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Utilizing sweet basil hydrodistillation by-products to boost soybean oil stability and combat induced-hyperlipidemia

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Introduction: The growing interest in natural antioxidants to enhance the stability of edible oils has led to exploring plant-based compounds that preserve oil quality and address critical health concerns, such as managing hyperlipidemia, a significant risk factor for cardiovascular diseases and metabolic dysfunction. This study aimed to evaluate the efficacy of basil (*Ocimum basilicum* L.) hydrodistillation aqueous extract (A-Bs) on stabilizing refined soybean oil (RSO) under accelerated storage (60 °C) and frying conditions and investigate the possible hypolipidemic effect of the fortified RSO in a hyperlipidemic mouse model induced by Triton WR-1339.

Methods: Total phenolic, flavonoid, and tannin contents of A-Bs were quantified, and phenolic composition was determined by HPLC-DAD. Antioxidant activity was evaluated using DPPH, FRAP, and TAC assays. Free fatty acids, peroxide value, tocopherol content, and malondialdehyde content were measured in treated and untreated oils. A-Bs applied to RSO (0.02 % and 1 %) under accelerated storage (60 °C) and frying conditions alongside synthetic BHA (0.02 %). Hypolipidemic effect was evaluated in hyperlipidemic mice receiving fortified RSO at 125 mg/kg and 250 mg/kg.

Results: Basil extract, rich in polyphenols and flavonoids, exhibited vigorous antioxidant activity. The A-Bs reduced the RSO oxidative degradation and preserved α-tocopherol, outperforming synthetic BHA. *In vivo*, the fortified RSO at 250 mg/kg body weight lowered plasma TC (76.6 %), TG (86.5 %), and glucose (44.1 %), reduced LDL-C, and elevated HDL-C.

Conclusion: This dual functionality of A-Bs could be considered a promising natural solution to extend food shelf life and prevent lipid-related metabolic disorders.

KEYWORDS

***Ocimum basilicum* L., antioxidants, oxidative stability, refined soybean oil, hypolipidemic effect, food supply**

1 Introduction

Lipid oxidation stands out as a significant catalyst for the degradation of vegetable oils during both processing and storage. This process significantly impacts various factors crucial to food quality and consumer satisfaction, including nutritional value, safety, color, and the emergence of undesirable flavors (German, 1999). Inevitable oxidation by-products exhibit high reactivity and might contribute to adverse effects on human health, such as allergic reactions, heart disease, atherosclerosis, and cancer (Redondo-Cuevas et al., 2019).

Various antioxidants are incorporated into edible oils during industrial procedures to prevent degradation. Synthetic antioxidants, including substances like BHA and BHT, are commonly employed. Despite their effectiveness in enhancing the oxidative stability of oils, several researchers have expressed concerns about the potential carcinogenic properties of these synthetic compounds (Baştürk et al., 2018). Recently, substantial attention has been directed toward identifying natural antioxidants that can be employed in food or medicinal contexts, replacing synthetic antioxidants that are limited by their adverse effects. Natural antioxidants protect the human body against free radicals, slow the advancement of various chronic diseases, and control lipid oxidation-induced degradation in food products (Muscolo et al., 2024). Additionally, aromatic plants can be included in oils to enhance their content with constituents known for their antimicrobial and antioxidant properties (Gülçin et al., 2007; Soares et al., 2020).

Basil (*O. basilicum* L.), belonging to the Lamiaceae family, is commonly used for medicinal purposes. It has been extensively researched, and its effectiveness in managing oxidation has been confirmed due to its high levels of phenolic and aromatic compounds (Nadeem et al., 2022). Additionally, basil's chemical composition, which is mainly composed of polyphenolic acids and flavonoids, finds significant value in the pharmaceutical and cosmetic sectors due to its culinary utilization (Zhakipbekov et al., 2024). These constituents highlight various beneficial properties, including antioxidant, antiviral, antibacterial, and therapeutic attributes (Ch et al., 2015). The distinct aroma of basil is attributed to specific volatile compounds, which can be categorized into two primary chemical groups: terpenoids and phenylpropanoids (Baştürk et al., 2018).

The effectiveness of plant extract antioxidants mainly depends on factors such as the method of extraction, the type of solvent used, and the concentration of phenolic compounds (Koraqi et al., 2023; Xu et al., 2017). Various techniques exist for extracting herbal compounds, with solvent extraction using a Soxhlet apparatus being one of the most prevalent methods (Abd El Azim, 2015; Baştürk et al., 2018; Moczkowska et al., 2020).

Beyond their role in preserving food systems, natural antioxidants like basil extracts also contribute to managing metabolic disorders associated with oxidative stress. Triton WR-1339, a nonionic detergent, inhibits lipoprotein lipase activity (Moumou et al., 2025), resulting in a rapid increase in plasma total cholesterol (TC) and triglycerides (TG) (Borensztajn et al., 1976) and changes in both high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels (Goldberg et al., 1990; Ishikawa and Fidge, 1979). Increased LDL-C and decreased HDL-C are key indicators of dyslipidemia, which is often linked to cardiovascular diseases (Miller, 2009; Zhao et al., 2021). This model provides an effective platform for evaluating the therapeutic potential of natural extracts in restoring lipid balance while showcasing their antioxidant benefits in food-related applications.

Fats and oils, especially those high in unsaturated fatty acids, can undergo oxidation reactions, forming dangerous secondary products when exposed to an oxidizing atmosphere during processing, storage, and cooking (Blasi and Cossignani, 2020). Various natural strategies have been explored to improve oils' oxidative stability, including using extracts from natural sources like rosemary (Song et al., 2023), basil (Khatib et al., 2021), pomegranate, orange, and beetroot leaves (Alizadeh et al., 2016), among others.

The present study features a unique integrated approach composed of three complementary components. First, evaluating the *in vitro* antioxidant profile provides the molecular basis for its biological activity. Second, oxidative stability tests in refined soybean oil demonstrate its potential as a natural preservative. Finally, using an animal model of hyperlipidemia confirms its biological and functional relevance under physiological conditions. The core innovation of this work is the valorization of a basil hydrodistillation by-product, an underused resource, and its application to stabilize refined soybean oil. This creates an innovative link between agro-industrial valorization and metabolic health.

2 Materials and methods

2.1 Chemicals and plant material

Dried basil leaves (*O. basilicum* L.) were sourced from an herbalist, and RSO (Refined Soybean Oil) was procured from a supermarket in Oujda, Morocco. The nutritional composition and health certification of the RSO are provided in Supplementary Table S1. All solvents and reagents were obtained from Sigma Aldrich, Germany, except the Folin–Ciocalteu reagent, which was obtained from Fluka Analytical, Switzerland. All reagents and solvents were of analytical grade.

2.2 Preparation of the basil extracts

Basil extracts were prepared by hydrodistillation using distilled water for 4 h. During the process, the essential oil was collected separately, while the remaining plant material and water condensate were treated as by-products. The aqueous fraction obtained after separation of the essential oil was filtered and designated as the initial aqueous extract (A-Bs). To concentrate the extract, the filtrate was subsequently evaporated in an oven at 45 °C until a dry residue was obtained.

2.3 Preparation of refined soybean oil added with basil extract and BHA

For the preparation of samples intended for the accelerated oxidation test (60 °C), the ultrasonic extraction was applied for 10 min, while for the frying stability test (performed at 180 ± 2 °C), the extraction was extended to 30 min at 30 ± 2 °C. The ultrasound operated at a frequency of 50/60 Hz and an intensity of approximately 0.0485 W/cm^2. A-Bs was added to different beakers containing RSO at concentrations of 0.02 and 1%. The beakers were then placed in the reactor, and the mixture was filtered to remove any traces of the extract. To compare the oxidative stability of RSO, butylated hydroxyanisole (BHA) was used at a concentration of 0.02%.

2.4 Total polyphenol content of basil extracts

The total polyphenol content (TPC) was determined using the methodology described by Moumou et al. (2025). The A-Bs extract was prepared as a solution at a concentration of 1 mg/mL in distilled water. Subsequently, 250 µL of Folin–Ciocalteu reagent and 500 µL of a 20% sodium carbonate solution were added to 500 µL of each sample. After thorough mixing, the samples were incubated in the dark for 30 min. The absorbance of the samples was measured at 765 nm using a UV–Vis spectrophotometer (RAY LLEIGH VIS-7220-G, Rayleigh Instruments, Beijing, China). A blank was prepared by replacing the sample with an equivalent volume of distilled water. The results were expressed as milligrams of rosmarinic acid per gram of dry extract (mg RAE/g). All measurements were performed in triplicate.

