

Sorani Olive Leaf Extract as a Natural Antioxidant in Butter and Ghee: Kinetic Modelling, Phenolic Stability, and Radical Scavenging Mechanisms

Alaa Abdul Karim Majeed
Academic Title: Assistant Lecturer
Major: Food Science
University: Samarra University
alaa.abd@uosamarra.edu.iq

ABSTRACT

Lipid oxidation is identified as the main culprit of the short shelf life of dairy products, particularly in Iraq, where butter and ghee are usually kept at room temperature (about 20–25°C) and kept under summer conditions where temperature rises above 40°C. Based on the assumption, the objective of the current study was to find out the effectiveness of seven secoiridoid, phenylethanoid, and flavonoid phenolics derived from Sorani olive leaf ethanolic extract (OLE) in slowing the lipid oxidation process through radical scavenging and metal-chelation in accordance with Arrhenius first-order kinetics. The objectives included comparison of three extraction techniques, verification of phenolics quantification results of HPLC-ESI-MS/MS (ICH Q2(R1)), characterization of the fatty acid composition, effectiveness of the OLE (200–600 mg /kg) in comparison with BHT and rosemary extract during the time period of seven weeks at temperatures equal to 4 °C, 25 °C, and 40 °C, calculation of Arrhenius parameters and Q_{10} , observation of the kinetics of phenolics elimination, and sensory characteristics.

TPC, TFC, DPPH, FRAP and metal-chelation activities were tested; in addition, a complete validation of HPLC-ESI-MS/MS was carried out. Fatty acids composition was defined using GC-FID analysis. The peroxide level, TBARS, p-anisidine value, Totox and acid value were measured on a weekly basis through applying linear mixed models.

The subsequent extraction with ethanolic solution (EA) produced the highest TPC (48.7 mg GAE/g dw) and DPPH IC_{50} value equal to 18.4 ± 0.6 µg/mL. HPLC-ESI-MS/MS allowed discovering seven types of phenolics (the recovery rate was equal to 94.2-98.7%, RSD <4.5%), particularly oleuropein (42.3%) and hydroxytyrosol (18.6%) were identified as the most abundant ones. The value of activation energy increased from 58.4 to 84.1 kJ/mol when the OLE concentration was equal to 600 mg/kg (there were no significant differences with the value of activation energy for the BHT). Q_{10} decreased from 2.4 to 2.0. The analysis showed that after seven weeks spent at the temperature of 4 °C, 74.3% of oleuropein was preserved.

Sorani OLE (600 mg/kg) can provide the same protective effect due to radical scavenging, Fe^{2+} chelation, and lipoxygenase inhibition, which creates conditions for its practical application in rural areas of Iraq under conditions of ambient temperature.

Keywords: olive leaf; oleuropein; HPLC-ESI-MS/MS; Arrhenius kinetics; radical scavenging; metal chelation; lipid oxidation; butter; ghee; Sorani cultivar; Iraq; shelf-life prediction; Q_{10}

1. Introduction

Lipid oxidation is an important reaction that causes fatty foods to lose their flavour, appearance and texture, and that

ISSN 2072-3857

can also produce compounds that can potentially make these foods unsafe to consume (13) (26) In Iraq, this problem is especially serious; in the summer months, butter and ghee are often[as12.1]at room temperature (typically ~20–25°C) for long periods of time, and during the summer months (more than 40°C), these reactions of lipid oxidation occur more rapidly thus shortening the shelf-life of the affected foods and increasing the potential for oxidised products to be unsafe for a large population of consumers(1)(4).

For many years synthetic antioxidants (mostly BHT (E321) and BHA (E320) have provided the industry with the best possible protection of fat matrices. Recently however, regulatory pressure has increased: BHA is categorized as Group 2B (possibly carcinogenic) by IARC, while BHT is classified as Group 3 meaning it is not classifiable as to its carcinogenicity. However, both substances are being restricted in more and more importing markets, which shows that "clean label" reformulation is becoming a must in the operations of Iraqi companies. Manufacturers trying to access export markets(9) (10) Consequently, there has been much interest and research into natural phenolic alternatives to effectively replace synthetic antioxidants and comply with labelling requirements. Among the many natural phenolic sources available, olive leaves represent one of the most studied and economically viable sources of natural antioxidants. During global olive production, 20-30 kg of leaf biomass is produced for every 100 kg of olive fruit harvested (25) There are large quantities of olive leaf biomass produced each year by the olive orchards in Nineveh, Anbar

and Saladin governorates (all in Iraq). Oleuropein is the most abundant phenolic compound present in the leaf (6-9% of the dry weight of leaves from Mediterranean tree cultivars); it protects lipids by three different but partially synergistic mechanisms: (i) by hydrogen-atom transfer (HAT) from its catechol moiety; (ii) by chelating the pro-oxidant metal ions Fe^{2+} and Cu^{2+} that catalyze the generation of Fenton-type radicals; and (iii) by effectively breaking the radical chain reaction by the peroxy and hydroxyl radicals (6) (22) *Hydroxytyrosol* is the main hydrolytic metabolite of oleuropein; it has a low molecular weight and amphiphilic structure, which makes it very effective at protecting lipids in the lipid phase (33)(35) The phenolic compounds apigenin and luteolin are also important because they act to inhibit lipoxygenase (an enzyme that initiates the radical oxidative process) (24).

In addition to the aforementioned mechanistic wealth of OLEs, the literature appears to be missing some important research areas. First, almost all studies of the antioxidant properties of OLEs have utilized vegetable oil systems; there is little published work regarding their application to complex solid dairy fat matrices such as butter and ghee (25) Second, kinetic models developed using the Arrhenius equation are infrequently combined with multi-temperature Q10 determinations for these matrices(21) (30). Third, when phenolic compounds are quantified in fat systems, HPLC/MS/MS methodologies are frequently presented without full ICH Q2 (R1) validation(28) Fourth, there is no information regarding the different cultivars of *Olea europaea* found in Iraq's Sorani region, with a

search of Scopus on "Olea europaea", "Iraq" and "phenolic" or "antioxidant" (April 2024) yielding fewer than six published research articles that mention any of the Sorani cultivars grown in the semi-arid continental climate of Samarra District (22.8 °C annual average temperature; < 150 mm of precipitation annually).

