment in mayonnaise and emulsion systems [28, 29]. Three dual-phase samples (LHE50, LHE100, LHE150) combined 2.5% OM with the same hydrophilic extract levels.

Oxidative stability monitoring of mayonnaise under accelerated aging conditions

Thermal incubation at 40 °C (preliminary assessment)

A preliminary thermal test, based on the Schaal oven principle, was conducted to accelerate lipid oxidation in mayonnaise and evaluate its susceptibility to oxidative degradation. Following a previously described protocol [30] samples were incubated at 40 °C. Peroxide value (PV) and titratable acidity (TA) were monitored at decreasing frequency over time : daily at first, then every two days, and later every five days. If thermal stress alone proved insufficient to generate measurable oxidation within a practically tractable timeframe, a $CuSO_4$-based catalytic approach was planned as a complementary strategy to initiate controlled oxidation while maintaining the mild thermal conditions required to preserve emulsion integrity.

$CuSO_4$-accelerated oxidation at 40 °C

$CuSO_4$ accelerates lipid oxidation by providing Cu^{2+} ions that catalyze the decomposition of lipid hydroperoxides into reactive radical species, thereby initiating and propagating lipid peroxidation chain reactions [31, 32].

The $CuSO_4$ concentration screening was performed separately using freshly prepared non-enriched mayonnaise samples to identify a $CuSO_4$ concentration capable of generating controlled and interpretable oxidation kinetics suitable for comparative evaluation of antioxidant efficacy.

To establish a controlled accelerated oxidation protocol, a fixed mass of mayonnaise (10 g) was mixed with a fixed volume of aqueous $CuSO_4$ solution (100 µL), while only the catalyst concentration was varied (2000, 100, 50, 20, 10 and 5 µM). PV was measured at t_0 and at defined intervals during incubation at 40 °C (6, 24, 48, 72 and 96 h). Semi-quantitative considerations, including the Cu^{2+}/lipid molar ratio and the relationship with endogenous antioxidants, were also used to confirm the catalytic rather than stoichiometric role of the selected concentration in the oxidation process. The concentration retained from this screening was subsequently applied uniformly to all thermally pre-incubated enriched formulations for comparative oxidative stability assessment.

Determination and monitoring of PV

PV was measured according to a standardized iodometric method [33]. The method was adapted for direct analysis of intact mayonnaise samples without prior lipid extraction in order to preserve the native emulsion matrix and to apply the same analytical procedure consistently to all formulations throughout the study. Briefly, 2 g of mayonnaise was mixed with 5 mL acetic acid–chloroform (3:2 v/v) and 1 mL saturated KI solution, left in the dark 3 min, then diluted with 30 mL water and a few drops of starch. The liberated iodine was titrated with 0.01 N sodium thiosulfate until the blue color disappeared. PV was monitored at defined intervals during incubation. Predictive stability modeling was performed using the Stability Study module of Minitab 18. A linear regression model including time, formulation, and their interaction was fitted to the experimental data, with a freely fitted (non-zero-constrained) intercept. Model performance was evaluated using goodness-of-fit statistics (R^2, adjusted R^2, and predicted R^2). The predicted time required to reach the predefined peroxide value threshold (10 meq O_2/kg), corresponding to the maximum acceptable peroxide value [34], was estimated by extrapolation of the fitted model beyond the experimental observation period using the Stability Study criterion. This predicted threshold time, together with the regression slope (oxidation rate), was used as a comparative indicator of oxidative stability among formulations.

Determination and monitoring of TA

TA was determined according to a standardized titration method [35]. For each analysis, 1 g of mayonnaise was dispersed in 30 mL of 55 °C water, phenolphthalein was added, and the solution was titrated with 0.1 N NaOH until a persistent pale pink endpoint. TA was monitored at defined intervals during incubation. Predictive stability modeling was performed using the Stability Study module of Minitab 18. A linear regression model was

fitted to the experimental data to estimate the predicted time required to reach the predefined titratable acidity threshold (4.5%), corresponding to the Codex Alimentarius standard [36]. This threshold time was estimated by extrapolation from the fitted model beyond the experimental observation period and was used only as a complementary model-derived indicator. Owing to the limited discriminant power of TA under the present experimental conditions, these predicted values were not intended as practical shelf-life predictions. The TA increase rate was determined from the slope of the fitted TA–time relationship.

Monitoring of conjugated dienes and trienes (K232 and K270)

Lipid oxidation was monitored by UV spectrophotometry at 232 nm and 270 nm to track primary and secondary oxidation. For each formulation, 10 g of mayonnaise was mixed with 100 µL $CuSO_4$, and 0.1 g samples were dissolved in 10 mL cyclohexane. The cyclohexane sample solutions were maintained at 40 °C throughout the oxidation experiment. At 10-min intervals, a fresh aliquot was withdrawn from the incubated solution and transferred to a quartz cuvette for immediate absorbance measurement at 232 and 270 nm at room temperature. The analytical reading was performed at ambient temperature, while oxidation proceeded continuously under controlled incubation at 40 °C. Specific extinction (Eλ) was calculated according to the following equation.

$$ E\lambda = {\text{ }}As{\text{ }}/{\text{ }}L{\text{ }} \times C $$

Where Eλ is the specific extinction at wavelength 232–270 nm, As is the absorbance of the sample solution, L is the optical path length (cm), and C is the sample concentration (g/L).

Two non-enriched controls were included: CTRL, incubated with $CuSO_4$ to assess oxidation, and $CTRL^-$, without $CuSO_4$ to verify baseline stability. Oxidative stability was expressed as lag time (minutes), defined as the time corresponding to the onset of the rapid increase phase in K232 or K270 absorbance curves, identified by consistent visual inspection as the first sampling point where a distinct change in slope occurred relative to the initial baseline region. Because absorbance measurements were collected at fixed 10-min intervals, lag times were recorded with a temporal resolution of 10 min. The same criterion was applied consistently to all samples, providing a quantitative measure of resistance to lipid oxidation.

Statistical analysis

All experimental data were obtained in triplicate and expressed as mean ± standard deviation (SD). The reported triplicates correspond to three technical replicates, with three independent analytical determinations performed on separate aliquots collected from the same mayonnaise batch for each formulation. One-way ANOVA followed by Fisher's LSD post hoc test ($\alpha = 0.05$) was performed using STATISTICA 7.1 (TIBCO Software Inc., Palo Alto, CA, USA) to compare lag-time values among formulations. Predictive modeling of oxidative stability parameters, including the estimated time to reach PV = 10 meq O_2/kg and TA = 4.5%, as well as the corresponding oxidation rates, was performed using Minitab® 18 (Minitab LLC, State College, PA, USA). Microsoft Excel 2010 was used for basic data processing and graph preparation.

Results and discussion

Bioactive composition and antioxidant potential of grape seed extract and macerated oil

Quantification of bioactive compounds

The results regarding the bioactive compound contents of the studied grape seed extracts are summarized in Table 1. Owing to the different extraction matrices involved, the various parameters were expressed according to the characteristics of each system and were primarily intended for individual extract characterization.

Table 1 Bioactive compounds and antioxidant capacity of hydrophilic extract and lipophilic OM

The hydrophilic extract showed a TPC of 1479 mg GAE/100 g DW, comparable to the values reported for *Tempranillo* (1048.59 mg GAE/100 g DW) and *Malvasia* (1534.85 mg GAE/100 g DW) grape seed extracts [37]. In contrast, our earlier work on the Algerian variety *Ahmar Bou-Amar* reported much higher levels, reaching 9656 mg GAE/100 g DW [19]. These differences emphasize the strong influence of varietal specificity on grape seed phenolic profiles. On the other hand, the lipophilic extract contained a TPC of 117 mg

GAE/kg OM. Although long-term OMs of grape seeds are scarcely documented and comparison is limited to grape seed oils, reported values fall in a similar range. For instance, values ranging from 59 to 115 mg GAE/kg have been reported for cold-pressed oils from nine grape varieties [38], while concentrations between 110 and 132 mg GAE/kg were observed depending on the extraction method [39].