2.5 Determination of flavonoid content of basil extracts

The flavonoid content (TFC) was estimated using aluminum chloride as a reagent, following the protocol described by Jay et al. (1975). A 1 mg/mL solution of A-Bs was prepared. Then, 1 mL of the test extract solution (1 mg/mL) was mixed with 0.5 mL of an $AlCl_3$ solution (containing 133 mg of $AlCl_3$ and 4% sodium acetate). The mixture was incubated at room temperature for 30 min in the dark, and the absorbance was measured at 430 nm using the same UV–Vis spectrophotometer. A blank was prepared by replacing the $AlCl_3$ solution with an equivalent volume of distilled water. The flavonoid content was determined using a rutin standard curve and expressed as milligrams of rutin per gram of dry extract (mg RE/g). All analyses were performed in triplicate.

2.6 Determination of condensed tannins

The quantification of condensed tannins (CT) was performed using the vanillin-HCl assay, following the procedure described (Priyanthi and Sivakanesan, 2021). A 1 mg/mL solution of A-Bs was prepared, and 200 µL of this sample was combined with 1.5 mL of a 4% vanillin solution in methanol and 750 µL of concentrated hydrochloric acid (HCl). After thorough vortexing, the mixture was left to incubate at room temperature for 15 min. The absorbance was then recorded at 500 nm using the same UV–Vis spectrophotometer. A blank control was prepared by substituting the sample with distilled water. The CT concentration was calculated based on a catechin standard curve and expressed as milligrams of catechin equivalents per gram of dry extract (mg CE/g). All analyses were conducted in triplicate.

2.7 High-performance liquid chromatography analysis of A-Bs

HPLC analysis of the phenolic acid-rich extract was conducted following the protocol outlined by Harnafi et al. (2013), using an Agilent 1,100 series chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a Diode Array Detector (DAD). Separation was achieved on a Hypersil ODS reverse-phase (RP18) analytical column (250 × 4 mm, particle size 5 µm) at 20 °C. The injection volume of the extract was 10 µL, and the flow rate was set at 1 mL/min. The mobile phase consisted of aqueous trifluoroacetic acid (pH 2.8) (solvent A) and acetonitrile (solvent B), applied using the following gradient program: 0–1 min: 0–3% B, 1–45 min: 3–40% B, 45–55 min: 40% B, 55–56 min: 0% B. Detection was carried out at 320 nm to target phenolic compounds. Compounds were identified using a phenolics database based on their retention times and UV–visible spectra.

2.8 Evaluation of antioxidant capacity

2.8.1 DPPH radical scavenging method

The radical scavenging activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical method (Moumou et al., 2025). A-Bs and BHA samples were prepared at various concentrations, ranging from 0.078 to 10 mg/mL. Then, 50 µL of each sample was mixed with 1.95 mL of a 0.004% DPPH solution and incubated in the dark for 30 min at room temperature. The absorbance of the mixtures was measured at 517 nm using the same UV–Vis spectrophotometer with a blank used as a reference. A lower absorbance value indicates a higher DPPH free radical scavenging activity.

The radical scavenging activity (inhibition %) was calculated using the formula mentioned below:

$$\text{Radical scavenging activity}\,(\%) = \left(\left(A_{\text{control}} - A_{\text{sample}}\right) / A_{\text{control}}\right) \text{x}100$$

Where $A_{control}$ is the blank absorbance, and A_{sample} is the tested sample absorbance.

The obtained inhibition values were represented on graphs against the extract concentrations, and the concentrations that transformed 50% of the color expression were determined as IC_{50} values.

2.8.2 Determination of malondialdehyde content (MDA)

This assay was performed to evaluate the antioxidant capacity of basil extracts by assessing their ability to inhibit lipid peroxidation through MDA formation. In this study, copper was introduced to accelerate oil oxidation. To assess the level of oil peroxidation, we quantified MDA, which is a secondary product of the peroxidation process. The MDA concentration evaluation was realized using the (Moumou et al., 2024) method. Fifty µL of RSO was combined with 50 µL of A-Bs and BHA at various concentrations, along with 50 µL of DMSO and 100 µL of $CuSO_4$ solution (0.33 mg/mL). The blend was mixed in test tubes and then incubated in a shaking oven for 24 h at 37 °C. The reaction was terminated by adding 0.5 mL of 20% trichloroacetic acid (TCA) and 0.5 mL of 0.8% thiobarbituric acid (TBA). Subsequently, the tubes were heated in a water bath at 95 °C for 15 min and then cooled to room temperature. Afterward, 2 mL of butanol was introduced, and the mixture was centrifuged at 4500 rpm for 10 min.

We measured the absorbance at 532 nm using the same UV–Vis spectrophotometer. For comparison, we prepared a negative control (C–) with 50 µL of RSO and 200 µL of DMSO and a positive control (C+) consisting of 50 µL of RSO incubated with 100 µL of copper sulfate and 100 µL of DMSO. MDA concentrations (nM) were

quantified using the MDA calibration curve. The percentage of inhibition of oil oxidation was determined using the following formula:

$$\text{Inhibition}(\%) = \left[(\text{MDA})_{C+} - (\text{MDA})_{\text{sample}} \right) / (\text{MDA})_{C+}] \times 100$$

Subsequently, curves were drawn against the extract concentrations to determine the IC_{50} by applying the equation of each curve.

2.8.3 FRAP assay

The ferric-reducing antioxidant power (FRAP) of A-Bs was evaluated based on its capacity to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), following the approach described in El Hachlafi et al. (2024). To prepare the test solutions, 1 mL of diluted A-Bs samples (0.078–10 mg/mL) was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%) and then incubated at 50 °C for 20 min. After incubation, 2.5 mL trichloroacetic acid (10%) was added to stop the reaction, and the mixture was centrifuged at 3500 rpm for 10 min to separate the supernatant. Then, 2.5 mL of supernatant was combined with 2.5 mL distilled water and 500 µL ferric chloride (0.1%), and the resulting color change was measured at 700 nm using the same UV–Vis spectrophotometer. Butylated hydroxyanisole (BHA) was used as a reference antioxidant, and results were expressed as mean ± standard deviation.

2.8.4 Total antioxidant capacity

The total antioxidant capacity (TAC) of A-Bs was assessed to determine its ability to neutralize free radicals and mitigate oxidative stress. This evaluation was conducted using the phosphomolybdenum method described by Elbouzidi et al. (2024). A 0.1 mL sample of 1 mg/mL A-Bs was mixed with 0.3 mL of TAC solution (containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate in a 1:1:1 ratio), then incubated at 95 °C for 90 min. The absorbance was read at 695 nm. A standard calibration curve was prepared using ascorbic acid, and the TAC values were expressed as ascorbic acid equivalents (AAE).

2.9 Evaluation of the thermal stability of refined soybean oil

The thermal stability of both the fortified and control samples of RSO was assessed under two conditions. First, for storage at 60 °C, oil samples with different levels of basil, 0.02% BHA, and a control sample were placed in closed vials and kept in an oven at a constant temperature of 60 °C ± 3 °C for 7 weeks. Second, for frying conditions, oil samples (control, 0.02% A-Bs, 1% A-Bs, and 0.02% BHA) were put in beakers on a hotplate set to the frying temperature and kept for 7 h and 30 min. Regular intervals of 7 days were chosen to analyze samples exposed to a temperature of 60 °C, while samples subjected to frying temperature were analyzed every 30 min. The heat stability of both tests was evaluated by measuring several parameters, including the free fatty acid content (FFA) to assess hydrolytic stability, peroxide value (PV), malondialdehyde (MDA) concentration, para-anisidine value (P-AV), and total oxidation value (TOTOX). The tocopherol measurement was also conducted for samples incubated at 60 °C only.

2.9.1 Free fatty acid content

The RSO's FFA content was measured with and without basil extracts using the ISO 660: 2020 method with slight modifications (ISO 660:2020(en), 2020). To begin with, a sample of the oil (1 ± 0.1 g) was weighed and placed into an Erlenmeyer flask. Next, 25 mL of a diethyl ether/ethanol mixture (1:1) was added and agitated until dissolved, followed by the addition of 1 mL of a phenolphthalein solution (1%, w/v). The mixture was then titrated slowly using a potassium hydroxide solution (0.1 N, w/v) until a color change occurred. The results were calculated using the formula below and expressed as a percentage mass fraction (g of oleic acid in 100 g of oil).

$$\text{FFA} = \left(\left(\text{V} - \text{V}_0 \right) \text{xCxM} \right) / 10\,\text{m}$$

Where V represents the titration volume of the sample, V_0 is the titration volume of the blank, C is the concentration of the potassium hydroxide (mol/L), M is the molar mass of oleic acid in (g/mol), and m is the mass of the sample portion in g.