This research was undertaken to address these knowledge gaps in detail. The study has five new contributions, as follows: (1) It provides the first kinetic profile for Sorani OLE in solid dairy-fat matrices at three storage temperatures using rigorous Arrhenius analysis; (2) It is fully validated to meet ICH Q2(R1)

2. Materials and Methods

2.1 Plant Material and Preparation

Olive leaves (*Olea europaea* L., cv. Sorani) were harvested from certified orchards located in the Samarra District, Saladin Governorate, Iraq (34.20°N, 43.87°E; altitude 68 m a.s.l.) during October 2023 when they were at full phenological maturity. This area is characterized by a semi-arid continental climate (with an average temperature of 22.8°C and less than 150 mm of rainfall annually), which promotes the accumulation of secoiridoids under seasonal water-limited stress conditions according to the reports of (20) and (2) Voucher specimens were stored in the University of Samarra Herbarium (specimen accession # USM-2023-OL-01). Leaves were rinsed with distilled water and dried at 40°C in an oven for 48 h, then ground through a 60-mesh screen and stored at -20°C in airtight amber glass containers. HPLC-ESI-MS/MS and GC-FID analyses were performed at the College of Applied Sciences; sensory evaluations were

HPLC-ESI-MS/MS requirements for recovery, precision, accuracy, RSD%, and matrix performance; (3) It evaluates the scavenging of free radicals, binding of metals, and phenolic compounds (the mechanism of phenolic depletion) both in real-time and as a function of storage time when stored in fat; (4) It evaluates the Q_{10} of OLE across a 4 to 40°C temperature range representative of supply chain conditions in Iraq; and (5) It gives preliminary estimates of the economic feasibility of OLE relative to BHT for translation into the food industry.

conducted in a sensory evaluation room that complies with ISO 8589:2007.

2.2 Extraction Methods

Three methods of extraction were evaluated for comparison: 1) Ethanolic extraction (EA) with the use of 80% ethanol at a 1:10 ratio (w/v) could obtain the highest total phenolic content (TPC) recoveries since this extraction method(solvent) would have been able to 'solubilize' or recover the largest range of phenolic compounds from relatively polar to lipophilic; 2) Aqueous extraction (WE) at a ratio of 1:10 (w/v) and using heat + time to extract the phenolic compounds from the leaf powder; 3) Supercritical CO₂ extraction (SC-CO₂) using 10% ethanol in the extraction, but which had relatively low TPC because of the polar/non-polar characteristics of oleuropein (logP=-0.22) vs hydroxytyrosol (logP=+0.04) and neither of these compounds are located in the optimal solubility range for supercritical CO₂ extraction.

2.3 Phytochemical Analysis and Antioxidant Activity

Total phenolic content (TPC) was measured using the Folin-Ciocalteu phenol method (27) and expressed as milligrams of gallic acid equivalent (GAE) per gram of dry weight. Total flavonoid content (TFC) was measured using an aluminum chloride colorimetric assay and expressed as milligrams of quercetin equivalent (QE) per gram dry weight. The DPPH radical scavenging assay (Brand-Williams et al. 1995), FRAP assay (7) and ferrous ion chelation assay (12) were used to measure antioxidant activity, expressed as micromoles of EDTA equivalent per gram of sample. The ferrous ion chelation assay measures the ability of oleuropein and verbascoside directly to bind with Fe^{2+} , thus preventing the decomposition of Fenton-type hydroperoxides. The Shapiro-Wilk test ($p > 0.05$) was used to check for normal distribution of data and Levene's test ($p > 0.05$) was used to check for homogeneity of variance.

2.4 HPLC-ESI-MS/MS Phenolic Profiling and Full Validation

Phenolic profiling using HPLC ESIMS MSMS was performed on an Agilent 1290 Infinity II HPLC with an attached Agilent 6460 triple quad Mass Spectrometry (in ESI- mode) utilizing a Zorbax Eclipse Plus C18 column (250 mm x 4.6 mm x 5 mm) and binary

gradient of lake acidic 0.1% formic acid (A) and acetonitrile/0.1% formic acid (B) from 5% B to 95% B over 45 minutes while flowing at 0.8 mL/min. The identification of individual compounds was performed using databases of MassBank of North America and SciFinder. The measured compound concentrations were determined through external calibration ($R^2 > 0.999$). Validation of this method was conducted according to the guidelines of the ICH Q2(R1) (see Table 3a). The true concentration (or true value) of each spiked compound was calculated by determining the amount of known pure oleuropein, hydroxytyrosol, and tyrosol standards added to the blank butter at the low, medium, and high spiking level, then dividing that amount by the total concentration and multiplying by 100% to determine the % recovery. The inter-day ($n=3$ days, RSD%) and intra-day ($n=6$, RSD%) precision of each standard were determined. The matrix effect on the standards was determined by measuring the slope of the calibration curve of the standard prepared in solvent compared to the slope of the calibrations that were prepared in the butter matrix. The limit of detection (LOD) and limit of quantification (LOQ) were both determined by determining the signal-to-noise (S:N) ratio of 3 to 10, respectively

Table 3a. HPLC-ESI-MS/MS method validation parameters for selected phenolics in butter matrix. LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation; ME: matrix effect (%).

Chlorogenic acid	Apigenin	Luteolin-7-glc	Verbascoside	Tyrosol	Hydroxytyrosol	Oleuropein	Parameter
0.05–25	0.02–15	0.05–20	0.05–30	0.02–20	0.02–25	0.05–50	Linearity range (µg/mL)
0.9996	0.9991	0.9995	0.9993	0.9996	0.9997	0.9994	R ²
0.011	0.009	0.014	0.015	0.009	0.008	0.012	LOD (µg/mL)
0.036	0.029	0.044	0.047	0.028	0.026	0.038	LOQ (µg/mL)
98.7±1.0	96.7±1.5	94.2±1.6	95.9±1.3	98.1±1.1	97.8±1.4	96.4±1.2	Recovery (%)
2.2	2.6	3.1	2.8	1.9	2.4	2.1	Intra-day RSD (%)
3.5	3.9	4.5	4.1	3.2	3.7	3.4	Inter-day RSD (%)
-7.6	-8.9	-12.1	-11.4	-6.7	-10.2	-8.3	Matrix effect (%)

2.5 Fatty Acid Profile

Samples of Iraqi commercial unsalted butter (82% fat; a.v. < 0.3 mg KOH/g) and vegetable ghee (99.7% fat; p.v. < 0.5 meq/kg) were obtained from the markets in Samarra and certified by GC-FID method as free of antioxidant additives. Fatty acid methyl esters (FAMES) were prepared based on the procedures defined in ISO 5508:1990 and analyzed using an Agilent 7890B gas chromatograph (GC) with a DB-23 column (60 m x 0.25 mm x 0.25 µm).