When expressed relative to seed powder dry weight, the lipophilic macerate contained 93 mg GAE/100 g DW, which is about 15 times lower than the 1479 mg GAE/100 g DW measured in the hydrophilic extract. This contrast highlights the role of solvent polarity. Polyphenols, being mainly hydrophilic, are extracted more efficiently in polar media [40]. Supporting this interpretation, grape seeds have been reported to contain approximately 60% of total berry phenolics, while only a limited fraction is recovered in oil matrices due to the low solubility of hydrophilic phenolics in lipophilic environments and partial retention within seed tissues [41]. Overall, OM captures only a limited portion of the seed's phenolic pool.

PA were detected only in the dry extract (2216 mg C3GE/100 g DW) and were absent from the OM, confirming the poor efficiency of lipid-based maceration in recovering hydrophilic phenolics, especially high-molecular-weight flavonoids such as condensed tannins. Our results showed higher concentrations of PA compared to values ranging from 312 to 882 mg/100 g DW reported across different grape varieties [42]. In contrast, they were closer to the concentration reported for the Violeta variety (2394.4 mg/100 g DW) [43], indicating comparable magnitudes. Variations across studies mainly reflect grape variety, extraction conditions, and seed maturity. Methodological differences also contribute: our values were expressed as catechin-3-glucoside equivalents via colorimetric assay, while others used HPLC with catechin, offering greater sensitivity. These factors together explain the wide range of condensed tannin concentrations reported.

TTP content obtained in this study reached 1620 mg α-TE/kg OM. Since tocopherols in long-term grape seed macerates with soybean oil have not been previously reported, comparison is limited. The tocopherols likely derive both from grape seed oil, naturally containing 143–359 mg/kg [44, 45], and from refined soybean oil, which retains substantial levels despite refining losses, with values around 778–1274 mg/kg [46]. Our values clearly exceed those ranges, confirming that maceration enriched soybean oil with grape-derived tocopherols and altered its isomeric profile. Grape seed oil is typically

dominated by α-tocopherol (84–91% of total) [44], whereas soybean oil contains mainly γ- and δ-tocopherol, with α-tocopherol only 9–14% [46]. The macerate not only raised overall tocopherol content but also increased α-tocopherol proportion, which is nutritionally significant as it is preferentially retained in human tissues via α-TTP and is the most potent antioxidant among tocopherol homologues [47, 48].

Antioxidant activity

The hydrophilic grape seed extract showed very high antioxidant activity (Table 1), with 8157 µmol TE/100 g DW for DPPH˙ scavenging and 39,714 µmol TE/100 g DW for FRAP. In contrast, the OM displayed much lower values, 163 µmol TE/kg OM (DPPH˙) and 1311 µmol TE/kg OM (FRAP). This difference reflects the composition of the extracts, particularly the abundance of proanthocyanidins in the hydrophilic fraction, which are well known for their radical-scavenging and reducing properties. Consistent with previous studies, antioxidant activity in grape seed extracts is strongly correlated with total phenolic content [49,50,51]. In contrast, the antioxidant activity of the lipophilic macerate is mainly linked to tocopherols, the dominant lipid-soluble antioxidants in oils, and to possible synergistic effects with small amounts of co-extracted phenolics. Similar synergistic effects between tocopherols and polyphenols have been reported to enhance lipid oxidation inhibition in complex food matrices [52, 53].

Selection of operating conditions for the $CuSO_4$-assisted accelerated oxidation protocol at 40 °C

Preliminary incubation of enriched mayonnaise formulations at 40 °C without $CuSO_4$ addition resulted in extremely slow oxidation kinetics, with no measurable PV detected during the first 40 days of storage using the adapted iodometric procedure. These observations confirmed that mild thermal conditions preserved emulsion integrity but were insufficient to induce measurable oxidation within a practical experimental timeframe. Consequently, a separate $CuSO_4$ concentration screening experiment was conducted using freshly prepared non-enriched mayonnaise samples.

The PV kinetics revealed that $CuSO_4$ concentration strongly influenced the oxidative response of mayonnaise under accelerated aging conditions. The detailed results are presented in Table 2, which summarizes the evolution of peroxide values over time for all tested $CuSO_4$ concentrations. This preliminary screening experiment was designed to identify a $CuSO_4$ concentration capable of generating controlled and interpretable

oxidation kinetics suitable for subsequent evaluation of antioxidant efficacy in mayonnaise systems.

Table 2 Preliminary screening of the effect of $CuSO_4$ concentration and oxidation time on peroxide value (PV, meq O_2/kg) of mayonnaise at 40 °C

At the highest concentration tested (2000 µM), oxidation was immediate, with PV already measurable at *t0* (1.9 meq O_2/kg). This value rose sharply after only 6 h (7.5 meq O_2/kg) and reached nearly 28 meq O_2/kg by 24 h, confirming that excessive pro-oxidant input abolishes the lag phase and triggers uncontrolled lipid oxidation. Such conditions are unsuitable for monitoring antioxidant effects, since the system is already oxidized before kinetic evaluation can begin.

Intermediate concentrations (100–20 µM) did not induce measurable oxidation at *t0*, but PV increased significantly after 6 h, with values ranging from 3.6 to 5.8 meq O_2/kg. After 24 h, PV values rose between 15 and 21 meq O_2/kg, indicating that although these concentrations avoid immediate oxidation, they still shorten the lag phase considerably. These conditions may therefore be too harsh to discriminate protective effects of natural antioxidants across time, since the oxidative burst occurs within the first day.

In contrast, low concentrations (10 and 5 µM) provided the most physiologically relevant conditions for accelerated monitoring. At 10 µM, PV remained undetected until 6 h, then increased moderately (1.7 meq O_2/kg) and reached ~13 meq O_2/kg at 24 h, demonstrating a controlled initiation of lipid peroxidation. At 5 µM, PV remained undetected up to 24 h, and oxidation was first detected at 48 h (1.55 meq O_2/kg), with gradual progression thereafter (2.67 meq O_2/kg at 96 h). This delayed onset represents a well-defined lag phase, allowing the subsequent propagation and termination stages to be clearly distinguished.

Taken together, these results show that 5 µM $CuSO_4$ was identified as the operating catalyst concentration that fulfilled the predefined performance criteria of the proposed protocol, as it ensured system sensitivity while maintaining an interpretable lag phase. Higher concentrations ($\geq$20 µM) acted as overly aggressive pro-oxidants, accelerating PV

accumulation too rapidly to allow meaningful evaluation of antioxidant efficacy. The selected operating condition at 5 µM therefore provided a reliable framework for subsequent comparative studies of grape seed extracts in mayonnaise. Accordingly, 5 µM should be regarded as the operating catalyst concentration established for the proposed protocol rather than as a mathematically determined optimum.

Beyond these empirical observations, semi-quantitative considerations support the operational suitability of the 5 µM concentration. Considering the high lipid content of mayonnaise (~73%) and the very low amount of Cu^{2+} introduced, the resulting Cu^{2+}/lipid ratio remained within a catalytic range, confirming the catalytic rather than stoichiometric role of Cu^{2+} in the oxidation process. Endogenous antioxidants such as tocopherols and egg yolk phospholipids are present in large excess relative to Cu^{2+}, contributing to the reproducible lag phase observed. Additionally, egg yolk phospholipids may partially modulate Cu^{2+} catalytic activity at the oil–water interface. As a hydrophilic ionic species, Cu^{2+} partitions preferentially in the aqueous phase but exerts its catalytic activity primarily at the oil–water interface, consistent with the delayed yet sustained oxidation kinetics observed at 5 µM. We therefore emphasize that the selected condition represents a structurally non-disruptive framework for mayonnaise, while acknowledging that validation in other emulsion systems would be required before generalization.