2.9.2 Evaluation of oxidative stability

2.9.2.1 Peroxide value

The PV of RSO with and without basil extracts was measured following the ISO 3960: 2017 method with minor adjustments (ISO 3960:2017, 2017). First, 1 ± 0.1 g of the oil sample was weighed, followed by adding 7.5 mL glacial acetic acid/chloroform (3:2), then stirred to dissolve, followed by 0.25 mL saturated potassium iodide (KI). After stirring for 30 min at room temperature, 30 mL of distilled water and 1 mL of 1% (w/v) starch solution were added to the mixture. The mixture was then slowly titrated with 0.01 M sodium thiosulfate solution until discoloration occurred. The results were calculated using the formula below and expressed in mEq O_2/kg of oil.

$$\text{PV} = \left(\left(\text{V} - \text{V}_0 \right) \text{xNx1000} \right) / \text{m}$$

Where V represents the titration volume of the sample, V0 is the titration volume of the blank, N is the normality of the sodium thiosulfate solution used, and m is the mass of the sample portion in g.

2.9.2.2 Para-anisidine value

The P-AV of RSO with and without basil extracts was measured according to the ISO 6885: 2016 method with slight modifications (ISO 6885:2016(en), 2016). 2 ± 0.1 g of oil sample was dissolved in 25 mL of isooctane. Then, 5 mL of the mixture was reacted with 1 mL of p-anisidine reagent (0.25 g/100 mL of glacial acetic acid). The same UV–Vis spectrophotometer was used to measure the absorbance of sample solutions with p-anisidine reagent (A_1) at 350 nm against the blank (isooctane with p-anisidine reagent: A_2). The P-AV was determined using the following formula:

$$\text{P} - \text{AV} = 25\text{x}\left(1{,}2\ \text{A}_1 - \text{A}_2 \right) / \text{m}$$

Where A_1 is the sample solution's p-anisidine reagent absorbance, A_2 is the blank absorbance (isooctane with p-anisidine reagent), and m is the mass of the sample portion in g.

2.9.2.3 TOTOX value

TOTOX was employed to assess lipid oxidative degradation. It is defined as the cumulative values of both PV and P-AV, representing the total oxidation value, and was calculated based on the subsequent formula:

$$\text{TOTOX value} = 2\,\text{PV} + \text{P} - \text{AV}$$

Where PV is the peroxide value, and P-AV is the anisidine value.

2.9.2.4 Determination of MDA content

A second MDA determination was performed to monitor the formation of secondary oxidation products during thermal oxidation of refined soybean oil. In this context, MDA concentrations (mM) reflect the extent of lipid oxidation rather than antioxidant capacity.

The procedure for estimating lipid oxidation was performed as described previously with slight modifications. An aliquot of 50 µL RSO with 0.5 mL TCA 20% and 0.5 mL of TBA 0.8% were thoroughly mixed in test tubes. This mixture was placed in a water bath at 95 °C for 15 min, followed by cooling to room temperature. The mixture was subsequently centrifuged at 4500 g/min for 10 min, and the optical density of the supernatant was determined at 532 nm using the same UV–Vis spectrophotometer, compared to a negative and positive control.

2.9.2.5 Tocopherols composition

The method developed by Rbah et al. (2025) was used to determine tocopherol using an HPLC apparatus (Shimadzu LC-6 AD system, Shimadzu, Kyoto, Japan) equipped with a DAD detector. Separation was carried out on a Uptisphere 120 Å NH2 silica column (4.6 × 250 mm, particle size: 5 µm) at a flow rate of 1 mL/min using a mixture of n-hexane and isopropanol (99/1; v/v). Tocopherol identification was done using tocopherol commercial standards (Sigma-Aldrich, St-Louis, USA) at wavelengths 292, 296, and 298 nm. Finally, the concentration of tocopherol was determined using an external calibration curve.

2.9.2.6 Rancimat test

The oxidative stability of RSO was evaluated using the Rancimat method, following the procedure outlined by Allay et al. (2024). The stability was determined by measuring the induction time (in hours) with a Metrohm 743 Rancimat instrument (Metrohm Co., Basel, Switzerland). The analysis was conducted under controlled conditions, using 3 g of RSO in three different formulations: non-fortified RSO, RSO supplemented with 0.02% (C1) and 1% A-Bs (C2), and RSO containing 0.02% BHA. The experiment was performed at 100 °C with an airflow rate of 20 L/h.

2.10 Acute hyperlipidemia mouse model induced by triton WR-1339

Male Swiss Albino mice (28–30 g), bred at the Faculty of Sciences, University Mohammed I in Oujda, Morocco, were housed in standard conditions at 22 °C, with a 12-h light/dark cycle and free access to food and water. The animal experiments adhered to the Care and Use of Laboratory Animals Guidelines set forth by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and approved by the Institutional Review Board (approval number: 044/2024).

The study involved four experimental groups ($n = 6$). The normolipidemic control (NC) group received distilled water via oral gavage. The hyperlipidemic control (HC) group was intraperitoneally injected with Triton WR-1339 (200 mg/kg) to induce acute hyperlipidemia and subsequently given distilled water orally. The treatment groups (D1 and D2) were also injected with Triton WR-1339 (200 mg/kg) to induce hyperlipidemia. Still, they were then administered RSO/A-Bs (RSO fortified with A-Bs) via oral gavage at doses of 125 mg/kg (D1) or 250 mg/kg (D2) body weight. After 24 h, blood samples were drawn from all animals using the retro-orbital bleeding technique under mild diethyl ether anesthesia. The collected blood was centrifuged at 2,500 rpm for 10 min to separate the plasma, which was then stored at 4 °C until analysis. According to the manufacturer's instructions, plasma levels of total cholesterol, triglycerides, glucose, HDL-cholesterol, and LDL-cholesterol were measured using enzymatic kits (Biosystems S. A., Barcelona, Spain).

2.11 Statistical analyses

To ensure the validity of all parameters, analyses were performed in triplicate, except for *in vivo* experiments, where each group consisted of six mice ($n = 6$). Data are presented as mean ± standard error of the mean (SEM). We then performed a statistical analysis using a one-way analysis of variance (ANOVA) with a threshold of $p < 0.05$ to determine statistical significance. We used GraphPad Prism 10.2.0 (GraphPad Software, San Diego, CA, USA) to create graphs and curves.

3 Results

3.1 Total polyphenol, flavonoid content, and condensed tannins of A-Bs

The study focused on analyzing A-Bs to evaluate its TPC, TFC, and CT, as detailed in Table 1. The extraction process yielded 17% of A-Bs. Analysis of the extract revealed a TPC of 191.72 ± 5.2 mg rosmarinic acid equivalents (RAE)/g, a TFC of 29.125 ± 0.79 mg rutin equivalents (RE)/g, and a CT of 2.06 ± 0.83 mg catechin equivalents (CE)/g of dry extract.

3.2 HPLC analysis of A-Bs

The HPLC chromatogram (Figure 1) revealed the presence of 19 phenolic compounds in the aqueous basil extract (A-Bs). The major identified compounds were caftaric acid (Peak 1), caffeic acid (Peak 2), chicoric acid (Peak 3), and rosmarinic acid (Peak 4) with relative area percentages of 35.03, 8.36, 21.15, and 16.33%, respectively.

3.3 Evaluation of antioxidant capacity

The antioxidant potential of A-Bs was assessed through various *in vitro* methods, with the key findings summarized in Table 2. A-Bs exhibited notable DPPH free radical scavenging activity, with a mean inhibitory concentration (IC_{50}) of 14.175 ± 0.24 µg/mL, which was higher than that of the reference antioxidant BHA

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TABLE 1 Total polyphenols, flavonoids, and condensed tannins content of the A-Bs Extracts.

Extract	Total Polyphenols*	Flavonoids**	Tannins***	Yield %
A-Bs	191.72 ± 6.84	29.125 ± 0.79	2.06 ± 0.83	17%

* mg of rosmarinic acid (RAE)/g of dry extract; ** mg of rutin (RE)/g of dry extract; *** mg of catechin (CE)/g of dry extract; A-Bs: first aqueous extract.