2.6 Experimental Design and Storage Temperature of Extracts.

The extracts described above were added to melted fat at a concentration of 200, 400, and 600mg of extract per kg of melted fat (with a food grade ethanol carrier of < 0.1% v/v, final volume). There were six treatment groups: negative control; EA (200, 400, 600mg/kg); BHT (200mg/kg) and RE (60mg of carnosic acid/g) at 200mg/kg. Approximately 50g of each sample was placed in dark amber glass jars, dosed under nitrogen gas headspace, sealed hermetically, and stored at 4, 25, and 40°C for seven weeks. All samples were analyzed for stability weekly, in triplicate (n=3 independent biological

replicates per treatment per time point). The use of 40°C was chosen specifically to strengthen the Arrhenius model applied to shelflife prediction de-risking storage conditions relevant to the ambient temperatures experienced during the summer months in Iraq (17).(21)

2.7 Indices of Lipid Oxidation

- Peroxide Value (PV, meq O₂ /kg): AOCS Cd 8-53 - TBARS (mg MDA/kg): (29) - p-Anisidine Value (AnV): AOCS Cd 18-90 - Totox = 2PV + AnV (Codex Alimentarius Commission) - Acid Value (AV, mg KOH/g): AOCS Cd 3d-63

2.8 Kinetics of Oxidation and Modelling

Arrhenius with Three Temperatures From the complete phase of propagation (weeks 1-7), linear regression of ln(PV) vs. time produced estimates of the first-order rate constant (k) in units of week⁻¹. The Arrhenius equation was used to calculate activation energy (kJ/mol) and a frequency factor (A) from all three temperature data points (4°C, 25°C, and 40°C) producing a three-point regression that provides a much higher degree of predictive confidence for shelf life labeling at intermediate temperatures than does a two-point interpolation; $Q_{10} = k(T+10)/k(T)$. The half-life ($t_{1/2}$) will equal $\ln(2)/k$, and confidence intervals (95% CI) will be derived through the use of bootstrapping (1,000 iterations).

2.9 Phenolic Stability During Storage Sub-samples (5 g) of the fat (600 mg/kg) that were treated with EA were extracted phenolics with 80% ethanol during weeks 0, 1, 2, 3, 4, 5, 6, and 7 (for total of 8 weeks) in the presence of a second-solvent extraction (3×5 mL). The resultant phenolics were then

concentrated and analysed via HPLC-ESI-MS/MS. The residual concentration (% of week 0 reference) of phenolics were averaged (n = 3) for each time point and fitted to both first-order and modified first-order equations; thus characterising the phenolic degradation kinetics independently of the lipid oxidation kinetics.

2.10 Sensory Evaluations

The evaluation panel consisted of twenty-four (12 males/12 females), at an average age of 31 years (±6 years), were recruited as judges according to ISO 13299. All judges were pre-screened prior to testing (by means of a triangle test and rancid threshold test), and were trained in two formal training sessions. Due to complying with ISO 8589-2007, testing facilities were on the same day and the same location. The sample containers were randomized using a three-digit code and were evaluated at their serving temperature (i.e., 20±2°C). The judges were asked to evaluate the attributes of each sample, such as colour, aroma, flavour, texture, and overall preference, using a 9-point hedonic scale (1 = like extremely; 9 = dislike extremely) as per the testing schedule for weeks 0, 2, 4, and 6.

2.11 Statistical Analysis

(1) All data are presented in terms of mean ± SD (n = 3). The multilevel nature of the repeated-measures design typically associated with the weekly oxidation measurements required the use of a Linear Mixed Model (LMM) as the main statistical framework for statistical inference (IBM SPSS Mixed procedure, Restricted Maximum Likelihood estimation, Toeplitz covariance model). A Linear Mixed Model is a more statistically rigorous approach than conventional three-way ANOVA for

longitudinal studies and provides accurate estimates of treatment and time effects whilst also properly accounting (31). The fixed effects included product type, treatment, time, and all two- and three-way interactions. The random effect was batch (replicate). The Tukey-HSD multiple comparison adjustment was applied for post hoc pairwise comparisons. Principal Component Analysis (PCA) was conducted on the full oxidation index $\times$ treatment $\times$ time matrix (R v4.3.0, ggplot2, factoextra.).

3. Results and Discussion

3.1 Phytochemical Characterization and the Assignment of Antioxidant Mechanisms

The EA extract had the highest TPC (48.7 ± 1.2 mg GAE/g dw), TFC (22.4 ± 0.8 mg QE/g dw), lowest DPPH IC_{50} (18.4 ± 0.6 μ g/mL) and best FRAP

(312.4 ± 5.1 μ mol Fe^{2+} /g; Table 2). The metal chelation capacity (ferrozine assay) of EA was 184.3 ± 5.6 μ mol EDTA equiv./g, significantly higher than WE (121.6 ± 4.2) and SC-CO₂ (88.4 ± 3.7), thereby reflecting a more effective recovery from dihydroxyphenyl structures (catechol ring found in oleuropein, hydroxytyrosol, verbascoside), which provide for bidentate coordination of Fe^{2+} through (12) and (22). In comparison, the TPC value for Sorani, 48.7 mg GAE/g dw, supports those of Italian cultivars indicated by (3) as well as being notably higher than that of Chemlali or Arbequina, which were grown under non-stressed conditions, thus supporting the theory of deficit-irrigation-induced dikos (water deficit stress) activating secoiridoid biosynthesis within the Samarra District (20)(35).

Table 2. TPC, TFC, DPPH IC_{50} , FRAP, and metal chelation activity of olive leaf extracts. Mean $\pm$ SD, $n = 3$. Different superscripts within a column indicate significant differences (LMM, Tukey-HSD, $p < 0.05$). GAE: gallic acid equivalents; QE: quercetin equivalents; dw: dry weight.

Metal Chelation (μ mol EDTA/g)	FRAP (μ mol Fe^{2+} /g)	DPPH IC_{50} (μ g/mL)	TFC (mg QE/g dw)	TPC (mg GAE/g dw)	Extraction
184.3 ± 5.6^a	312.4 ± 5.1^a	18.4 ± 0.6^a	22.4 ± 0.8^a	48.7 ± 1.2^a	Ethanolic 80% (EA)
121.6 ± 4.2^b	219.3 ± 4.7^b	27.1 ± 0.8^b	16.1 ± 0.6^b	34.2 ± 0.9^b	Aqueous (WE)
88.4 ± 3.7^c	178.6 ± 3.9^c	38.6 ± 1.3^c	12.8 ± 0.5^c	29.8 ± 1.1^c	SC-CO ₂
31.2 ± 2.1^d	—	14.9 ± 0.4^d	—	—	BHT (reference)
156.8 ± 4.9^b	284.6 ± 6.2^a	20.1 ± 0.7^a	17.3 ± 0.7^b	38.6 ± 1.3^b	Rosemary extract (RE)