Previous studies have shown that sub-micromolar to low-micromolar Cu^{2+}/lipid ratios are sufficient to catalyze lipid peroxidation in emulsified systems, confirming the catalytic rather than stoichiometric role of transition metals in oxidation processes [54]. This interpretation is consistent with earlier reports indicating that very low Cu^{2+}/lipid molar ratios are adequate to initiate oxidation in oil-in-water emulsions, due to the interfacial localization of transition metals and their catalytic activity [55]. Moreover, studies on model phospholipid bilayers have demonstrated that Cu^{2+} binds to phospholipids such as phosphatidylethanolamine and catalyzes lipid peroxidation even at low Cu^{2+}/lipid ratios [56]. Although these findings were obtained in simplified membrane systems, they support the mechanistic rationale that low Cu^{2+} concentrations can effectively initiate oxidation in emulsions containing phospholipids, such as mayonnaise.

Oxidative stability monitoring of mayonnaise under accelerated aging conditions

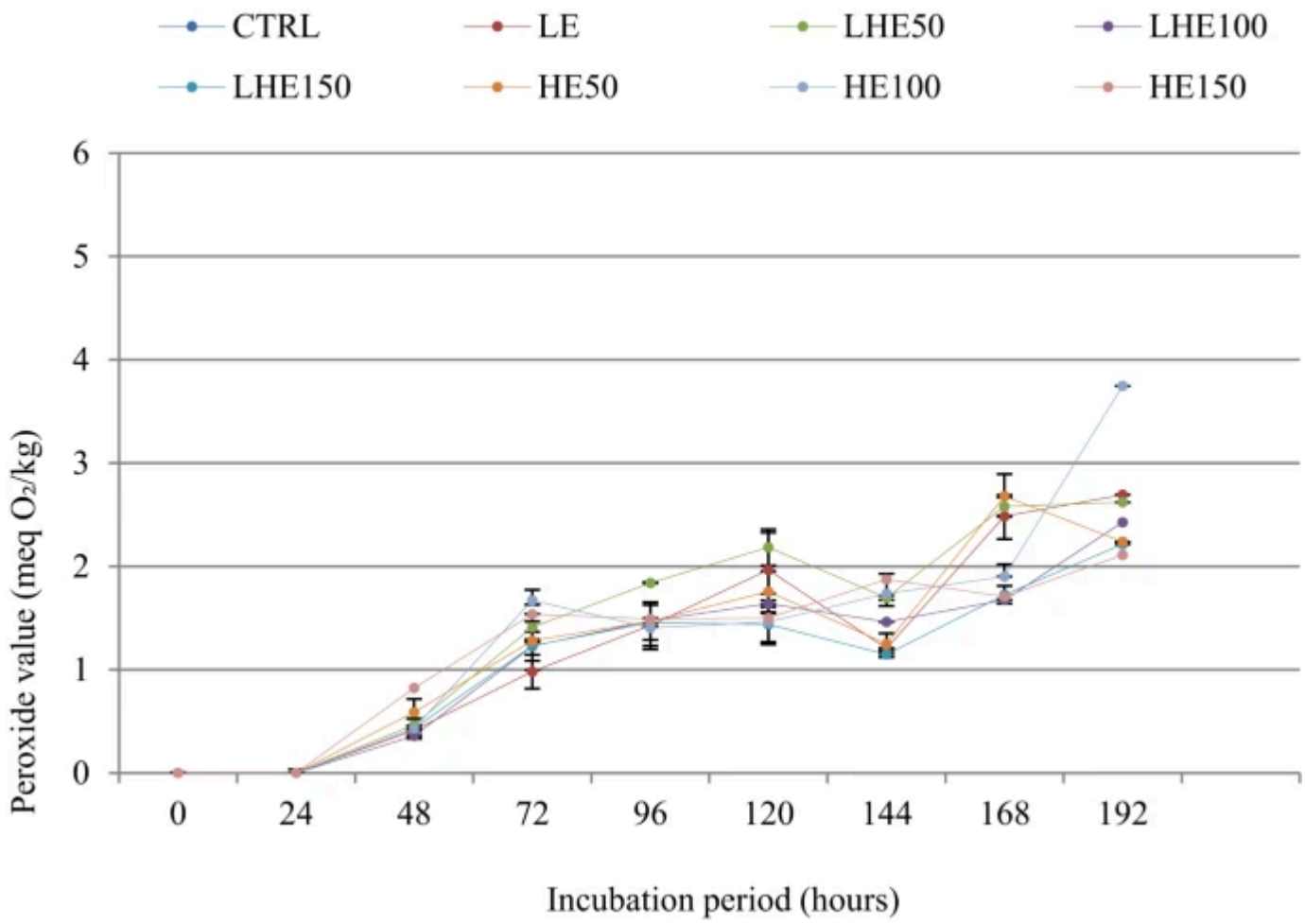
To overcome the long induction time observed under thermal stress alone, a novel oxidation protocol was developed using copper sulfate ($CuSO_4$) as a catalytic pro-oxidant. After 45 days of incubation at 40 °C, $CuSO_4$ was added at a concentration of 5 µM (0.1 mL per 10 g of mayonnaise) to initiate accelerated oxidative conditions. This setup enabled controlled lipid peroxidation and allowed for a comparative assessment of antioxidant efficacy across formulations.

Oxidative stability assessment via PV modeling

Throughout the thermal incubation at 40 °C, PV was monitored regularly, with no detectable PV up to day 40, indicating no measurable primary oxidation under thermal stress alone. In parallel, no visible signs of physical instability, such as phase separation or oil release, were observed throughout the 45-day incubation period, supporting the suitability of 40 °C as a structurally non-disruptive condition for mayonnaise systems. It should be noted that droplet size distribution, zeta potential, and microstructural characterization were not assessed in the present study. Such analyses would provide complementary information regarding emulsion physical stability and its relationship with oxidation kinetics.

Under these structurally preserved thermal conditions, $CuSO_4$ addition at day 45, PV was monitored daily to assess the onset and propagation phases. Lipid peroxidation was initiated in all formulations within approximately 48 h, with significant differences in hydroperoxide accumulation rates among samples (Fig. 1).

Fig. 1



Changes in peroxide value of mayonnaise during incubation at 40 °C under $CuSO_4$-induced oxidation. *CTRL* Control with $CuSO_4$, *LE* Lipophilic extract (2.5% OM), *LHE50–LHE150* Dual-phase enrichment (2.5% OM +50–150 ppm hydrophilic extract), *HE50–HE150* Hydrophilic extract (50–150 ppm). Data are expressed as means ± SD

The CTRL was the most prone to oxidation, exceeding 5.77 meq O_2/kg after 168 h, whereas enriched formulations showed greater resistance with LE reaching ~2.7 meq O_2/kg at 192 h. Aqueous-phase samples (HE50, HE100, HE150) also improved stability, with HE150 being the most effective (~1.9 meq O_2/kg at 168 h). The strongest protection was observed in dual-phase systems (LHE50, LHE100, LHE150), which exhibited significantly lower PVs ($p < 0.05$); notably, LHE100 and LHE150 remained below 2.7 meq O_2/kg after 192 h and showed latency phases during the first 72–96 h.

The accelerated oxidation in CTRL is attributable to the catalytic action of transition metals, particularly Cu^{2+} from $CuSO_4$, which promote hydroperoxide formation and decomposition into reactive radicals [57]. Copper is especially pro-oxidant under acidic conditions typical of oil-in-water emulsions. Metal-catalyzed oxidation depends on pH,

metal concentration, and fatty acid composition, and can only be partially limited by antioxidants such as tocopherols or citric acid. Processing and heating further enhance this effect [58, 59], explaining why $CuSO_4$ efficiently overcame the lag phase and triggered oxidation in our system.

It should be noted that peroxide values were determined directly in the mayonnaise matrix using an adapted iodometric procedure. Consequently, the PV values reported in the present study should be interpreted within the context of this analytical protocol and should not be assumed to be directly equivalent to peroxide values obtained on extracted lipid fractions according to ISO 3960.

To complement PV measurements, predictive modeling using the Minitab Stability Study module estimated the time required for each formulation to reach the predefined peroxide value threshold (10 meq O_2/kg). The linear regression model showed an excellent goodness-of-fit (R^2 = 90.87%, adjusted R^2 = 90.18%, predicted R^2 = 89.54%), explaining more than 90% of the variability in peroxide formation and providing a robust basis for estimating the predicted threshold times. These model-predicted threshold times served as a comparative indicator of oxidative stability among formulations and are presented in Table 3.