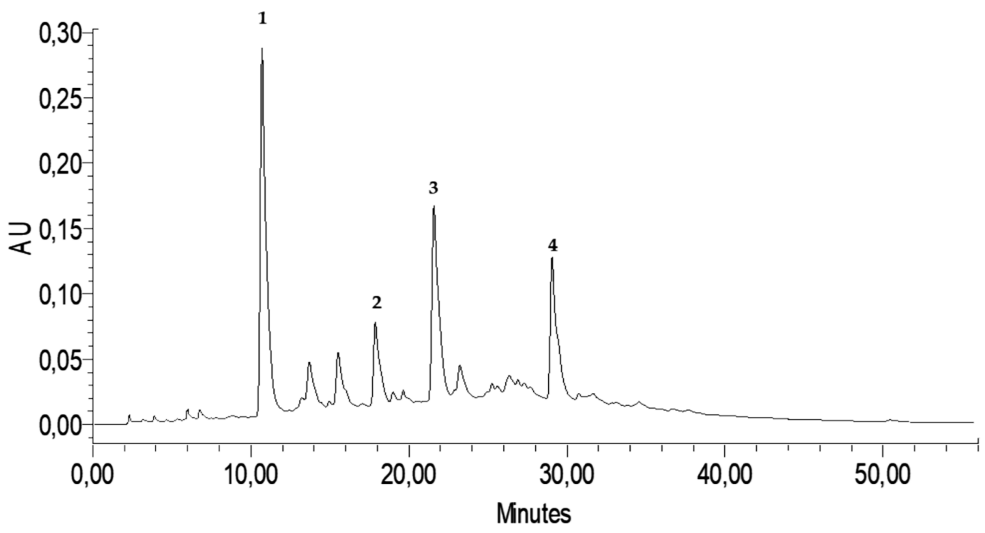


FIGURE 1
HPLC phenolic profile of the A-Bs; 1: Caftaric acid; 2: Caffeic acid; 3: Chicoric acid; 4: Rosmarinic acid.

TABLE 2 Antioxidant activity of A-Bs and BHA.

Extract type	DPPH scavenging IC_{50} (μg/mL)	MDA content IC_{50} (mg/mL)	FRAP IC_{50} (mg/mL)	Total antioxidant capacity *
A-Bs	14.175 ± 0.24[a]	1.49 ± 0.11[a]	0.881 ± 0.016[a]	0.285 ± 0.002[a]
BHA	6.453 ± 0.31[b]	1.23 ± 0.18[b]	0.229 ± 0.008[b]	0.891 ± 0.005[b]

A-Bs: Aqueous extract. Tukey's multiple range test ($p < 0.05$) uses small letter superscripts to indicate significant differences. *TAC is expressed in mg of ascorbic acid equivalents per milligram of dry extract.

(IC_{50} = 6.45 ± 0.31 μg/mL). A-Bs demonstrated a moderate antioxidant effect in the FRAP assay, with an IC_{50} of 0.881 ± 0.016 mg/mL, exceeding BHA's IC_{50} of 0.229 ± 0.008 mg/mL. Furthermore, A-Bs moderately reduced malondialdehyde (MDA) concentration, with an IC_{50} of 1.49 ± 0.11 mg/mL, compared to 1.23 ± 0.18 mg/mL for BHA. The total antioxidant capacity (TAC) of A-Bs was measured at 0.285 ± 0.002 mg of AA equivalent per mg of dry extract, compared to 0.891 ± 0.005 mg of AA equivalent per mg for BHA.

3.4 Evaluation of the thermal stability of refined soybean oil

3.4.1 Free fatty acid content

Tables 3, 4 present the acid values of fortified and unfortified RSO samples after accelerated storage at 60 °C and frying temperature, measured via titration to assess free fatty acid levels from oil degradation. Unfortified RSO (C-) showed consistent stability over 4 weeks, maintaining an acidity of 0.28 ± 0.0005%, similar to BHA-fortified oil. Fortified samples (C + and C1; 0.02%) exhibited rising acidity from the third week (0.56 ± 0.003%), while C2 (1%) showed an increase in the fourth week, with reversible effects observed at high concentrations (0.843 ± 0.001%).

A thermal oxidation test at frying temperature revealed that higher extract concentrations reduced free fatty acid content ($p < 0.05$), confirming a concentration-dependent reduction in acidity. Notably, C2 demonstrated greater stability in FFA compared to BHA, suggesting its potential as an effective natural alternative for enhancing RSO stability under thermal stress.

3.4.2 Evaluation of oxidative stability

3.4.2.1 Peroxide value

Tables 3, 4 illustrate the variations in peroxide value (PV) under two thermal oxidation conditions: at 60 °C (Table 3) and frying temperature (Table 4). At 60 °C, PV improved over 7 weeks, with all samples initially showing PV below 10 mEq/kg. The oxidation rate increased, peaking around the sixth week before declining, likely due to the volatilization of lipid hydroperoxide breakdown products. Degradation was consistent across all stages, with the positive control (C+) exhibiting the highest PV, followed by C1 (0.02%), C2 (1%), and BHA in descending order ($p < 0.05$). C + showed a significant rise in PV at frying temperature, while C2 and BHA were more effective than C1 in preventing hydroperoxide formation and delaying primary oxidation ($p < 0.05$). Notably, BHA demonstrated the best overall stability for RSO, though C2's performance highlighted the effectiveness of higher extract concentrations in inhibiting lipid oxidation.

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TABLE 3 FFA, PV, P-AV value, TOTOX value, and MDA concentration in RSO fortified with A-Bs during accelerated storage at 60 °C.