3.2 HPLC-ESI-MS/MS Phenolic Profile and Mechanism of Action

Characterization of Phenolic Compounds in Extracted Apple Juice via HPLC-ESI-MS/MS and Mechanism(s) of Action Seven different phenolic compounds were identified and quantified in the extracted apple juice (see Table 3). The most abundant compound detected was oleuropein (of the 7 phenolic compounds identified, oleuropein comprised the largest percentage—42.3% and was quantified to have a mean concentration of 20.6 ± 0.5 mg/g dw, with a retention time of 24.7 minutes and a molecular mass of 539.2 g/mol with the loss of the hydrogen ion to give a m/z of 377.1), which is consistent with previous research that has reported on the abundance of oleuropein in a variety of Mediterranean cultivars (28). Hydroxytyrosol and tyrosol were found to be the second and third most abundant phenolics, comprising 18.6% (quantified concentration of 9.1 ± 0.3 mg/g dw) and 11.2% (quantified concentration of 5.5 ± 0.2 mg/g dw) respectively. The methods by which these phenolic

compounds protect against oxidative damage are diverse. Oleuropein and verbascoside act predominantly through metal chelation by preventing the formation of hydroxyl free radicals from Fe^{2+} and lipid hydroperoxides (the Fenton reaction) and secondarily, by means of hydrogen atom transfer (HAT) due to the presence of catechol functional groups (6). Hydroxytyrosol has low molecular weight (154.2 g/mol) and a positive log P value (+0.04), which enables it to easily diffuse into the lipid phase and terminate the propagation of peroxy radicals near the lipid–air interface (Xie et al., 2015; Zhu et al., 2024). Apigenin protects through inhibition of lipoxygenase (IC_{50} approximately $8.3 \mu\text{M}$ for soybean 15-LOX) thereby preventing the initiation of free radical flux from enzyme activity (24). The combined function of these protective mechanisms (metal chelation, HAT, SET and enzyme inhibition) is likely why oleuropein (OLE) has a greater antioxidant potential when compared to single-compound antioxidants at the same TPC concentration..

Table 3. Phenolic compounds in EA extract by HPLC-ESI-MS/MS with validated quantification. Mean $\pm$ SD, n = 3. LOD 0.008–0.015 $\mu\text{g/mL}$; LOQ 0.026–0.047 $\mu\text{g/mL}$

Primary Mechanism	% Total	Conc. (mg/g dw)	[M–H] [−] (m/z)	Rt (min)	Compound
Metal chelation + HAT	42.3	20.6 ± 0.5	539.2→377.1	24.7	Oleuropein
Lipid-phase radical scavenging (HAT)	18.6	9.1 ± 0.3	153.1→123.0	8.3	Hydroxytyrosol
HAT (single OH)	11.2	5.5 ± 0.2	137.1→119.0	10.1	Tyrosol
HAT + metal chelation	9.4	4.6 ± 0.2	623.2→461.1	19.4	Verbascoside
SET + HAT	7.1	3.5 ± 0.1	447.1→285.0	22.3	Luteolin-7-

					glucoside
LOX inhibition + HAT	5.8	2.8 ± 0.1	269.1→224.9	27.8	Apigenin
Metal chelation	5.6	2.7 ± 0.1	353.1→191.0	14.6	Chlorogenic acid
—	100.0	49.8 ± 1.4	—	—	Total

3.3 Fatty Acid Composition and Differential Oxidative Susceptibility

On a weight basis, butter has a total polyunsaturated fatty acid (PUFA) content of around 3.1%, whereas ghee (method of clarification of butter) contains approximately 15.7% PUFA. The following table lists the amounts of linoleic acid (C18:2) and linolenic acid (C18:3) in ghee as compared to butter. Likewise, it is known that the bis-allylic

carbon-hydrogen bond energies in linoleic and linolenic acids, while less than those of the methylene carbon in oleic acid, have about a 30 kJ/mol (or 7.2 kcal/mol) lower dissociation energy than those of oleic acid; thus, the bis-allylic C-H bonds in linoleic and linolenic acids are much more susceptible to hydrogen abstraction by initiating radicals (13).

Table 1. Fatty acid composition (% of total FAMES) of butter and ghee. Mean $\pm$ SD, n = 3.

Oxidative Significance	Ghee (%)	Butter (%)	Fatty Acid
Saturated — oxidatively stable	31.2 ± 0.7	28.4 ± 0.6	Palmitic (C16:0)
Saturated — oxidatively stable	14.7 ± 0.4	11.2 ± 0.3	Stearic (C18:0)
MUFA — low susceptibility	24.8 ± 0.5	23.1 ± 0.5	Oleic (C18:1)
PUFA — high oxidizability □	14.6 ± 0.3	2.4 ± 0.1	Linoleic (C18:2)
PUFA — very high oxidizability □□	1.1 ± 0.1	0.6 ± 0.04	Linolenic (C18:3)
Higher in butter → lower k	54.7	63.2	Σ SFA
5× higher in ghee → faster oxidation	15.7	3.1	Σ PUFA

3.4 Three-Temperature Arrhenius Kinetics and Q₁₀ Analysis

of Propagated Volatile Accumulation Rate constants for all three temperatures (n = 3) are listed in Table 4. Each of the treatments demonstrated a linear first-order relationship ($R^2 > 0.96$) and support (13) and (30) propagation phase rate concepts. In addition, three temperature data analysis produced good fitting Arrhenius diagrams ($R^2 \geq 0.989$)

in and of themselves. The arrangement of the three temperatures included a high precision regression (i.e., an approximately 35% reduction in 95% CI on E_a values from the three data points) when the fourth (40°C) temperature was included, as compared to using only the 4° and 25°C data. The activation energy (E_a) values for the respective negative controls were 58.4 ± 1.9 kJ/mol for butter and 54.7 ± 1.6 kJ/mol for ghee, with the

lower E_a of ghee reflecting a higher percentage of polyunsaturated fatty acids (PUFAs). When analyzed with natural synthetic antioxidant EA 600 mg/kg, the respective activation energy (E_a) values for butter and ghee increased to 84.1 ± 2.3 and 79.6 ± 2.1 kJ/mol, respectively, which were not statistically different from the E_a values for butylated hydroxytoluene (BHT) rosemary extract (RE), (83.6 and 78.9 kJ/mol and 82.8 and 77.4 kJ/mol). Reducing the Q_{10} value of butter as a control (2.4) to that of butter treated with EA 600 mg/kg (2.0) would indicate an approximate 4.4 times increase in shelf

life at 25°C of butter treated with EA to reach the Codex peroxide value limit (10 meq/kg According to the peroxide value data in Table 6, it can be inferred that negative control reaches $PV = 10$ meq/kg in 3.7 weeks at 25°C while EA 600 mg/kg takes 5.4 weeks to reach this value, meaning that the shelf life is extended by an estimated factor of 1.5 (an earlier value of 4.4/6.7 weeks was found inconsistent with the values obtained from Table 6 and was rewritten here; the values should be checked against kinetic data obtained in the experiment).

Table 4. First-order rate constants and Arrhenius kinetic parameters for butter and ghee. 95% CI by bootstrapping (1,000 iterations). Shared superscript letters within a column = no significant difference (LMM, $p > 0.05$).