Table 3 ANOVA of stability models for peroxide value and titratable acidity

Time ($p < 0.0001$) and the *time × formulation interaction* ($p < 0.0001$) were the main factors affecting peroxide formation, whereas the main effect of formulation alone was weak ($p = 0.565$). This indicates that the antioxidant effect of grape seed extracts is time-dependent and formulation-specific, influencing oxidation kinetics rather than shifting all PV curves uniformly. Regression slopes (oxidation rates) complemented the "time-to-threshold" values, integrating the speed of peroxidation with resistance to critical PV levels.

Predicted times to reach PV = 10 meq O_2/kg varied widely among formulations (95% confidence), confirming strong formulation-dependent antioxidant effects. CTRL had the lowest tolerance (286.58 h) and the fastest PV formation rate (0.03194 µEq g^{-1} h^{-1}). LE

nearly doubled endurance (593.34 h) and reduced the oxidation rate by over 50%. Dual-phase systems (LHE50, LHE100, LHE150) further improved performance, reaching 574.26 h, 670.45 h, and 722.01 h, with slopes decreasing from 0.01463 to 0.01123 μEq g^{-1} h^{-1}, making LHE150 ~ 2.5× more resistant than CTRL.

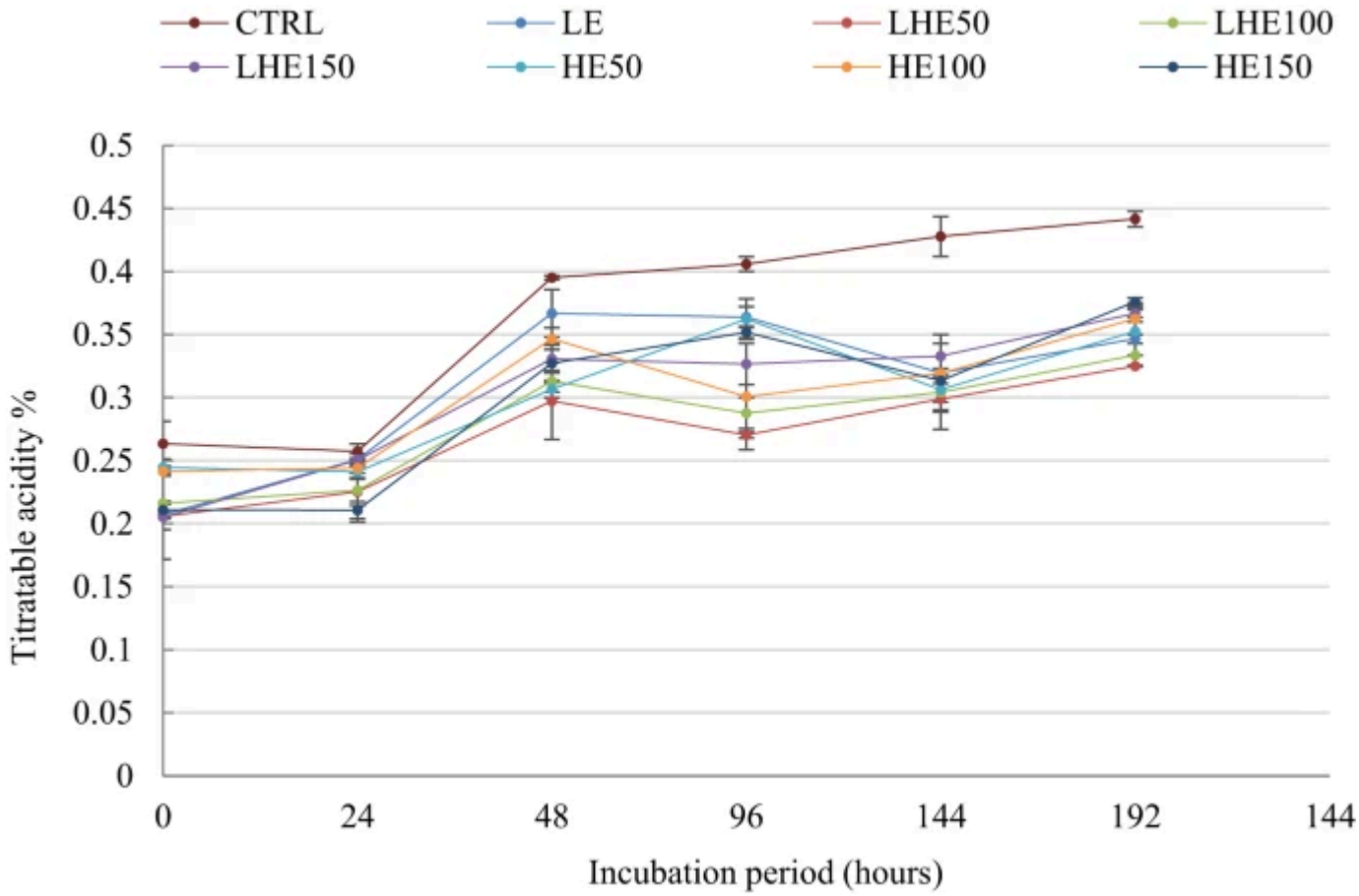
Hydrophilic extracts also enhanced stability: HE50 and HE150 had extended threshold times (628.28 h and 735.50 h) and low oxidation rates (0.01315 and 0.01073 μEq g^{-1} h^{-1}), with HE150 showing the lowest rate overall. HE100 provided moderate protection (539.08 h; 0.01596 μEq g^{-1} h^{-1}).

Overall, predicted threshold times and oxidation rates align, revealing an antioxidant hierarchy: dual-phase enrichment (LHE100, LHE150) and high-dose hydrophilic extract (HE150) offered the strongest protection, increasing oxidative endurance 2.3–2.6 fold relative to the CTRL. This highlights that distributing antioxidants in both lipid and aqueous phases maximizes defense against Cu^{2+}-driven lipid peroxidation in mayonnaise emulsions.

Oxidative stability assessment via TA modeling

TA was monitored as a complementary indicator of lipid degradation, reflecting the accumulation of free fatty acids [60] and secondary oxidation products [61]. During the first 45 days at 40 °C, all formulations, including controls and enriched samples, remained stable (TA ≈ 0.22%, $p > 0.05$), confirming the physicochemical resilience of the emulsions under thermal stress (Fig. 2). After $CuSO_4$ addition, TA increased across all samples, but no significant differences were observed among formulations ($p > 0.05$). The time×formulation interaction was not significant ($p = 0.050$), and all formulations shared an identical acidification rate (0.000654%·h^{-1}, $R^2 = 0.63$), confirming that TA lacks discriminant power for comparing antioxidant efficacy under the present experimental conditions. Full TA modeling data are provided in Table S3.

Fig. 2



Changes in titratable acidity of mayonnaise during incubation at 40 °C under $CuSO_4$-induced oxidation. *CTRL* Control with $CuSO_4$, *LE* Lipophilic extract (2.5% OM), *LHE50–LHE150* Dual-phase enrichment (2.5% OM +50–150 ppm hydrophilic extract), *HE50–HE150* Hydrophilic extract (50–150 ppm). Data are expressed as means ± SD

UV absorbance monitoring (K232 and K270)

Table 4 shows lag times at 232 nm and 270 nm for control and enriched mayonnaise. Two controls were used: $CTRL^-$ (non-enriched, no $CuSO_4$) as baseline, and CTRL (non-enriched, with $CuSO_4$) as pro-oxidant reference. $CTRL^-$ had the longest lag times (233.33 min at 232 nm; 240 min at 270 nm), while CTRL had the shortest (16.66 min; 10 min), confirming its high susceptibility to $CuSO_4$-induced oxidation ($p < 0.05$). Among enriched samples, LHE100 showed the greatest oxidative resistance, with lag times matching $CTRL^-$ at 232 nm (230 min) and surpassing all others at 270 nm (170 min). LHE150 (130 min at both wavelengths) and HE150 (≈87 min) followed, forming distinct statistical groups and confirming dose-dependent antioxidant efficacy. LE strongly inhibited secondary oxidation, reaching 240 min at 270 nm, equivalent to $CTRL^-$ and confirming lipophilic antioxidant efficacy. HE100 (110 min) and LHE50 (70–80 min)

showed intermediate stability, while HE50 (30 min) had the shortest lag times, close to CTRL, indicating low antioxidant levels were insufficient against $CuSO_4$-induced stress.