Oil constants					
Time (Weeks)	C-	C+	C1	C2	BHA
FFA (g of oleic acid/100 g of oil)					
0	0.28 ± 00^{a}	0.28 ± 00^{a}	0.28 ± 00^{a}	0.28 ± 00^{a}	0.28 ± 00^{a}
1	0.28 ± 0.001^{c}	0.279 ± 0.0005^{a}	0.281 ± 0.0044^{bc}	0.28 ± 0.002^{bc}	0.28 ± 0.009^{bc}
2	0.27 ± 0.0005^{a}	0.279 ± 0.0005^{a}	0.279 ± 0.009^{a}	0.281 ± 0.001^{a}	0.28 ± 0.002^{a}
3	0.27 ± 0.0008^{a}	0.559 ± 0.0011^{b}	0.56 ± 0.001^{b}	0.278 ± 0.007^{a}	0.278 ± 0.004^{a}
4	0.28 ± 0.0009^{a}	0.56 ± 0.0005^{b}	0.56 ± 0.001^{b}	0.84 ± 0.001^{c}	0.28 ± 0.006^{a}
PV (mEq O2/kg of oil)					
0	3.72 ± 00^{a}	3.72 ± 00^{a}	3.72 ± 00^{a}	3.72 ± 00^{a}	3.72 ± 00^{a}
1	4.53 ± 0.47^{a}	18.89 ± 3.56^{b}	18.21 ± 2.3^{b}	16.55 ± 2.31^{b}	11.57 ± 0.89^{ab}
2	2.96 ± 0.007^{a}	25.82 ± 1.18^{bc}	25.82 ± 0.09^{bc}	26.95 ± 1.18^{bc}	23.18 ± 0.66
3	6.28 ± 0.33^{a}	36.18 ± 0.89^{b}	35.24 ± 0.89^{b}	34.30 ± 0.23^{b}	26.86 ± 0.53^{b}
4	9.39 ± 0.3^{a}	58.44 ± 7.39^{b}	54.69 ± 1.06^{b}	44.46 ± 0.58^{b}	44.06 ± 1.32^{b}
5	8.91 ± 0.99^{a}	68.15 ± 2.21^{c}	60.13 ± 2.21^{bc}	62.7 ± 1.29^{bc}	48.00 ± 2.93^{b}
6	17.81 ± 4.85^{a}	125.129 ± 2.11^{e}	94.40 ± 2.11^{d}	67.72 ± 2.39^{c}	55.20 ± 1.92^{b}
7	3.28 ± 0.32^{a}	83.99 ± 0.288^{d}	61.85 ± 3.63^{c}	56.40 ± 5.88^{c}	37.6 ± 4.17^{b}
P-AV value					
0	4.57 ± 00^{a}	4.57 ± 00^{a}	4.57 ± 00^{a}	4.57 ± 00^{a}	4.57 ± 00^{a}
1	7.79 ± 00^{a}	7.79 ± 00^{a}	6.71 ± 1.07^{a}	7.79 ± 00^{a}	7.79 ± 00^{a}
2	6.83 ± 0.35^{a}	10.53 ± 0.36^{b}	7.01 ± 0.53^{a}	8.82 ± 0.36^{ab}	7.45 ± 0.64^{a}
3	5.36 ± 0.2^{a}	8.29 ± 0.2^{d}	7.54 ± 0.24^{c}	7.26 ± 0.06^{c}	6.57 ± 0.2^{b}
4	6.58 ± 0.29^{a}	12.14 ± 0.26^{c}	9.57 ± 1.05^{b}	7.82 ± 0.14^{ab}	9.44 ± 0.24^{c}
5	8.05 ± 0.31^{a}	13.06 ± 0.25^{c}	9.77 ± 1.22^{ab}	12.84 ± 0.62^{c}	12.34 ± 0.35^{bc}
6	4.65 ± 0.045^{a}	17.1 ± 0.49^{c}	16.82 ± 1.27^{c}	9.08 ± 0.008^{b}	9.21 ± 0.06^{b}
7	6.11 ± 0.06 ^{b}	14.34 ± 0.05^{f}	9.21 ± 0.35^{d}	9.98 ± 0.007^{de}	3.54 ± 0.36^{a}
TOTOX value					
0	12.01 ± 00^{a}	12.01 ± 00^{a}	12.01 ± 00^{a}	12.01 ± 00^{a}	12.01 ± 00^{a}
1	16.85 ± 0.95^{a}	45.58 ± 7.12^{b}	43.15 ± 5.21^{b}	40.90 ± 4.62^{b}	30.94 ± 1.78^{ab}
2	12.76 ± 0.37^{a}	62.18 ± 2.68^{bc}	58.67 ± 0.7^{bc}	62.74 ± 2.4^{bc}	53.82 ± 1.06^{b}
3	17.93 ± 0.52^{a}	80.65 ± 1.69^{b}	78.02 ± 1.55^{b}	75.87 ± 0.4^{b}	60.30 ± 1.21^{b}
4	25.38 ± 0.33^{a}	129.0 ± 15.03^{b}	118.97 ± 3.08^{b}	96.76 ± 1.32^{b}	97.56 ± 2.78^{b}
5	25.88 ± 2.25^{a}	149.36 ± 17.9^{c}	130.04 ± 3.56^{bc}	138.24 ± 3.21^{bc}	108.35 ± 5.72^{b}
6	40.28 ± 9.68^{a}	267.36 ± 1.16	205.63 ± 2.94^{f}	144.53 ± 4.8^{c}	119.61 ± 3.78^{b}
7	12.69 ± 0.7^{a}	182.34 ± 0.62^{d}	132.91 ± 6.92^{c}	122.79 ± 11.75^{c}	78.75 ± 8.34^{b}
MDA concentration (nM /mg oil)					
0	28.73 ± 00^{a}	28.73 ± 00^{a}	28.73 ± 00^{a}	28.73 ± 00^{a}	28.73 ± 00^{a}
1	47.46 ± 1.58^{a}	108.88 ± 1.4^{a}	91.89 ± 5.67^{a}	98.73 ± 1.03^{a}	87.36 ± 1.92^{a}
2	70.37 ± 6.39^{a}	174.40 ± 1.03^{a}	161.64 ± 6.58^{a}	151.34 ± 0.86^{a}	128.8 ± 0.73^{a}
3	56.64 ± 2.86^{a}	132.12 ± 1.67^{bc}	137.91 ± 3.65^{bc}	182.38 ± 3.36^{d}	133.88 ± 3.27^{bc}
4	47.38 ± 1.27^{a}	183.56 ± 5.83^{d}	120.14 ± 0.36^{bc}	123.58 ± 0.34^{c}	124.47 ± 1.55^{c}

FFA: free fatty acid; PV: peroxide value; P-AV value: anisidine Index; TOTOX value: total oxidation value; MDA: malondialdehydes; C-: unfortified RSO with no thermal treatment; C+: unfortified RSO with thermal treatment; C1: RSO with 0.02% of A-Bs; C2: RSO with 1% of A-Bs; BHA: RSO with 0.02% of BHA. Small letter superscripts show significant differences between samples during the same week ($p < 0.05$).

3.4.2.2 Para-anisidine value

The results detailing the P-AV (p-Anisidine value) of RSO samples after accelerated storage at 60 °C for 7 weeks are presented in Table 3. The P-AV quantifies secondary oxidation products, offering insights into oxidative degradation under high-temperature conditions. The findings reveal that the positive control (C+)

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TABLE 4 FFA, PV, and MDA concentration in RSO fortified with A-Bs during frying.

Oil constants					
Time (hours)	C-	C+	C1	C2	BHA
FFA (g of oleic acid/100 g of oil)					
5 h 30 min	0.27 ± 0.0004^{a}	0.54 ± 0.002^{c}	0.27 ± 0.003^{a}	0.55 ± 0.001^{c}	0.49 ± 0.007^{b}
6 h	0.27 ± 0.0004^{a}	0.55 ± 0.003^{c}	0.81 ± 0.03^{d}	0.554 ± 0.003^{c}	0.52 ± 0.006^{b}
6 h 30 min	0.27 ± 0.0004^{a}	0.84 ± 0.001^{c}	0.55 ± 0.002^{b}	0.556 ± 0.003^{b}	0.53 ± 0.01^{b}
7 h	0.27 ± 0.0004^{a}	0.82 ± 0.004^{c}	0.83 ± 0.005^{c}	0.551 ± 0.001^{b}	0.66 ± 0.07^{b}
7 h 30 min	0.27 ± 0.0004^{a}	0.27 ± 0.003^{a}	0.84 ± 0.001^{c}	0.553 ± 0.001^{b}	0.659 ± 0.08^{b}
PV (mEq O_2/kg of oil)					
5 h 30 min	3.7 ± 0.0003^{a}	10.68 ± 0.53^{b}	12.18 ± 0.64^{b}	3.92 ± 0.28^{a}	4.44 ± 0.38^{a}
6 h	3.7 ± 0.0003^{a}	21.18 ± 0.89^{d}	10.59 ± 0.71^{c}	6.49 ± 0.35^{b}	4.81 ± 0.29^{ab}
6 h 30 min	3.7 ± 0.0003^{a}	9.46 ± 0.27^{b}	14.36 ± 0.25^{c}	5.60 ± 0.36^{a}	5.36 ± 0.91^{a}
7 h	3.7 ± 0.0003^{a}	11.42 ± 0.29^{d}	11.81 ± 0.03^{d}	8.32 ± 0.21^{c}	4.76 ± 0.01^{b}
7 h 30 min	3.7 ± 0.0003^{a}	9.91 ± 0.02^{c}	10.46 ± 0.27^{c}	8.15 ± 0.09^{bc}	6.78 ± 1.21^{b}
MDA concentration (nM /mg oil)					
5 h 30 min	27.91 ± 0.0003^{a}	172.76 ± 0.56^{e}	105.44 ± 3.74^{d}	68.55 ± 4.08^{b}	88.7 ± 4.43^{c}
6 h	27.91 ± 0.0003^{a}	194.70 ± 8.83^{d}	108.20 ± 3.01^{c}	83.23 ± 4.68^{b}	79.55 ± 3.87^{b}
6 h 30 min	27.91 ± 0.0003^{a}	175.59 ± 8.57^{e}	98.50 ± 3.36^{c}	78.60 ± 1.72^{b}	118.95 ± 1.03^{d}
7 h	27.91 ± 0.0003^{a}	102.01 ± 3.92^{bc}	113.50 ± 4.35^{cd}	94.77 ± 4.41^{b}	117.98 ± 3.23^{d}
7 h 30 min	27.91 ± 0.0003^{a}	111.34 ± 1.72^{b}	127.51 ± 5.68^{c}	108.55 ± 5.73^{b}	145.72 ± 0.94^{d}

PV: Peroxide value. FFA: free fatty acid; MDA: malondialdehydes; C-: unfortified RSO that was not fortified for accelerated oxidative treatment; C+: fortified RSO with accelerated oxidative treatment; C1: RSO with 0.02% of A-Bs; C2: RSO with 1% of A-Bs; BHA: RSO with 0.02% of BHA. Small letter superscripts show significant differences between different samples during the same week ($p < 0.05$).

exhibited significantly higher degradation, with a 53 ± 0.05% increase in P-AV compared to the room-temperature-stored sample (C–), which had the lowest P-AV. In contrast, fortified samples, particularly C2 (1%) and BHA, demonstrated resistance to the formation of secondary oxidation products, with no significant difference between them ($p > 0.05$). The oxidation rate peaked in the sixth week for C1 (0.02%), showing a 1.63 ± 3.34% reduction compared to C+, while C2 and BHA peaked in the fifth week, with reductions of 24.91 ± 2.08% and 27.83 ± 0.67%, respectively, compared to C+, followed by a decline. These results highlight the effectiveness of C2 and BHA in mitigating secondary oxidation, with BHA showing the most robust stabilization of RSO under accelerated storage conditions.