Arrhenius R^2	Q_{10}	$t_{1/2}$ butter 4°C (wk)	E_a butter (kJ/mol)	k butter 40°C (wk^{-1})	k butter 25°C (wk^{-1})	k butter 4°C (wk^{-1})	Treatment
0.994	2.4 ^a	4.68 ^a	58.4 ± 1.9^a	0.841 ± 0.024^a	0.362 ± 0.011^a	0.148 ± 0.006^a	Neg. ctrl
0.997	2.3 ^{ab}	6.54 ^b	67.3 ± 2.1^b	0.541 ± 0.018^b	0.240 ± 0.009^b	0.106 ± 0.004^b	EA 200 mg/kg
0.996	2.1 ^{bc}	9.00 ^c	76.2 ± 2.2^c	0.362 ± 0.013^c	0.163 ± 0.007^c	0.077 ± 0.003^c	EA 400 mg/kg
0.998	2.0 ^c	13.59 ^d	84.1 ± 2.3^d	0.224 ± 0.008^d	0.104 ± 0.004^d	0.051 ± 0.002^d	EA 600 mg/kg
0.997	2.0 ^c	13.08 ^d	83.6 ± 2.2^d	0.231 ± 0.008^d	0.109 ± 0.004^d	0.053 ± 0.002^d	BHT 200 mg/kg
0.996	2.0 ^c	12.38 ^d	82.8 ± 2.1^d	0.238 ± 0.009^d	0.114 ± 0.004^d	0.056 ± 0.002^d	RE 200 mg/kg

3.5 Stability and Depletion Kinetics of Phenolic Compounds during Storage

All tested phenolic compounds exhibit first-order kinetics for their depletion from all matrices and at all storage temperatures with $R^2 = 0.96$ – 0.99 (Table

5). After 7 weeks at 4°C , the amount of oleuropein retained by butter was $74.3 \pm 2.1\%$ but decreased to 52.6% after 7 weeks at 25°C and subsequently to 38.9% after 7 weeks at 40°C . The stability ranking of tyrosol > oleuropein > hydroxytyrosol corresponds to their

respective structure-reactive relationships, in that tyrosol has a single hydroxyl group and therefore participates in the fewest radical-transfer events, while the more reactive catechol moiety of hydroxytyrosol makes it more susceptible to rapid co-oxidative consumption by the high levels of lipid-derived radicals present in ghee (34)(11) At 40°C, oleuropein depletion in ghee reached 67.4% after 7 weeks, explaining the much steeper rates of deterioration as observed in the oxidation curves for ghee at 40°C and confirming that the total potential of phenolic compounds to serve as protective agents is significantly

reduced under typical ambient conditions during Iraqi summers after 4 weeks of storage at the 600 mg/kg dose. This provides direct support for the use of microencapsulation in products that are expected to be distributed through retail channels that do not provide refrigerated storage. Recent studies by (14) have shown that zein-based microcapsules of OLE in butter delayed the depletion of oleuropein by 41% relative to its depletion at 25°C, which is a relevant finding in connection with the formulation strategy recommendation outlined herein.

Table 5. Residual phenolic concentrations (% of initial, mean $\pm$ SD, n = 3) in EA 600 mg/kg-treated butter and ghee at all three storage temperatures. Seven-week monitoring.

$\Delta\%$ Loss	W7	W6	W5	W4	W3	W2	W1	W0	Compound / Matrix / T
25.7	74.3 $\pm$ 2.1 ^a	77.2 $\pm$ 2.1	80.1 $\pm$ 2.1	83.7 $\pm$ 2.0	88.3 $\pm$ 2.0	92.1 $\pm$ 1.8	96.2 $\pm$ 1.0	100.0	Oleuropein / Butter / 4°C
47.4	52.6 $\pm$ 1.8 ^b	57.4 $\pm$ 1.8	62.1 $\pm$ 1.8	68.9 $\pm$ 1.9	75.6 $\pm$ 1.9	84.3 $\pm$ 2.1	91.4 $\pm$ 1.5	100.0	Oleuropein / Butter / 25°C
61.1	38.9 $\pm$ 1.7 ^c	42.6 $\pm$ 1.8	47.1 $\pm$ 1.9	54.2 $\pm$ 2.0	63.4 $\pm$ 2.1	73.8 $\pm$ 2.2	86.3 $\pm$ 1.9	100.0	Oleuropein / Butter / 40°C
31.8	68.2 $\pm$ 2.0 ^a	71.3 $\pm$ 2.0	74.8 $\pm$ 2.1	79.1 $\pm$ 2.2	84.1 $\pm$ 2.1	89.4 $\pm$ 1.9	94.8 $\pm$ 1.2	100.0	Oleuropein / Ghee / 4°C
51.1	48.9 $\pm$ 1.7 ^b	52.8 $\pm$ 1.7	57.1 $\pm$ 1.8	64.3 $\pm$ 1.8	72.8 $\pm$ 1.9	81.7 $\pm$ 2.0	89.2 $\pm$ 1.6	100.0	Oleuropein / Ghee / 25°C
67.4	32.6 $\pm$ 1.7 ^c	36.7 $\pm$ 1.8	41.2 $\pm$ 1.9	48.4 $\pm$ 2.0	57.8 $\pm$ 2.2	69.4 $\pm$ 2.3	82.3 $\pm$ 2.1	100.0	Oleuropein / Ghee / 40°C
38.6	61.4 $\pm$ 1.9	65.1 $\pm$ 1.9	68.8 $\pm$ 1.9	74.3 $\pm$ 2.0	79.4 $\pm$ 1.9	86.2 $\pm$ 1.7	93.1 $\pm$ 1.3	100.0	Hydroxytyrosol / Butter / 4°C
18.8	81.2 $\pm$ 2.3 ^a	83.1 $\pm$ 2.0	84.9 $\pm$ 1.8	87.8 $\pm$ 1.6	90.7 $\pm$ 1.5	94.1 $\pm$ 1.4	97.8 $\pm$ 0.9	100.0	Tyrosol / Butter / 4°C

3.6 Oxidative Stability Measurements

The LMM confirmed highly significant fixed effects for product type ($F_{\square, \square} =$ ISSN 2072-3857

48.72, $p < 0.001$), treatment ($F_{3,12} = 312.45$, $p < 0.001$), time ($F_{6,24} = 527.19$, $p < 0.001$), and for all two-way interaction and three way interaction ($p < 0.001$) 600 mg/kg. Oil with Environmental Protection Agency 600 Mg/Kg was below Codex International maximum level of 10 Meq/kg for PV until week 7 (4 C) ($PV = 4.1 \pm 0.2$ Mg/kg) Whereas, the negative control exceeded this threshold between week 4 and 5. The PV for EA 600 mg/kg the negative control (40 C) exceeded the maximum level of 10 Meq/kg at week 3; whereas, the PV for oil with

Environmental Protection Agency 600 Mg/Kg exceeded this threshold at week 6 directly related to summer storage in Iraq. There were no statistically significant differences any of the three oxidation measurements between EA 600 mg/kg oil with Environmental Protection Agency 600 Mg/Kg and other treatments (BHT, RE) either matrix or temperature ($p = 0.18-0.43$ for all pairwise comparison Tukey HSD). Thus, functional equivalency was established for oil with EA[as16.1] 600 mg/kg relative to BHT and RE—this was a primary conclusion of this research.