Table 4 Integrated oxidative stability profile of enriched mayonnaise under metal-catalyzed oxidation

Comparative evaluation of oxidative stability among enriched mayonnaise formulations

Combining PV kinetics, TA modeling, and UV lag times provides a multidimensional view of Cu^{2+}-accelerated oxidation in mayonnaise. Each marker captured distinct oxidation stages, and their agreement confirmed the efficacy of grape seed extracts. This integrated approach revealed differences in oxidative resistance among formulations and emphasized the importance of antioxidant localization and concentration for emulsion stability.

To better contextualize the following discussion, the estimated TPC delivered to the mayonnaise matrix for each formulation was calculated from the TPC of the grape seed powder, the hydrophilic extraction yield (34.53%), and the amount of dry hydrophilic extract incorporated into each formulation. The results are presented in Table 5. The LE formulation delivered 2.93 mg GAE/kg mayonnaise exclusively from the lipophilic fraction, while HE50, HE100, and HE150 delivered 2.14, 4.28, and 6.42 mg GAE/kg mayonnaise, respectively, from the hydrophilic fraction. Dual-phase formulations (LHE50–LHE150) provided the highest combined phenolic loads (5.07–9.35 mg GAE/kg mayonnaise), consistent with their superior antioxidant performance observed in PV kinetics and UV lag times. These results indicate that oxidative stability depended not only on total phenolic load, but also on antioxidant localization and phase distribution. For instance, LE (2.93 mg GAE/kg) exhibited stronger inhibition of secondary oxidation than HE100 (4.28 mg GAE/kg), underscoring the importance of lipophilic partitioning at the oil–water interface.

Table 5 Estimated TPC delivered to the mayonnaise matrix by each enrichment

formulation, calculated from characterized extract values (Sect. 3.1)

CTRL's high oxidative susceptibility was consistently confirmed by PV, K232, and K270, demonstrating the intrinsic vulnerability of emulsified lipids in the absence of antioxidants, particularly under Cu^{2+}-catalyzed conditions that accelerate both initiation and propagation of lipid peroxidation [57]. LE showed limited impact on primary oxidation but strongly inhibited secondary oxidation, with lag times at 270 nm comparable to $CTRL^{-}$ and minimal triene formation. This illustrates the efficiency of oil-phase enrichment, where lipophilic antioxidants (tocopherols, lipid-soluble polyphenols) act mainly during propagation by stabilizing aldehydes and ketones. Their localization at the oil–water interface enhances radical scavenging in the lipid domain, though limited aqueous solubility reduces initiation-phase activity [62, 63]. Similar effects were reported in grape seed extract-enriched emulsions, which reduced peroxide and diene formation while preserving tocopherols [28, 64].

The HE150 formulation, rich in hydrophilic antioxidants, effectively delayed primary oxidation, though its suppression of secondary oxidation was weaker than LE due to limited interfacial residence of polyphenols [65]. Low-concentration systems (HE50, LHE50) offered minimal protection, consistent with the importance of antioxidant concentration and interfacial distribution in determining antioxidant efficacy in emulsions [66]. Dual-phase formulations (LHE100, LHE150) provided the strongest protection, maintaining low PVs and long lag times at 232 nm and 270 nm. Their effectiveness arises from co-localization of hydrophilic and lipophilic antioxidants at the oil–water interface, optimizing radical interception.

LHE100 showed the most balanced antioxidant performance, whereas LHE150 exhibited the longest predicted time required to reach the selected peroxide value threshold Collectively, these findings indicate that the dual-phase formulations provided the highest overall oxidative stability among the tested formulations. Although the present study did not directly evaluate antioxidant partitioning at the oil–water interface, the superior performance of the LHE formulations is consistent with the literature-supported hypothesis that antioxidant efficacy depends strongly on interfacial partitioning and may be enhanced by the complementary distribution of hydrophilic and lipophilic antioxidants [63, 66]. Synergistic interactions between antioxidants may also have contributed to the

enhanced antioxidant efficacy [67], further supporting the superior stability of the LHE systems.

In addition to radical scavenging activity, grape seed polyphenols may also contribute to oxidative protection through interactions with Cu^{2+} ions, potentially reducing their pro-oxidant catalytic activity. Catechins, gallic acid derivatives, and proanthocyanidins possess metal-chelating properties through their ortho-dihydroxy groups and can form complexes with transition metal ions such as Cu^{2+}, thereby modulating their redox activity [68, 69]. Such interactions may reduce the amount of catalytically active copper available to promote lipid hydroperoxide decomposition through Fenton-like reactions, contributing to lag-phase extension [70, 71]. Previous studies have shown that flavonoid efficiency in Cu^{2+}-induced oxidation systems depends on both molecular structure and interfacial partitioning behavior [72]. Therefore, the delayed oxidation observed in enriched formulations likely resulted from the combined action of radical scavenging and partial modulation of Cu^{2+} catalytic activity at the oil–water interface. However, the relative contribution of these mechanisms could not be fully distinguished within the present experimental design, which represents an inherent limitation of metal-catalyzed oxidation systems applied to polyphenol-rich antioxidants. Future studies specifically designed to distinguish radical scavenging from metal chelation would further clarify the relative contribution of these mechanisms to the antioxidant activity of grape seed polyphenols.

Overall, antioxidant efficacy depended on solubility, interfacial localization, and phase distribution. Hydrophilic extracts (HE150) were most effective during initiation, lipophilic systems (LE) during propagation, and dual-phase formulations (LHE100, LHE150) provided the most balanced protection with long induction periods, low PVs, and delayed oxidation thresholds. Considering all oxidative stability indicators collectively, the results consistently indicated the following antioxidant performance trend: LHE100 ≈ LHE150 > HE150 > LE > HE100 > LHE50 > HE50 > CTRL. This hierarchy was derived from the convergent interpretation of the complementary oxidative stability parameters (PV formation rate, predicted time to reach PV = 10 meq O_2/kg, K232 lag time, and K270 lag time), all of which consistently identified the dual-phase formulations as the most effective antioxidant systems. These findings confirm the superiority of dual-phase enrichment for improving oxidative resistance under Cu^{2+}-induced oxidative stress.

Although PV and UV indices (K232 and K270) provided useful information regarding oxidation progression and oxidative stability, more specific characterization of secondary oxidation products using techniques such as gas chromatography would further strengthen the evaluation of lipid oxidation mechanisms. In addition, direct determination of the peroxide value of the oil macerate and the corresponding unmacerated soybean oil before incorporation into mayonnaise would provide complementary information regarding the initial oxidative status of the lipophilic extract preparation. The use of technical replicates from a single mayonnaise batch per formulation allowed assessment of analytical repeatability but did not establish inter-batch reproducibility of the formulation effects.

Conclusion

This study demonstrates the effectiveness of a $CuSO_4$-assisted accelerated oxidation protocol combining mild thermal conditions (40 °C) with a controlled catalytic Cu^{2+} concentration to assess oxidative stability in oil-in-water emulsions such as mayonnaise. Unlike conventional high-temperature accelerated tests, which may destabilize emulsion structure and alter oxidation mechanisms, the proposed protocol preserved emulsion integrity while generating clear and consistent oxidation kinetics within a manageable experimental timeframe. The study also demonstrates that oxidation development in mayonnaise under moderate thermal conditions alone is extremely slow, whereas the proposed $CuSO_4$-assisted protocol enables oxidative stability assessment within a practical and experimentally manageable timeframe. At 5 µM $CuSO_4$, oxidation developed after a controlled induction phase, enabling reliable differentiation among antioxidant formulations.