3.4.2.3 Total oxidation value

The TOTOX value, which combines PV and P-AV to assess oil oxidation, indicates greater oxidative stability. Table 3 reveals that increasing the concentration of A-Bs reduces the TOTOX value. The C+ sample showed an 84.93 ± 2.05% increase in TOTOX value compared to C–, while C1 (0.02%) had the highest TOTOX value among the samples, followed by C2 (1%) and BHA in descending order. During heating, the oxidation rate increased, peaking around the sixth week for all samples ($p < 0.05$), followed by a decline. BHA was the most effective antioxidant, reducing oxidation products by 55.26% ± 1.6, followed by C2 (45.94% ± 1.56) and C1 (23% ± 1.43). These results demonstrate that the effectiveness of A-Bs in reducing oxidation under accelerated conditions depends on their concentration, with higher concentrations providing greater stability.

3.4.2.4 Effect of basil extract on MDA formation in RSO

Malondialdehyde (MDA), a secondary oxidation product formed from the oxidation of polyunsaturated fatty acids, was measured to assess oxidative degradation in RSO under accelerated conditions at 60 °C and frying temperature, as shown in Tables 3, 4. At 60 °C, MDA formation in the positive control (C+) increased significantly by 61.66 ± 1.1% compared to the unfortified sample (C-) ($p < 0.05$). In the second week, C1 (0.02%) showed the highest reduction in MDA content (11.94 ± 1.98%), while C2 (1%) exhibited minimal inhibition, with only a 0.64 ± 3.88% decrease compared to C+. During the thermal oxidation test at frying temperature, MDA levels were monitored for over 7 h and 30 min. A clear trend emerged, showing that MDA levels decreased as the concentration of the extract increased. C2 demonstrated greater effectiveness in reducing MDA compared to BHA and C1, highlighting the plant extract's potential antioxidative properties in enhancing RSO stability under high-temperature conditions. These results suggest that higher extract concentrations, particularly C2, can significantly inhibit secondary oxidation, offering a promising natural alternative to synthetic antioxidants like BHA.

3.4.2.5 Tocopherol content

Table 5 presents the tocopherol content in RSO before oxidative treatment (T0) and after 7 weeks of heating at 60 °C with A-Bs, emphasizing the role of A-Bs in maintaining oxidative stability. A significant decrease in tocopherol content was observed during heating, regardless of A-Bs concentration ($p < 0.05$). After 7 weeks, C2 (1%) retained the highest total tocopherol content, followed by BHA, while C1 (0.02%) was less effective in preserving tocopherols.

Regarding α-tocopherol, C1 showed a significant preventive effect, recording the highest value (27.15 ± 1.08 mg/kg RSO), surpassing C2. However, BHA remained the most effective in preserving α-tocopherol. Interestingly, C2 exhibited lower α-tocopherol content than C+, leading to unexpected degradation, potentially due to the pro-oxidant effects of tocopherols at high concentrations.

For γ-tocopherol, C2 was the most effective in preventing degradation (210.56 ± 1.3 mg/kg RSO), followed by BHA (190.68 ± 0.65 mg/kg RSO), while C1 resulted in greater loss compared to C+. Similarly, δ-tocopherol content was best preserved by C2 (131.38 ± 11.31 mg/kg RSO).

3.4.2.6 Rancimat test

The oxidative stability (Figure 2) of RSO, both unfortified and fortified (0.02% BHA, 0.02% (C1) and 1% A-Bs (C2)), evaluated by Rancimat tests, shows a slight difference between samples ($p < 0.05$). Among the RSO samples, the highest oil stability (OS) value was observed for RSO fortified with BHA (13.81 ± 0.11 h), whereas the lowest OS value was for unfortified RSO (13.07 ± 0.1 h). RSO/A-Bs exhibited an OS value of 13.690 ± 0.01 h, placing it in the middle with no significant difference compared to the sample fortified with BHA.

3.5 Hypolipidemic effect of the fortified RSO in triton WR-1339 hyperlipidemic mice

The Triton-induced hyperlipidemic model (HC) caused severe metabolic dysregulation (Figure 3), elevating plasma total cholesterol (TC), triglycerides (TG), and glucose levels by 368, 3,371, and 80.4%, respectively, compared to NC. Additionally, LDL-C levels in HC increased by 304.7%, while HDL-C decreased by −7.7% relative to NC, confirming a significant lipid imbalance.

Administration of RSO/A-Bs at the higher dose (D2; 250 mg/kg) significantly counteracted these effects, reducing TC and TG by −76.6% and −86.5% relative to HC, while restoring glucose to near-normal levels (269.06 vs. 266.67 mg/dL). Additionally, D2 exhibited a 71.4% inhibition of LDL-C accumulation, lowering it close to NC values (only 15.7% higher than NC). In comparison, HDL-C increased by 28.2% relative to HC, surpassing even NC levels by 18.3%. In contrast, the lower dose (D1; 125 mg/kg) produced minimal improvements, with only 8.4% (TC), 15.1% (TG), and 12.5% (glucose) reductions compared to HC. Despite these limited effects, TC and TG levels in D1 remained markedly elevated at 328 and 2,846% above NC values, highlighting unresolved hyperlipidemia. Notably, glucose levels in D1 showed a significant difference compared to NC. Still, no significant difference was observed between D1 and HC, suggesting that the lower dose (D1) moderately affected glucose regulation. However, it was not enough to completely reverse the glucose elevation induced by the high-lipid diet. Likewise, LDL-C in D1 remained 317% higher than NC. At the same time, HDL-C showed only a slight recovery, remaining 11.6% lower than NC, reinforcing the limited efficacy of the lower dose in restoring lipid balance.

4 Discussion

Oil oxidation significantly deteriorates edible oils' quality and shelf life, driven by free radical chain reactions and metal-catalyzed degradation (Blasi and Cossignani, 2020; German, 1999). In this study, A-Bs has demonstrated its efficacy as a natural antioxidant, showing strong potential in limiting oxidation during storage (60 °C) and frying conditions. The extract's high total phenolic (191.72 ± 5.2 mg/g) and flavonoid (29.125 ± 0.79 mg/g) content support its dual antioxidant action (radical scavenging and metal chelation), which synergistically modulates oxidative pathways, as evidenced by its low IC_{50} values in DPPH, FRAP, and TAC assays (Pulido et al., 2000; Rice-Evans et al., 1996; Xu et al., 2017).

The FRAP assay showed that A-Bs exhibited a dose-dependent reduction effect, emphasizing its vigorous electron-donating activity. This process, critical for neutralizing free radicals and terminating oxidation chain reactions, involves the reduction of Fe^{3+}-TPTZ complexes to Fe^{2+} by polyphenols such as rosmarinic and caffeic acid (Pulido et al., 2000; Rice-Evans et al., 1996; Świderski et al., 2024). These results are consistent with the radical scavenging activity observed in the DPPH assay, where hydroxyl (-OH) groups donate electrons or hydrogen atoms to neutralize free radicals, thereby stabilizing reactive species (Gülçin et al., 2007; Osei Akoto et al., 2020; Vlase et al., 2014; Warsi and Sholichah, 2017). The combined effects of hydrogen atom donation (DPPH) and electron transfer (FRAP) highlight A-Bs' ability to influence oxidative processes at different phases (Andrés et al., 2023; Muscolo et al., 2024).

Complementing these results, the TAC assay quantified A-Bs' total antioxidant capacity, reflecting the cumulative effects of phenolic acids, flavonoids, and synergistic interactions (Ruskovska et al., 2020; Xu et al., 2017). For instance, rosmarinic acid's conjugated structure enhances electron delocalization (Hieu Truong et al., 2022), while chicoric acid's esterified caffeoyl groups amplify metal chelation (Świderski et al., 2020; Zhu et al., 2018). These combined interactions likely contribute to A-Bs' effectiveness in suppressing malondialdehyde (MDA) formation and lowering TOTOX values, a composite indicator of primary and secondary oxidation, in oil samples during accelerated

TABLE 5 Tocopherols content of RSO after 7 weeks of accelerated storage at 60 °C (mg/kg oil).