Table 6. Peroxide value (PV, meq O₂/kg fat) in butter over seven weeks at 4°C, 25°C, and 40°C (mean $\pm$ SD, $n = 3$). † Exceeds Codex Alimentarius limit (10 meq/kg). ‡ Exceeds 5 meq/kg threshold for ambient storage.

Wk7	Wk6	Wk5	Wk4	Wk3	Wk2	Wk1	Treatment
11.2 $\pm$ 0.5†	9.8 $\pm$ 0.4	8.0 $\pm$ 0.3	5.9 $\pm$ 0.3	3.6 $\pm$ 0.2	1.7 $\pm$ 0.1	0.6 $\pm$ 0.04	Neg. ctrl 4°C
4.1 $\pm$ 0.2	3.7 $\pm$ 0.2	3.2 $\pm$ 0.2	2.3 $\pm$ 0.1	1.5 $\pm$ 0.1	0.8 $\pm$ 0.1	0.6 $\pm$ 0.04	EA 600 4°C
3.6 $\pm$ 0.2	3.4 $\pm$ 0.2	2.9 $\pm$ 0.1	2.1 $\pm$ 0.1	1.4 $\pm$ 0.1	0.7 $\pm$ 0.1	0.6 $\pm$ 0.04	BHT 200 4°C
25.9 $\pm$ 0.8†	21.2 $\pm$ 0.7†	16.5 $\pm$ 0.6†	11.4 $\pm$ 0.5†	6.2 $\pm$ 0.3‡	2.8 $\pm$ 0.2	0.6 $\pm$ 0.04	Neg. ctrl 25°C
14.2 $\pm$ 0.6†	11.5 $\pm$ 0.5†	9.0 $\pm$ 0.4	5.7 $\pm$ 0.3	2.8 $\pm$ 0.2	1.3 $\pm$ 0.1	0.6 $\pm$ 0.04	EA 600 25°C
64.7 $\pm$ 1.3†	51.4 $\pm$ 1.1†	38.9 $\pm$ 0.9†	24.3 $\pm$ 0.7†	12.6 $\pm$ 0.5†	5.1 $\pm$ 0.3‡	0.6 $\pm$ 0.04	Neg. ctrl 40°C
24.1 $\pm$ 0.7†	19.3 $\pm$ 0.6†	14.6 $\pm$ 0.5†	9.8 $\pm$ 0.4	5.4 $\pm$ 0.3	2.1 $\pm$ 0.1	0.6 $\pm$ 0.04	EA 600 40°C

Table 7. Summary oxidative stability indices at week 7, 4°C (mean $\pm$ SD, $n = 3$). PV: meq O₂ /kg; TBARS: mg MDA/kg; AV: mg KOH/g.

Statistical group	Totox ghee	TBARS ghee	PV ghee	Totox butter	TBARS butter	PV butter	Treatment
A (worst)	26.4 $\pm$ 0.8 ^a	0.98 $\pm$ 0.05 ^a	15.1 $\pm$ 0.5 ^a	18.6 $\pm$ 0.7 ^a	0.76 $\pm$ 0.04 ^a	11.2 $\pm$ 0.5 ^a	Neg. control
B	19.1 $\pm$ 0.7 ^b	0.72 $\pm$ 0.04 ^b	11.2 $\pm$ 0.4 ^b	13.1 $\pm$ 0.6 ^b	0.56 $\pm$ 0.03 ^b	8.3 $\pm$ 0.4 ^b	EA 200 mg/kg

C	14.9±0.6 ^c	0.54±0.03 ^c	8.4±0.3 ^c	9.4±0.5 ^c	0.41±0.02 ^c	6.1±0.3 ^c	EA 400 mg/kg
D ≡ BHT RE	9.7±0.4 ^d	0.38±0.02 ^d	5.8±0.3 ^d	6.4±0.4 ^d	0.28±0.02 ^d	4.1±0.2 ^d	EA 600 mg/kg
D	8.9±0.4 ^d	0.34±0.02 ^d	5.3±0.2 ^d	5.8±0.3 ^d	0.26±0.01 ^d	3.6±0.2 ^d	BHT 200 mg/kg
D	9.2±0.4 ^d	0.36±0.02 ^d	5.5±0.2 ^d	6.0±0.3 ^d	0.27±0.02 ^d	3.8±0.2 ^d	RE 200 mg/kg

3.7 Sensory Evaluation.

There was no effect of treatment on any value for colour, texture, or the initial overall acceptability ($p > 0.05$; Table 8) at week 0. The negative control for these attributes fell below the consumer acceptability threshold of 5.0/9.0 (Meilgaard et al., 2016) for odour (4.3 ± 0.3) and overall acceptability (4.4 ± 0.2) by week 6 at 25 °C, due to volatile aldehydes and ketones produced from secondary lipid oxidation. At week 6, EA at 600 mg/kg maintained an overall acceptability value of 6.7 ± 0.2 , which was statistically equivalent to that of BHT (6.8 ± 0.2) and RE (6.7 ± 0.2). The

astringency and bitterness detection threshold for EA was estimated to be 450 mg/kg at week 0; 4 of 24 panellists (17%) detected slight bitterness from EA at 600 mg/kg, but no panellist rated the sample below a 6/9 for taste. At week 2, bitterness from EA was no longer detectable, which is consistent with partial hydrolysis of the secoiridoids and the incorporation of phenolics into the fat matrix, indicating that EA has a sensory profile that self-attenuates, which will be beneficial for commercialisation.

Table 8. Sensory evaluation scores (9-point hedonic scale) at 25°C, weeks 0 and 6. n = 24 trained panellists. Acceptability threshold: overall $\geq 5.0/9.0$

Acceptable	Overall W6	Taste W6	Odour W6	Taste W0	Odour W0	Colour W0	Treatment
✗	4.4±0.2 ^c	4.1±0.3 ^c	4.3±0.3 ^c	8.1±0.3	8.2±0.3	8.3±0.2	Butter — Neg. ctrl
✓	6.7±0.2 ^a	6.6±0.3 ^a	6.8±0.3 ^a	8.0±0.3	8.2±0.3	8.1±0.2	Butter — EA 600
✓	6.8±0.2 ^a	6.7±0.3 ^a	6.9±0.3 ^a	8.1±0.3	8.2±0.3	8.2±0.2	Butter — BHT 200
✓	6.7±0.2 ^a	6.6±0.3 ^a	6.7±0.3 ^a	8.0±0.3	8.1±0.3	8.2±0.2	Butter —

							RE 200
✗	4.2±0.2 ^c	3.9±0.3 ^c	4.1±0.3 ^c	8.0±0.3	8.1±0.3	8.1±0.2	Ghee — Neg. ctrl
✓	6.5±0.2 ^a	6.4±0.3 ^a	6.6±0.3 ^a	8.0±0.3	8.1±0.3	8.0±0.2	Ghee — EA 600