The integrated analytical approach based on PV kinetics, UV indices (K232, K270), lag-time analysis, and predictive modeling allowed precise discrimination of oxidative stability among enriched formulations. PV and UV lag times proved to be the most sensitive indicators, whereas TA showed limited responsiveness to formulation effects. Antioxidant efficacy was strongly influenced by phenolic reactivity, solubility, interfacial localization, and concentration balance.

Dual-phase formulations (LHE100 and LHE150) provided the greatest oxidative protection, with longer induction periods, lower hydroperoxide accumulation, and

extended UV lag times. HE150 also showed substantial protective effects, whereas low-dose systems provided limited stabilization, emphasizing the importance of balanced antioxidant partitioning between aqueous and lipid phases.

Overall, this study establishes a matrix-adapted accelerated oxidation protocol for oxidative stability assessment in mayonnaise. The proposed approach offers a practical and structurally non-disruptive alternative to conventional high-temperature oxidation tests, while dual-phase enrichment with natural grape seed extracts appears to be a promising clean-label strategy for improving oxidative stability in mayonnaise and warrants further evaluation in other emulsion-based food systems.

The predicted peroxide value and titratable acidity threshold times should be interpreted as model-based comparative estimates obtained by extrapolation beyond the experimental observation period, and their predictive relevance warrants further experimental validation. Further studies correlating the accelerated oxidation kinetics obtained under $CuSO_4$ stress conditions with long-term storage under real conditions (20–25 °C) would further strengthen the predictive relevance and practical applicability of the proposed model.

Data availability

Raw datasets are available from the corresponding author upon reasonable request.

Abbreviations

BHA: Butylated hydroxyanisole

BHT: Butylated hydroxytoluene

CTRL: Control sample

$CuSO_4$: Copper sulfate

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

FRAP: Ferric reducing antioxidant power

HE: Hydrophilic extract

K232: Specific extinction coefficient at 232 nm

K270: Specific extinction coefficient at 270 nm

LE: Lipophilic extract

LHE: Dual-phase extract formulation

OM: Oil macerate

PA: Proanthocyanidins

PV: Peroxide value

TA: Titratable acidity

TTP: Total tocopherols

References

1. Shahidi F, Hossain A (2022) Role of lipids in food flavor generation. Molecules 27:5014. https://doi.org/10.3390/molecules27155014

2. Bravo-Díaz C (2022) Lipid oxidation in food and biological systems: A physical chemistry perspective, 2nd edn. Springer Nature, Cham. https://doi.org/10.1007/978-3-030-87222-9

3. Wąsowicz E, Gramza A, Hęś M, Jeleń HH, Korczak J, Małecka M, Mildner-Szkudlarz S, Rudzińska M, Samotyja U, Zawirska-Wojtasiak R (2004) Oxidation of lipids in food.

Pol J Food Nutr Sci 13/54(Special Issue 1):87–100

4. Ghorbani Gorji S, Smyth HE, Sharma M, Fitzgerald M (2016) Lipid oxidation in mayonnaise and the role of natural antioxidants: A review. Trends Food Sci Technol 56:88–102. https://doi.org/10.1016/j.tifs.2016.08.002

5. Hennebelle M, Villeneuve P, Durand E, Lecomte J, van Duynhoven J, Meynier A, Yesiltas B, Jacobsen C, Berton-Carabin C (2024) Lipid oxidation in emulsions: new insights from the past two decades. Prog Lipid Res 94:101275. https://doi.org/10.1016/j.plipres.2024.101275

6. Bao Y, Pignitter M (2023) Mechanisms of lipid oxidation in water-in-oil emulsions and oxidomics-guided discovery of targeted protective approaches. Compr Rev Food Sci Food Saf 22:2678–2705. https://doi.org/10.1111/1541-4337.13158

7. Codex Alimentarius Commission (1995) General Standard for Food Additives (CXS 192–1995). FAO/WHO, Rome

8. Gutiérrez-Del-Río I, López-Ibáñez S, Magadán-Corpas P, Fernández-Calleja L, Pérez-Valero Á, Tuñón-Granda M, Miguélez EM, Villar CJ, Lombó F (2021) Terpenoids and polyphenols as natural antioxidant agents in food preservation. Antioxidants (Basel) 10:1264. https://doi.org/10.3390/antiox10081264

9. Reguengo LM, Salgaço MK, Sivieri K, Maróstica Júnior MR (2022) Agro-industrial by-products: valuable sources of bioactive compounds. Food Res Int 152:110871. https://doi.org/10.1016/j.foodres.2021.110871

10. Chedea VS, Tomoiagă LL, Macovei ȘO, Măgureanu DC, Iliescu ML, Bocsan IC, Buzoianu AD, Voșloban CM, Pop RM (2021) Antioxidant/pro-oxidant actions of polyphenols from grapevine and wine by-products-base for complementary therapy

in ischemic heart diseases. Front Cardiovasc Med 8:750508. https://doi.org/10.3389/fcvm.2021.750508

11. Andrade MA, Lima V, Sanches Silva A, Vilarinho F, Castilho MC, Khwaldia K, Ramos F (2019) Pomegranate and grape by-products and their active compounds: Are they a valuable source for food applications? Trends Food Sci Technol 86:68–84. https://doi.org/10.1016/j.tifs.2019.02.010

12. Silva A, Martins R, Silva V, Fernandes F, Carvalho R, Aires A, Igrejas G, Falco V, Valentão P, Poeta P (2024) Red grape by-products from the demarcated Douro region: chemical analysis, antioxidant potential and antimicrobial activity against food-borne pathogens. Molecules 29:4708. https://doi.org/10.3390/molecules29194708

13. de Menezes ML, Johann G, Diório A, Schuelter Boeing J, Visentainer JV, Raimundini Aranha AC, Curvelo Pereira N (2023) Comparison of the chemical composition of grape seed oil extracted by different methods and conditions. J Chem Technol Biotechnol 98:1103–1113. https://doi.org/10.1002/jctb.7314

14. Dimić I, Teslić N, Putnik P, Bursać Kovačević D, Zeković Z, Šojić B, Mrkonjić Ž, Čolović D, Montesano D, Pavlić B (2020) Innovative and conventional valorizations of grape seeds from winery by-products as sustainable source of lipophilic antioxidants. Antioxidants (Basel) 9:568. https://doi.org/10.3390/antiox9070568

15. Laqui-Estaña J, Obreque-Slier E, García-Nauto N, Saldaña E (2024) Advances in grape seed oil extraction techniques and their applications in food products: a comprehensive review and bibliometric analysis. Foods 13:3561. https://doi.org/10.3390/foods13223561

16. De Leonardis A, Macciola V, Iftikhar A, Lopez F (2022) Characterization, sensory and oxidative stability analysis of vegetable mayonnaise formulated with olive leaf

vinegar as an active ingredient. Foods 11:4006. https://doi.org/10.3390/foods11244006

17. Ramanathan L, Das NP, Li QT (1994) Studies on lipid oxidation in fish phospholipid liposomes. Biol Trace Elem Res 40:59–70. https://doi.org/10.1007/bf02916821

18. Schaich KM (1992) Metals and lipid oxidation: Contemporary issues. Lipids 27:209–218. https://doi.org/10.1007/BF02536181

19. Medouni-Adrar S, Boulekbache-Makhlouf L, Cadot Y, Medouni-Haroune L, Dahmoune F, Makhoukhe A, Madani K (2015) Optimization of the recovery of phenolic compounds from Algerian grape by-products. Ind Crops Prod 77:123–132. https://doi.org/10.1016/j.indcrop.2015.08.039

20. D'Eusanio V, Malferrari D, Marchetti A, Roncaglia F, Tassi L (2023) Waste by-product of grape seed oil production: chemical characterization for use as a food and feed supplement. Life (Basel) 13:326. https://doi.org/10.3390/life13020326

21. Konuskan DB, Kamiloglu O, Demirkeser O (2019) Fatty acid composition, total phenolic content and antioxidant activity of grape seed oils obtained by cold-pressed and solvent extraction. Indian J Pharm Educ Res 53:144–150. https://doi.org/10.5530/ijper.53.1.19