Experimental group	Total tocopherols	α-tocopherol	γ-tocopherol	δ-tocopherol
C−	537.7 ± 6.41^{e}	65.6 ± 0.57^{f}	351.98 ± 10.95^{f}	129.75 ± 0.6^{c}
C+	321.82 ± 2.64^{b}	24.72 ± 1.05^{bc}	166.52 ± 7.29^{b}	124.62 ± 0.68^{c}
C1	247.37 ± 0.14^{a}	27.15 ± 1.08^{cd}	117.4 ± 0.76^{a}	103.53 ± 0.1^{a}
C2	365.5 ± 3.04^{c}	22.63 ± 1.07^{ab}	210.65 ± 1.3^{d}	131.38 ± 1.04^{c}
BHA	331.72 ± 3.62^{b}	28.34 ± 0.15^{d}	190.68 ± 0.65^{c}	108.10 ± 5.39^{ab}

C−: unfortified RSO with no thermal treatment; C+: unfortified RSO with thermal treatment; C1: RSO with 0.02% of A-Bs; C2: RSO with 1% of A-Bs; BHA: RSO with 0.02% of BHA. Small letter superscripts show significant differences between samples during the same week ($p < 0.05$).

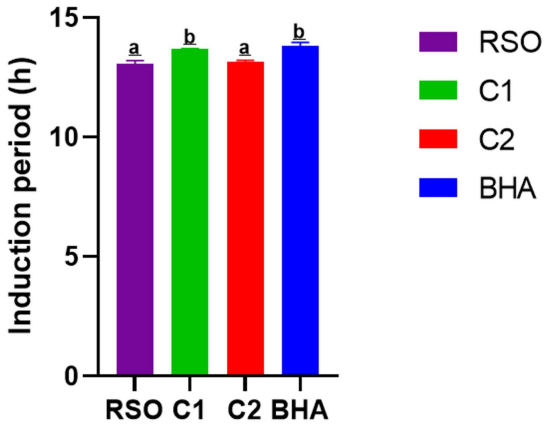


FIGURE 2
Induction period (h) of unfortified and fortified RSO. C1 and C2 refers to RSO fortified with 0.02 and 1% of A-Bs, and BHA refers to RSO fortified with 0.02% BHA. Small letter superscripts indicate significant differences between samples ($p < 0.05$). The same letter indicates no significant difference.

storage and frying tests (BEN Moumen et al., 2015; ISO 3960:2017, 2017; ISO 6885:2016(en), 2016).

Under accelerated storage conditions (60 °C), autoxidation predominates, characterized by slow yet persistent radical formation, leading to hydroperoxide accumulation (Blasi and Cossignani, 2020; German, 1999). Trace metals such as Fe^{2+} and Cu^{2+} can accelerate degradation via Fenton reactions (Kremer, 1999). Their concentrations in refined, bleached, and deodorized (RBD) soybean oil are typically very low (Flider and Orthoefer, 1981). However, A-Bs may still exert protective effects through metal chelation, binding these ions via hydroxyl and carbonyl groups to inhibit hydroperoxide decomposition (Boulebd et al., 2022; Gülçin and Alwasel, 2022). This aligns with the observed reduction in MDA levels, a marker of secondary oxidation. The extract's polyphenols, such as rosmarinic and chicoric acid, form stable metal complexes, delaying oxidation progression (Kowalczyk et al., 2024; Palierse et al., 2020; Song et al., 2023; Świderski et al., 2024). However, prolonged exposure at 60 °C necessitates higher A-Bs concentrations (C2; 1%) to saturate the system, as lower doses (C1; 0.02%) show limited efficacy in counteracting sustained radical generation.

Under frying temperatures, oxidative mechanisms undergo a profound shift. The thermolytic breakdown of triglycerides releases free fatty acids (FFA) (Tarmizi et al., 2016), while rapid radical generation surpasses the capacity of endogenous antioxidants. Remarkably, A-Bs outperformed synthetic BHA in this high-heat environment, reducing FFA and MDA at the C2 concentration (Abdo et al., 2023; Khatib et al., 2021). This superiority stems from the thermal stability of key polyphenols (rosmarinic acid, stable up to 180 °C) (Moczkowska et al., 2020; Song et al., 2023) and the potential degradation of compounds like chicoric acid, which may lead to structural modifications affecting its antioxidant activity (Lee and Scagel, 2013; Zhu et al., 2018). The rise in radical formation at frying temperatures further amplifies A-Bs' efficacy, creating more targets for its polyphenols to neutralize and maximizing their hydrogen-donating potential (Senanayake, 2018; Zhou and Elias, 2012).

The Rancimat assay further validated A-Bs' protective role, showing an induction period of 13.69 ± 0.01 h for fortified rapeseed oil (RSO), comparable to BHA (13.81 ± 0.11 h). This extended stabilization period reflects A-Bs' ability to synergize with endogenous tocopherols, potentially through radical scavenging and interactions with oxidized tocopherols. Research on catechins and green tea polyphenols suggests similar interactions could occur with other phenolic acids in A-Bs (Neunert et al., 2015; Pazos et al., 2007; Zhou et al., 2005). While α-tocopherol degrades rapidly above 110 °C (Dolde and Wang, 2011; Sabliov et al., 2009), A-Bs at high concentration preserved total tocopherol levels compared to the oxidized control (C+), showing a protective effect similar to BHA. Under these conditions (60 °C), γ- and δ-tocopherols were maintained better than α-tocopherol, consistent with previous observations in thermally stressed oils.

The reduced TOTOX values highlight its dual capacity to inhibit primary peroxides and secondary aldehydes, outperforming synthetic alternatives in comprehensive oxidation control.

The Triton WR-1339 model is widely used to investigate acute lipid and glucose metabolism disturbances (McPherson et al., 1975; Millar et al., 2005). Inhibiting lipoprotein lipase interferes with normal lipid breakdown, leading to a significant increase in plasma TC and TG, alongside secondary hyperglycemia (Borensztajn et al., 1976; Sheorain et al., 2017; Weickert and Pfeiffer, 2006). These metabolic disruptions are similar to those observed in obesity and diabetes, where elevated lipids and glucose levels contribute to oxidative stress through an overproduction of reactive species (RSO), further destabilizing metabolic balance (Furukawa et al., 2004; Găman et al., 2020; Vilas-Boas et al., 2021).

Treatment with RSO/A-Bs at a dosage of 250 mg/kg (D2) effectively mitigated the metabolic disturbances induced by Triton, significantly reducing TC and TG levels while approaching normal glucose concentrations. These notable lipid-regulating effects suggest that RSO with A-Bs may counteract Triton's inhibition of lipoprotein lipase, thereby facilitating lipid metabolism and hepatic uptake (Duan et al., 2022; Moon et al., 2022). Additionally, restoring glucose balance indicates enhanced insulin sensitivity or a reduction in gluconeogenic pathways (Chen et al., 2023; DeFronzo, 2010). In contrast, the metabolic improvements at a lower dosage (D1; 125 mg/kg) were significantly less pronounced, highlighting a dose-dependent therapeutic effect (Tallarida, 2000).

Beyond these lipid-lowering effects, RSO/A-Bs also influenced high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels, critical indicators of lipid homeostasis (Harnafi et al., 2013; Moumou et al., 2025). LDL-C, often called "bad" cholesterol, is responsible for transporting cholesterol from the liver to peripheral tissues; elevated levels can lead to atherosclerosis and cardiovascular diseases (Ruskovska et al., 2020). In contrast, HDL-C, known as "good" cholesterol, promotes reverse cholesterol transport, removing excess cholesterol from the bloodstream and decreasing cardiovascular risk (Muscolo et al., 2024; Ruskovska et al., 2020). The observed decrease in LDL-C and increase in HDL-C following RSO/A-Bs treatment highlights their potential cardiovascular benefits, supporting their role in managing dyslipidemia and metabolic disorders (Harnafi et al., 2013; Moumou et al., 2024; Zhakipbekov et al., 2024).