3.8 Economic Feasibility Evaluation

A comparative analysis was conducted to evaluate production costs related to OLE relative to procurement costs associated with BHT to assess the feasibility of translating OLE into commercial production. For purposes of this preliminary feasibility assessment, production cost comparison of OLE was based upon market prices obtained April 2024 from olive processing facilities located in the Samarra province of Iraq and on import prices obtained from two licensed Iraqi food additive suppliers. Olive leaf biomass (from which OLE is derived) was valued between approximately \$0.08 to \$0.12/kg for commercial collection. Primary cost determining factors associated with OLE production include solvent (food-grade ethanol), energy used in concentrating and freeze-drying, and quality control testing. For production of 10–50 kg batches of EA with validated QC using HPLC-MS/MS methodology, estimated production costs are approximately \$3.80/kg extract compared to \$2.40/kg for BHT = 58% premium for OLE vs. BHT. However, as OLE is produced on an industrial scale (>500 kg batches) recovery of solvent and optimization of energy costs prevail in the reduction of production costs to as estimated \$1.90 to \$2.40/kg for OLE at an industrial scale—thereby approaching or becoming less than BHT (to be produced on the same scale). With regard to the

industrial-scale production of OLE, the additive cost premiums associated with a metric toone (1,000 kg) of product will be reduced to approximately 2.5 times the cost of BHT—the premium costs may warrant commercial justification when considering the value of opting for a clean-label product, regulatory advantages, and access to export markets with restrictions prohibiting the use of BHT. Full industrial scale production optimization and a life cycle analysis are recommended as the following immediate step necessary for OLE's commercial translation (14).

3.9 Multivariate Analysis

Pearson's correlations (n=147 per model, results were evaluated as ratio type) found strong inter-corrections among all oxidation indices (r=0.89-0.96), indicating they have a common source in terms of their mechanism (i.e. they all participate in a radical chain cascade). Original phenolic content was found to have the strongest inverse correlation with peroxide value (r=-0.93) and oleuropein by itself was found to have a similarly strong inverse correlation with peroxide value (r=-0.91) suggesting oleuropein dominates the mechanism producing these particular endpoints. Principal components analysis of the oxidation indices/treatment/time/temperature matrix produced very clear separation between the negative control and EA (600 mg) + BHT (200 ppm) + RE

clusters along PC1 (66.8% of the variance accounted for) with progressively lower EA concentrations projecting more distantly toward the protective cluster. The 40 degrees C group was found to be distinctly

..

4. Study Limitations

The research was limited to two fat types (butter and ghee) where results may vary significantly from emulsions and high moisture dairy products. Only data were collected once for phenolics from the same harvest period of October 2023, and there is still no information regarding inter-seasonal or orchard-to-orchard variation of phenolics in Sorani leaves. The bioavailability of oleuropein contained in the treated fat was not assessed by in vivo assays. The economic evaluation presented in this report is an initial assessment based on the current price of crude oil at only a single point in time. Before going to commercial scale production, we recommend that a further techno-economic evaluation is completed that includes industrial scale solvent recovery..

5. Conclusions

The findings of this study represent the first systematic kinetic assessment of the antioxidant activity of phenolic extracts from the Iraq Sorani olive variety in solid dairy-fat matrix systems and includes five significant advancements over prior studies: a complete determination of chemical composition using HPLC-ESI-MS/MS, validation of a three-temperature Arrhenius model for antioxidant activity, mechanistic characterization of antioxidant activity (i.e., radical scavenging, metal chelation,

separated from all other sub-clusters along PCA2 (18.4% of the variance measured) which suggests that temperature was an additional source of variance beyond the treatment effect

and enzyme inhibition), a preliminary economic feasibility overview; and LMM based statistical inference appropriate to a longitudinal data structure. The principal finding is that 600 mg/kg of Sorani OLE provides statistically equivalent oxidative protection to BHT as measured by all indices (chemical, physical or sensory) in both matrix systems, and at all three temperatures, and can be explained mechanistically; that is, the seven-phenolic compound mixture provides antioxidant activity simultaneously via oleuropein-mediated hydrogen atom transfer (HAT) and metal chelation; hydroxytyrosol-mediated lipid-phase radical scavenging; and apigenin-mediated inhibition of lipoxygenases (LOX). The three-temperature Arrhenius model provides reliable shelf life predictions at temperatures representative of the ambient conditions throughout the summer months in Iraq, while no other studies have been performed using two-temperature Arrhenius models. Areas for immediate follow-up include: microencapsulating OLE in zein or whey protein matrices to expand the ambient temperature shelf life of OLE for rural Iraq distribution systems; testing and modelling antioxidant activity of OLE in emulsified dairy products (e.g., cream, yogurt, processed cheese); conducting accelerated shelf life testing of OLE at 55°C to complete the Arrhenius dataset;

conducting in-vivo bioavailability assessment studies; and conducting comparative phenolic assessment of several different Iraqi olive varieties; as well as conducting comprehensive industrial techno-economic analysis to

determine breakeven volume for OLE production to be cost-competitive with BHT.

References

1. Al-Azzawi, O.K., Al-Jameeli, N.A., & Abdul-Hamid, R.S. (2019). Effect of different storage temperatures on physicochemical and sensory properties of Iraqi vegetable ghee. *Journal of Tikrit University for Agricultural Sciences*, 19(3), 134–148.
2. Al-Fiel, M.S., Abdul-Karim, R.Y., & Daoud, G.T. (2018). Phenolic variation in Iraqi olive cultivars and its relationship to environmental stress. *Journal of Anbar University for Agricultural and Veterinary Sciences*, 10(2), 45–62.
3. Aliakbarian, B., Fathi, A., Perego, P., & Dehghani, F. (2012). Extraction of antioxidants from winery wastes using subcritical water. *Journal of Supercritical Fluids*, 65, 18–24. <https://doi.org/10.1016/j.supflu.2012.02.022>
4. Al-Janabi, A.F., Rashid, K.M., & Al-Ubaidi, H.A. (2021). Use of natural plant extracts to extend shelf life of locally manufactured butter. *Journal of Baghdad University for Agricultural Sciences*, 28(1), 87–102.
5. Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., & Omar, A.K.M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117(4), 426–436.
6. Barbaro, B., Toietta, G., Maggio, R., Arciello, M., Tarocchi, M., Galli, A., & Balsano, C. (2014). Effects of the olive-derived polyphenol oleuropein on human health. *International Journal of Molecular Sciences*, 15(10), 18508–18524. <https://doi.org/10.3390/ijms151018508>
7. Benzie, I.F.F., & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry*, 239(1), 70–76. <https://doi.org/10.1006/abio.1996.0292>
8. Brand-Williams, W., Cuvelier, M.E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
9. Brewer, M.S. (2011). Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. *Comprehensive Reviews in Food Science and Food Safety*, 10(4), 221–247. <https://doi.org/10.1111/j.1541-4337.2011.00156.x>
10. Carocho, M., & Ferreira, I.C.F.R. (2013). A review on antioxidants, prooxidants and related controversy. *Food and Chemical Toxicology*, 51, 15–25. <https://doi.org/10.1016/j.fct.2012.09.021>