22. Velioglu YS, Mazza G, Gao L, Oomah BD (1998) Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agric Food Chem 46:4113–4117. https://doi.org/10.1021/jf9801973

23. Škerget M, Kotnik P, Hadolin M, Hraš AR, Simonič M, Knez Ž (2005) Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chem 89:191–198. https://doi.org/10.1016/j.foodchem.2004.02.025

24. Saeed AM, Hamzah MJ, Ali NJM (2018) Sensitive spectrophotometric method for determination of vitamins (C and E). Int J Pharm Sci Res 9(8):3373–3377. https://doi.org/10.13040/IJPSR.0975-8232.9

25. Milardović S, Iveković D, Grabarić BS (2006) A novel amperometric method for antioxidant activity determination using DPPH free radical. Bioelectrochemistry 68:175–180. https://doi.org/10.1016/j.bioelechem.2005.06.005

26. Oyaizu M (1986) Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet 44:307–315. https://doi.org/10.5264/eiyogakuzashi.44.307

27. Chatterjee D, Bhattacharjee P (2015) Use of eugenol-lean clove extract as a flavoring agent and natural antioxidant in mayonnaise: product characterization and storage study. J Food Sci Technol 52:4945–4954. https://doi.org/10.1007/s13197-014-1573-6

28. Altunkaya A, Hedegaard RV, Harholt J, Brimer L, Gökmen V, Skibsted LH (2013) Oxidative stability and chemical safety of mayonnaise enriched with grape seed extract. Food Funct 4:1647–1653. https://doi.org/10.1039/c3fo60204d

29. Razem M, Suhag R, Buddhika A, Ferrentino G (2026) Enrichment of mayonnaise with plant-based antioxidants enhances phenolic bioaccessibility and oxidative stability. Food Chem X 33:103475. https://doi.org/10.1016/j.fochx.2025.103475

30. Makhoul H, Ghaddar T, Toufeili I (2006) Identification of some rancidity measures at the end of the shelf life of sunflower oil. Eur J Lipid Sci Technol 108:143–148.

https://doi.org/10.1002/ejlt.200500262

31. Osborn-Barnes HT, Akoh CC (2003) Copper-catalyzed oxidation of a structured lipid-based emulsion containing alpha-tocopherol and citric acid: influence of pH and NaCl. J Agric Food Chem 51:6851–6855. https://doi.org/10.1021/jf0261553

32. Fomuso LB, Corredig M, Akoh CC (2002) Metal-catalyzed oxidation of a structured lipid model emulsion. J Agric Food Chem 50:7114–7119. https://doi.org/10.1021/jf020256j

33. ISO (2007) ISO 3960:2007. Animal and vegetable fats and oils—Determination of peroxide value—Iodometric (visual) endpoint determination. International Organization for Standardization, Geneva

34. Codex Alimentarius Commission (1999) Codex standard for named vegetable oils. Codex Stan 210–1999. FAO/WHO, Rome

35. ISO (2009) ISO 660:2009. Animal and vegetable fats and oils—Determination of acid value and acidity. International Organization for Standardization, Geneva

36. Codex Alimentarius Commission (1987) Report of the fifteenth session of the Coordinating Committee for Europe (Thun, Switzerland, 16–20 June 1986). ALINORM 87/19. FAO/WHO, Rome

37. Berradre M, González C, Sulbarán B, Fernández V (2013) Polyphenols content and antioxidant activity of grape seed extract (*Vitis vinifera*) Malvasia and Tempranillo variety. Rev Fac Agron (LUZ) 30:619–631

38. Bail S, Stuebiger G, Krist S, Unterweger H, Buchbauer G (2008) Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total

phenols and antioxidant capacity. Food Chem 108:1122–1132. https://doi.org/10.1016/j.foodchem.2007.11.063

39. Ubaid M, Sainia CS (2025) Evaluation of different green technological approaches for the extraction of oil from grape seeds: a comparative study. Grasas Aceites 75:2173. https://doi.org/10.3989/gya.1200232.2173

40. Ghaffar N, Perveen A (2025) Solvent polarity effects on extraction yield, phenolic content, and antioxidant properties of Malvaceae family seeds: a comparative study. N Z J Bot 63:627–637. https://doi.org/10.1080/0028825X.2024.2392705

41. Downey MO, Harvey JS, Robinson SP (2003) Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. Aust J Grape Wine Res 9:15–27. https://doi.org/10.1111/j.1755-0238.2003.tb00228.x

42. Ju Y-L, Yang L, Yue X-F, He R, Deng S-L, Yang X, Liu X, Fang Y-L (2021) The condensed tannin chemistry and astringency properties of fifteen Vitis davidii Foex grapes and wines. Food Chem X 11:100125. https://doi.org/10.1016/j.fochx.2021.100125

43. Rebello LPG, Lago-Vanzela ES, Barcia MT, Ramos AM, Stringheta PC, Da-Silva R, Castillo-Muñoz N, Gómez-Alonso S, Hermosín-Gutiérrez I (2013) Phenolic composition of the berry parts of hybrid grape cultivar BRS Violeta (BRS Rubea × IAC 1398-21) using HPLC-DAD-ESI-MS/MS. Food Res Int 54:354–366. https://doi.org/10.1016/j.foodres.2013.07.024

44. Göktürk Baydar N, Özkan G, Sema Çetin E (2007) Characterization of grape seed and pomace oil extracts. Grasas Aceites 58:29–33. https://doi.org/10.3989/gya.2007.v58.i1.5

45. Cravotto C, Rapinel V, Nguyen-Thanh B, Bonet-García R, Bartier M, Claux O, Jacques L, Tabasso S, Barrajón-Catalán E, Fabiano-Tixier A-S (2025) Sustainable grape seed oil processing: green solvent extraction and byproduct valorisation. Food Bioprod Process 149:428–438. https://doi.org/10.1016/j.fbp.2024.12.006

46. Wen Y, Xu L, Xue C, Jiang X, Wei Z (2020) Assessing the impact of oil types and grades on tocopherol and tocotrienol contents in vegetable oils with chemometric methods. Molecules 25:5076. https://doi.org/10.3390/molecules25215076

47. Brigelius-Flohé R, Traber MG (1999) Vitamin E: function and metabolism. FASEB J 13:1145–1155. https://doi.org/10.1096/fasebj.13.10.1145

48. Mukai K, Noborio S, Nagaoka S-I (2005) Why is the order reversed? peroxyl-scavenging activity and fats-and-oils protecting activity of vitamin E. Int J Chem Kinet 37:605–610. https://doi.org/10.1002/kin.20114

49. Yilmaz Y, Toledo RT (2004) Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. J Agric Food Chem 52:255–260. https://doi.org/10.1021/jf030117h

50. Rockenbach II, Gonzaga LV, Rizelio VM, Gonçalves AEDS, Genovese MI, Fett R (2011) Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (*Vitis vinifera* and *Vitis labrusca*) pomace from Brazilian winemaking. Food Res Int 44:897–901. https://doi.org/10.1016/j.foodres.2011.01.049

51. Zhang F, Zhang Z, Wen Y, Wu Q, Zhang L, Li S, Yang H (2023) The action potential of antioxidant grape seed proanthocyanidin as a rumen modifier to mitigate rumen methanogenesis in vitro. Fermentation 9:513. https://doi.org/10.3390/fermentation9060513

52. Szydłowska-Czerniak A, Trokowski K, Karlovits G, Szłyk E (2010) Determination of antioxidant capacity, phenolic acids, and fatty acid composition of rapeseed varieties. J Agric Food Chem 58:7502–7509. https://doi.org/10.1021/jf100852x

53. Zhang Y, Wang X, Zeng Q, Deng Y, Xie P, Zhang C, Huang L (2023) A new insight into synergistic effects between endogenous phenolic compounds additive and α-tocopherol for the stability of olive oil. Food Chem 427:136667. https://doi.org/10.1016/j.foodchem.2023.136667