RSO/A-Bs may also help counteract oxidative stress by stabilizing lipid and glucose levels, as hyperlipidemia and hyperglycemia are significant contributors to the generation of reactive oxygen species (ROS) (Amiya, 2016; Rani et al., 2016). By reducing lipid peroxidation substrates (TC, TG) and limiting glucose-derived advanced glycation end products (AGEs), which contribute to oxidative stress and

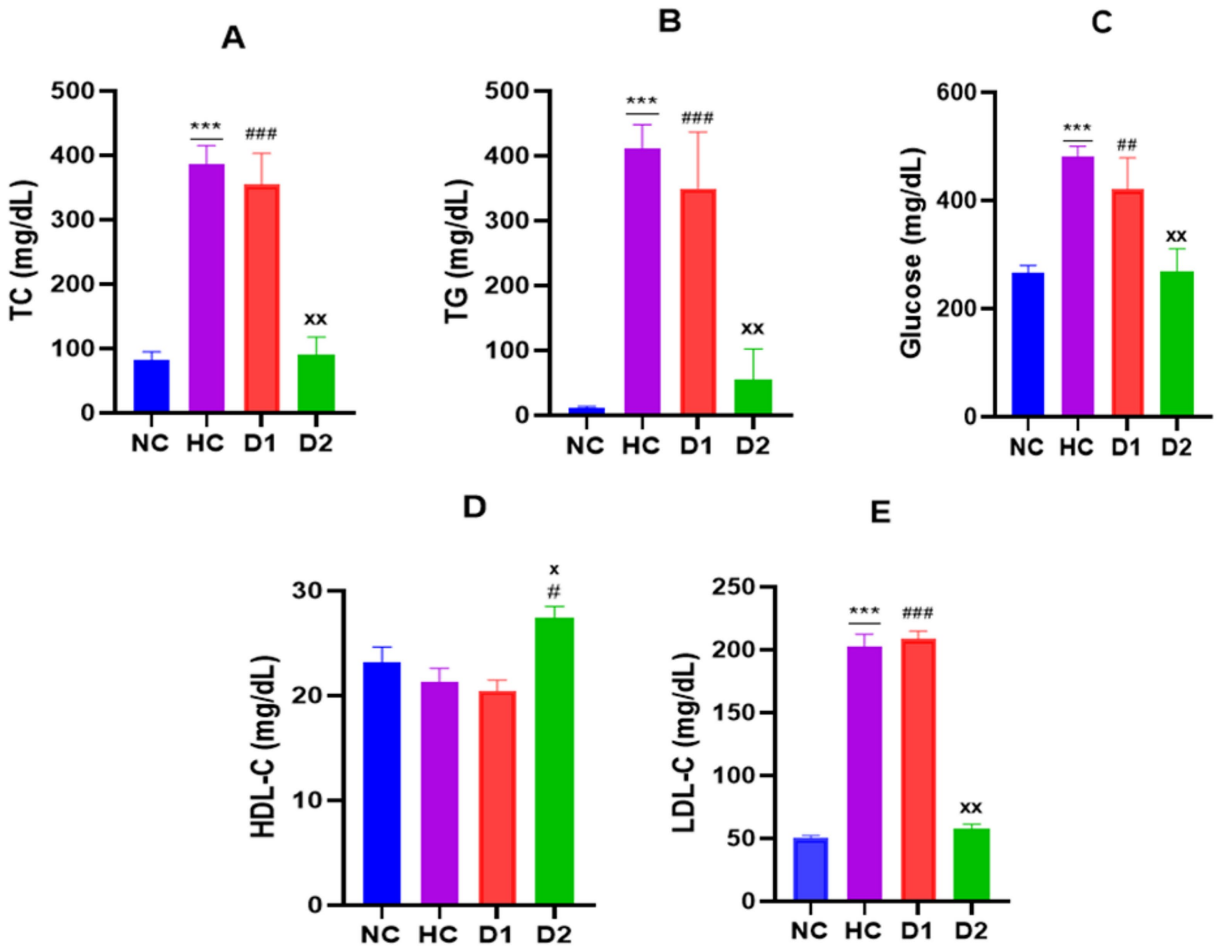


FIGURE 3
Effect of A-Bs on plasma TC **(A)**, TG **(B)**, glucose **(C)**, HDL-C **(D)**, and LDL-C **(E)** levels in triton-induced hyperlipidemic mice. NC: normal control; HC: hyperlipidemic control; D1: treated group with 125 mg/kg; D2: treated group with 250 mg/kg. Statistical significance: ***$p < 0.001$ (HC vs. NC); $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ (D1/D2 vs. NC); $^{X}P < 0.01$, $^{XX}P < 0.001$ (D1/D2 vs. HC).

metabolic dysfunction (Tentolouris et al., 2023; Chilelli et al., 2016; Singh et al., 2014), RSO/A-Bs could disrupt the pathological relationship between oxidative stress and metabolic dysfunction. However, it is essential for future research to directly evaluate oxidative stress markers, such as malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx), to validate this hypothesis (Cota-Magaña et al., 2024; Montazerifar et al., 2012; Valko et al., 2007).

These findings demonstrate that the biological effects observed *in vivo* are consistent with the antioxidant and stabilizing properties of A-Bs in oils, reinforcing their dual functionality in both food preservation and metabolic health.

5 Conclusion

This study presents an innovative application of basil hydrodistillation by-products (A-Bs) as multifunctional agents, contributing to both food stability and metabolic health. Using residues from the distillation process, we demonstrate that A-Bs improve the oxidative stability of refined soybean oil (RSO) under thermal conditions and provide lipid-lowering effects. At a dosage of 250 mg/kg, RSO/A-Bs significantly reduced plasma total cholesterol (TC) by 76.6%, triglycerides (TG) by 86.5%, and glucose levels by 44.1% in a Triton WR-1339-induced hyperlipidemia model. Additionally, RSO/A-Bs substantially lowered low-density lipoprotein cholesterol (LDL-C) while increasing high-density lipoprotein cholesterol (HDL-C). Incorporating A-Bs into RSO significantly reduced lipid peroxidation markers (FFA, PV, MDA) while preserving tocopherol content, positioning them as a promising natural alternative to synthetic antioxidants like BHA. Beyond their role in food protection, the observed dose-dependent hypolipidemic effects highlight their potential as nutraceutical candidates for addressing metabolic disorders such as obesity, which are closely associated with oxidative stress and lipid imbalances. This research aligns with circular economy principles by transforming plant by-products into valuable resources, promoting sustainability while enhancing functional applications. Future studies should aim to optimize the large-scale extraction of A-Bs, assess their long-term safety, and investigate their mechanisms of action. These efforts will facilitate the integration of these extracts into functional foods or therapeutic solutions. This innovative approach advances sustainable food systems and contributes to the development of natural interventions for managing metabolic health.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal studies were approved by the animal experiments adhered to the Care and Use of Laboratory Animals Guidelines set forth by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and approved by the Institutional Review Board of the Faculty of Sciences, Oujda, Morocco. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

AT: Conceptualization, Writing – original draft, Writing – review & editing. YR: Validation, Writing – original draft. MM: Formal analysis, Investigation, Writing – original draft. AH: Formal analysis, Writing – original draft. TA: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. HA: Resources, Validation, Writing – original draft. RA: Formal analysis, Writing – original draft. KR: Writing – original draft. HB: Conceptualization, Writing – original draft, Writing – review & editing. DM: Data curation, Writing – original draft. SA: Data curation, Writing – original draft. HH: Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2025.1668207/full#supplementary-material

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Glossary

A-Bs - Hydrodistillation Aqueous Basil Extract

AE-Bs - Ethyl Acetate Basil Extract

BHA - Butylated hydroxyanisole

BHT - Butylated Hydroxytoluene

DMSO - Dimethyl Sulfoxide

DPPH - 2.2-diphenyl-1-picrylhydrazyl

EtOAc - Ethyl Acetate

FFA - Free Fatty Acids

GAE - Gallic Acid Equivalent

H-Bs - Hexane Basil Extract

HDL-C - high-density lipoprotein cholesterol

HPLC - High-Performance Liquid Chromatography

IC_{50} - Median inhibitory concentration

LDL-C - low-density lipoprotein cholesterol

M-Bs - Methanol Basil Extract

MDA - Malondialdehyde

n-BuOH - n-Butanol

P-AV - Para-anisidine Value

POB - Pomegranate, Orange, and Beetroot extract

PV - Peroxide Value

QE - Quercetin Equivalent

ROS - Reactive Oxygen Species

RSO - Refined Soybean Oil

RSO/A-Bs - Refined Soybean Oil fortified with A-Bs

SEM - Standard Error of the Mean

TBA - Thiobarbituric Acid

TBHQ - Tert-Butylhydroquinone

TC - Total Cholesterol

TCA - Trichloroacetic Acid

TFC - Total Flavonoid Content

TG - Triglycerides

Tm-Bs - Trichloromethane Basil Extract

TOTOX - Total Oxidation Value

TPC - Total Polyphenol Content