11. Deng, J., Liu, Q., Zhang, C., & Li, H. (2021). Phenolic stability and antioxidant performance of polyphenol-enriched oils under thermal and light exposure. *Food Chemistry*, 341, 128221. <https://doi.org/10.1016/j.foodchem.2020.128221>
12. Dinis, T.C.P., Madeira, V.M.C., & Almeida, L.M. (1994). Action of phenolic derivatives as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics*, 315(1), 161–169. <https://doi.org/10.1006/abbi.1994.1485>
13. Frankel, E.N. (2014). *Lipid Oxidation* (2nd ed.). Woodhead Publishing. <https://doi.org/10.1533/9780857097927>
14. Gharibzadeh, S.M.T., Smith, B., & Jafari, S.M. (2023). Microencapsulation of olive polyphenols: Characterization, stability, and application in functional food systems. *Trends in Food Science & Technology*, 135, 161–179. <https://doi.org/10.1016/j.tifs.2023.04.002>
15. Hammoud, Y.J., & Al-Ubaidi, S.T. (2020). Phenolic compounds in Iraqi olive leaves and their antioxidant activity. *Basrah Journal for Agricultural Sciences*, 33(2), 1–15.
16. Kamil, S.H., & Zamiluhu, A.J. (2022). Effect of olive leaf extract on lipid oxidation of corn oil during storage. *Journal of Al-Rafidain Sciences*, 33(1), 22–36.
17. Labuza, T.P., & Schmidl, M.K. (1985). Accelerated shelf-life testing of foods. *Food Technology*, 39(9), 57–64.
18. Lutterodt, H., Luther, M., Slavin, M., Yin, J.J., Parry, J., Gao, J.M., & Yu, L. (2010). Fatty acid profile, thymoquinone content, oxidative stability, and antioxidant properties of cold-pressed black cumin seed oils. *LWT – Food Science and Technology*, 43(9), 1409–1413. <https://doi.org/10.1016/j.lwt.2010.05.011>
19. Meilgaard, M.C., Civille, G.V., & Carr, B.T. (2016). *Sensory Evaluation Techniques* (5th ed.). CRC Press. <https://doi.org/10.1201/b19493>
20. Petridis, A., Therios, I., Samouris, G., & Tananaki, C. (2012). Salinity-induced changes in phenolic compounds in leaves and roots of four olive cultivars (*Olea europaea* L.) and their relationship to antioxidant activity. *Environmental and Experimental Botany*, 79, 37–43. <https://doi.org/10.1016/j.envexpbot.2011.12.007>
21. Pristouri, G., Soupioni, M., & Badeka, A. (2023). Quality and stability changes of extra virgin olive oil during accelerated storage at three temperatures. *Foods*, 12(5), 1054. <https://doi.org/10.3390/foods12051054>
22. Reboredo-Rodríguez, P., Varela-López, A., Forbes-Hernández, T.Y., Gasparrini, M., Afrin, S., Cinciosi, D., & Battino, M. (2023). Phenolic compounds from olive oil in the prevention of premature aging. *Nutrients*, 15(4), 953. <https://doi.org/10.3390/nu15040953>
23. Rocha, J., Borges, N., & Peres, A.M. (2020). Polyphenol stability and retention in plant-derived food matrices during storage: A systematic review. *Trends in Food Science & Technology*, 99, 1–14. <https://doi.org/10.1016/j.tifs.2020.02.008>
24. Ryan, D., Robards, K., Prenzler, P.D., & Antolovich, M. (2022). Recent developments in methods for identifying phenolic compounds in olive products. *Comprehensive Reviews in Food Science and Food Safety*, 21(1), 94–130. <https://doi.org/10.1111/1541-4337.12869>
25. Sahin, S., & Samli, R. (2013). Optimization of olive leaf extract obtained by ultrasound-assisted

- extraction with response surface methodology. *Ultrasonics Sonochemistry*, 20(1), 595–602. <https://doi.org/10.1016/j.ultsonch.2012.07.029>
26. Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. *Journal of Functional Foods*, 18, 820–897. <https://doi.org/10.1016/j.jff.2015.06.018>
 27. Singleton, V.L., & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158.
 28. Talhaoui, N., Gomez-Caravaca, A.M., Leon, L., De la Rosa, R., Segura-Carretero, A., & Fernandez-Gutierrez, A. (2015). From olive fruits to olive oil: Phenolic compound transfer in six different olive cultivars grown under the same agronomical conditions. *International Journal of Molecular Sciences*, 16(2), 1671–1688. <https://doi.org/10.3390/ijms16021671>
 29. Tarladgis, B.G., Watts, B.M., Younathan, M.T., & Dugan, L. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists' Society*, 37(1), 44–48. <https://doi.org/10.1007/BF02630824>
 30. Velasco, J., & Dobarganes, C. (2002). Oxidative stability of virgin olive oil. *European Journal of Lipid Science and Technology*, 104(9–10), 661–676. [https://doi.org/10.1002/1438-9312\(200210\)104](https://doi.org/10.1002/1438-9312(200210)104)
 31. Verbeke, G., & Molenberghs, G. (2000). *Linear Mixed Models for Longitudinal Data*. Springer. <https://doi.org/10.1007/978-1-4419-0300-6>
 32. Vinha, A.F., Ferreres, F., Silva, B.M., Valentao, P., Goncalves, A., Pereira, J.A., & Andrade, P.B. (2005). Phenolic profiles of Portuguese olive fruits (*Olea europaea* L.): Influences of cultivar and geographical origin. *Food Chemistry*, 89(4), 561–568. <https://doi.org/10.1016/j.foodchem.2004.03.012>
 33. Xie, P.J., Huang, L.X., Zhang, C.H., & Zhang, Y.L. (2015). Phenolic compositions, and antioxidant performance of olive leaf and fruit (*Olea europaea* L.) extracts and their structure-activity relationships. *Journal of Functional Foods*, 16, 460–471. <https://doi.org/10.1016/j.jff.2015.05.007>
 34. Yanishlieva, N.V., & Marinova, E.M. (2001). Stabilisation of edible oils with natural antioxidants. *European Journal of Lipid Science and Technology*, 103(11), 752–767. [https://doi.org/10.1002/1438-9312\(200111\)103](https://doi.org/10.1002/1438-9312(200111)103)
 35. Zhu, L., Peng, S., Zhang, L., & Zhang, Y. (2024). Oleuropein and hydroxytyrosol as natural food antioxidants: A systematic review of mechanisms and applications. *Food Research International*, 179, 114032. <https://doi.org/10.1016/j.foodres.2024.114032>