54. Chan PC, Peller OG, Kesner L (1982) Copper(II)-catalyzed lipid peroxidation in liposomes and erythrocyte membranes. Lipids 17:331–337. https://doi.org/10.1007/bf02535190

55. Berton-Carabin CC, Ropers M-H, Genot C (2014) Lipid oxidation in oil-in-water emulsions: Involvement of the interfacial layer. Compr Rev Food Sci Food Saf 13:945–977. https://doi.org/10.1111/1541-4337.12097

56. Poyton MF, Sendecki AM, Cong X, Cremer PS (2016) Cu(2+) binds to phosphatidylethanolamine and increases oxidation in lipid membranes. J Am Chem Soc 138:1584–1590. https://doi.org/10.1021/jacs.5b11561

57. Wang G, Wang T (2008) Oxidative stability of egg and soy lecithin as affected by transition metal ions and pH in emulsion. J Agric Food Chem 56:11424–11431. https://doi.org/10.1021/jf8022832

58. Kanner J (2010) Metals and food oxidation. In: Decker EA, Elias RJ, McClements DJ (eds) Oxidation in Foods and Beverages and Antioxidant Applications: Understanding Mechanisms of Oxidation and Antioxidant Activity. Woodhead Publishing, Cambridge, pp 36–56. https://doi.org/10.1533/9780857090447.1.36

59. Mancuso JR, McClements DJ, Decker EA (2000) Iron-accelerated cumene hydroperoxide decomposition in hexadecane and trilaurin emulsions. J Agric Food Chem 48:213–219. https://doi.org/10.1021/jf990757d

60. Sakaino M, Sano T, Kato S, Shimizu N, Ito J, Rahmania H, Imagi J, Nakagawa K (2022) Carboxylic acids derived from triacylglycerols that contribute to the increase in acid value during the thermal oxidation of oils. Sci Rep 12:12460. https://doi.org/10.1038/s41598-022-15627-3

61. Geng L, Liu K, Zhang H (2023) Lipid oxidation in foods and its implications on proteins. Front Nutr 10:1192199. https://doi.org/10.3389/fnut.2023.1192199

62. Frankel EN, Huang S-W, Kanner J, German JB (1994) Interfacial phenomena in the evaluation of antioxidants: Bulk oils vs emulsions. J Agric Food Chem 42:1054–1059. https://doi.org/10.1021/jf00041a001

63. Laguerre M, Lecomte J, Villeneuve P (2007) Evaluation of the ability of antioxidants to counteract lipid oxidation: existing methods, new trends and challenges. Prog Lipid Res 46:244–282. https://doi.org/10.1016/j.plipres.2007.05.002

64. Jang S, Lee J, Choi W-S (2015) Oxidative stability of grape seed oil by addition of grape seed extract. J Korean Soc Food Sci Nutr 44:1813–1818. https://doi.org/10.3746/jkfn.2015.44.12.1813

65. Losada-Barreiro S, Paiva-Martins F, Bravo-Díaz C (2024) Analysis of the efficiency of antioxidants in inhibiting lipid oxidation in terms of characteristic kinetic parameters. Antioxid (Basel) 13:593. https://doi.org/10.3390/antiox13050593

66. Costa M, Losada-Barreiro S, Paiva-Martins F, Bravo-Díaz C (2017) Physical evidence that the variations in the efficiency of homologous series of antioxidants in

emulsions are a result of differences in their distribution. J Sci Food Agric 97:564–571. https://doi.org/10.1002/jsfa.7765

67. Zhang Z, Li X, Sang S, McClements DJ, Chen L, Long J, Jiao A, Jin Z, Qiu C (2022) Polyphenols as plant-based nutraceuticals: health effects, encapsulation, nano-delivery, and application. Foods 11:2189. https://doi.org/10.3390/foods11152189

68. Mira L, Fernandez MT, Santos M, Rocha R, Florêncio MH, Jennings KR (2002) Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. Free Radic Res 36:1199–1208. https://doi.org/10.1080/1071576021000016463

69. Yao S-P, Wang W-Z, Li X-Y, Liu X-Y, Han R-M, Zhang J-P (2024) Effects of metal ions on antioxidant activity of red wine. ChemistrySelect 9:e202400226. https://doi.org/10.1002/slct.202400226

70. Andrés CMC, Pérez de la Lastra JM, Juan CA, Plou FJ, Pérez-Lebeña E (2023) Polyphenols as antioxidant/pro-oxidant compounds and donors of reducing species: relationship with human antioxidant metabolism. Processes 11:2771. https://doi.org/10.3390/pr11092771

71. Simunkova M, Barbierikova Z, Jomova K, Hudecova L, Lauro P, Alwasel SH, Alhazza I, Rhodes CJ, Valko M (2021) Antioxidant vs. prooxidant properties of the flavonoid, kaempferol, in the presence of Cu(II) ions: a ROS-scavenging activity, Fenton reaction and DNA damage study. Int J Mol Sci 22:1619. https://doi.org/10.3390/ijms22041619

72. Brown JE, Khodr H, Hider RC, Rice-Evans CA (1998) Structural dependence of flavonoid interactions with Cu2 + ions: implications for their antioxidant properties. Biochem J 330(Pt 3):1173–1178. https://doi.org/10.1042/bj3301173

Acknowledgements

We are grateful to the Ministry of Higher Education and Scientific Research of Algeria, particularly DGRSDT.

Funding

Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. This research has not received any specific grant from any organization.

Author information

Authors and Affiliations

Université de Bejaia, Faculté des Sciences de la Nature et de la Vie, Laboratoire de Biomathématiques Biophysique, Biochimie et de Scientométrie (L3BS), 06000., Bejaia, Algeria
Sonia Medouni-Adrar, Aida Mekhoukhe, Ouahiba Soufi-Maddi & Sabine Chabane

Université de Bejaia, Faculté des Sciences de la Nature et de la Vie, 06000, Bejaia, Algeria
Amina Kessaissia & Amel Aidli-Ouadah

Centre de Recherche en Technologies Agro-alimentaires, Route de Targa Ouzemmour, Campus Universitaire, 06000, Bejaia, Algeria
Lamia Medouni-Haroune

Université de Bejaia, Faculté des Sciences de la Nature et de la Vie, Laboratoire de Biochimie Appliquée, 06000, Bejaia, Algeria
Salima Dib-Djafri

Complexe agro-alimentaire de CEVITAL, Agro-industrie, Nouveau Quai Port de Bejaia, 06000, Bejaia, Algeria
Athmane Azouaou & Samir Hadjal

Department of Food and Health, Instituto de la Grasa-CSIC, Campus Universidad Pablo de Olavide, Edificio 46, 41013, Seville, Spain

Javier S. Perona

Contributions

SMA : Conceptualization, Recipe development, Writing—original draft, Formal analysis, Statistical analysis, Writing—review and editing, Supervision. AK: Investigation, Writing—review and editing. LMH: Conceptualization, Methodology, Writing—review and editing. AAO: Investigation. AM : Formal analysis. OSM: Conceptualization. SC: Investigation. SDD: Investigation. AA: Formal analysis. SH: Resources. JSP: Supervision, Writing—review and editing.

Corresponding author

Correspondence to Javier S. Perona.

Ethics declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Additional information

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary Information

Below is the link to the electronic supplementary material.

Supplementary Material 1 (download DOCX ⭳)

Rights and permissions

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Reprints and permissions

About this article

Cite this article

Medouni-Adrar, S., Kessaissia, A., Medouni-Haroune, L. *et al.* Development of a copper-based accelerated oxidation protocol for evaluating the oxidative stability of mayonnaise enriched with hydrophilic and lipophilic grape seed extracts. *Eur Food Res Technol* **252**, 397 (2026). https://doi.org/10.1007/s00217-026-05317-z

Received	Revised	Accepted
23 March 2026	27 August 2026	17 September 2026

Published	Version of record
02 October 2026	02 October 2026

DOI
https://doi.org/10.1007/s00217-026-05317-z

Keywords

Accelerated oxidation protocol · Mayonnaise · Grape seed extract · Dual-phase antioxidant · Oxidative stability · Copper-catalyzed oxidation