ORIGINAL ARTICLE
http://journal.pan.olsztyn.pl

Polish Journal of Food and Nutrition Sciences
2025, Vol. 75, No. 3, 245–260
DOI: 10.31883/pjfns/208802
ISSN 1230-0322, e-ISSN 2083-6007

# Functional Olive Oils Infused with Mediterranean Herbs Enhance Cheese Preservation and Nutritional Profile

Souha Ammar[1], Hamza Gadhoumi[2], Nejia Farhat[1], Asma Mejri[1], Salma Nait-Mohamed[1], Kamel Hessini[3], Moufida Saidani Tounsi[2], Nabil Ben Youssef[1], Fethi Ben Slama[4,5], Hedia Manai-Djebali[1]*

[1]Laboratory of Olive Biotechnology, LR15CBBC05, Biotechnology Center of Borj-Cedria, BP 901, 2050, Hammam-Lif, Tunisia
[2]Laboratory of Medicinal and Aromatic Plants, LR15CBBC06, Biotechnology Center of Borj-Cedria, BP 901, 2050, Hammam-Lif, Tunisia
[3]Department of Biology, College of Sciences, Taif University, P.O. Box 11099, Taif, 21944, Saudi Arabia
[4]Department of Nutrition, Higher School of Health Sciences and Techniques of Tunis, 4021 Tunis, Tunisia
[5]Biochemistry and Biotechnology Research Laboratory, LR01ES05, Faculty of Sciences of Tunis, 2092 Manar II, Tunis, Tunisia

The demand for functional foods has driven research into bioactive-enriched dietary products. In this study, olive oil was enriched with phenolic-rich herbs, including oregano, rosemary, basil, and thyme, using an optimized ultrasound-assisted extraction (UAE) process. The resulting infused oils were evaluated for their phenolic content, antioxidant capacity, oxidative stability, and effects on cheese preservation. Rosemary-infused oil exhibited the highest total phenolic content (556.1 mg GAE/kg) and oxidative stability in the Rancimat test (induction time of 82.25 h), while oregano- and basil-infused oils had the strongest DPPH radical scavenging capacity ($IC_{50}$ of 2.36–2.38 mg/mL). Cheese stored in these plant-infused oils showed reduced lipid oxidation and microbial growth over 21 days. The lowest content of thiobarbituric acid reactive substances (TBARS) was recorded in the cheese immersed in rosemary- and thyme-infused oils (0.53 and 0.66 mg MDA/kg cheese, respectively); whereas the untreated cheese had TBARS value of 2.98 mg MDA/kg cheese. Microbiological analysis revealed a reduction in counts of coliforms and yeasts and molds, particularly in the cheese treated with oregano- and basil-infused oils. These results highlight the potential of bioactive-enriched olive oils as natural preservatives and functional ingredients, extending cheese shelf life while enhancing its nutritional value. This study provides a foundation for developing innovative functional foods that integrate plant-derived antioxidants and antimicrobials into widely consumed products.

**Keywords:** cheese, functional food, medicinal plants, oil enrichment

## INTRODUCTION

The concept of functional foods, *i.e.*, foods that offer additional health benefits besides providing basic nutrients, has gained widespread attention in recent years due to the increasing consumer demand for health-promoting and disease-preventing dietary products [Deshmukh & Gutte, 2024]. Dairy products, especially cheese, are among the most widely consumed foods due to their rich nutrient profile, including proteins, calcium and vitamins [Araujo *et al*., 2024]. The incorporation of bioactive ingredients into cheese can enable the development of new functional foods, products with improved nutritional properties and adapted to contemporary dietary trends.

The traditional high-fat diet, often characterized by high levels of unhealthy fats and sugars, has been implicated in causing various health issues, including obesity, metabolic disorders, and cardiovascular diseases [Navarro *et al*., 2024]. In contrast, emerging dietary approaches emphasize a healthier high-fat paradigm, focusing on high-fat products, such as extra virgin

***Corresponding Author:**
tel.: +216 79 325 855; fax: +216 79 325 638; e-mail: **hedia_manai@yahoo.fr / Hedia.Manai@cbbc.rnrt.tn** (Dr. H. Manai-Djebali)

**Submitted:** 16 March 2025
**Accepted:** 30 July 2025
**Published on-line:** 13 August 2025

olive oil containing omega-3 fatty acids and natural bioactive compounds such as phenolics [Tsimihodimos & Psoma, 2024]. These compounds have been shown to elicit numerous health benefits due to anti-inflammatory, antioxidant, and cardiovascular protective effects [Gabbia, 2024]. The diet with extra virgin olive oil promotes better metabolic health, improves energy balance, and protects against the adverse effects of traditional high-fat diets [Tsimihodimos & Psoma, 2024].

Oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), basil (*Ocimum basilicum* L.), and thyme (*Thymus vulgaris* L.) are aromatic herbs widely known for their high content of phenolic compounds and essential oils, which exhibit strong antioxidant, antimicrobial, and anti-inflammatory activities [Delgado *et al*., 2023; Nieto, 2020; Zhakipbekov *et al*., 2024]. These herbs have traditionally been used in Mediterranean cuisine, and their integration into food products has gained scientific attention for their ability to enhance both flavor and health benefits [Delgado *et al*., 2023]. The enrichment of olive oil with these herbs has been shown to extend its shelf life and improve its bioactive properties [Barreca *et al*., 2021; Özcan *et al*., 2022; Yfanti *et al*., 2024]. This enrichment is most often performed by maceration of herbs in oil. The additional use of advanced techniques, such as ultrasound assistance, allows for the extraction of higher levels of bioactive compounds. Ultrasound treatment is known to improve the extraction efficiency by disrupting the plant cell walls, thereby enhancing the transfer of phenolic compounds and essential oils into the olive oil matrix [Ioannis *et al*., 2024].

In the context of functional foods, combining enriched olive oil with cheese presents a novel approach to creating a product that not only provides essential nutrients but also elicits enhanced health benefits. Cheese is an excellent vehicle for delivering bioactive compounds, as its fat matrix can effectively encapsulate and preserve these compounds during storage. Additionally, the inclusion of herbs with antimicrobial properties in the olive oil may contribute to the microbial stability of cheese, extending its shelf life without the need for artificial preservatives. This is particularly relevant for fresh cheeses, which are more susceptible to microbial spoilage due to their higher moisture content [Klisović *et al*., 2022].

This study focused on enriching extra virgin olive oil with phenolic-rich Mediterranean herbs, namely oregano, rosemary, basil, and thyme, and assessing the functional properties of the resulting formulations. The enriched oils were evaluated for their phenolic content, antioxidant capacity, and key quality parameters. In the second stage of the study, these bioactive oils were used to preserve fresh cheese, with particular attention given to their ability to preserve the product's physicochemical quality and microbiological safety during 21 days of storage. By determining phenolic content, antioxidant potential, and quality of herb-infused oils, as well as tracking changes in the nutritional value and oxidative and microbial stability of cheeses, we aimed to demonstrate the functional value of herb-infused oils as natural preservatives and carriers of health-promoting compounds in dairy applications.

## MATERIAL AND METHODS

### Materials

Oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), basil (*Ocimum basilicum* L.), and thyme (*Thymus vulgaris* L.), were commercially sourced from the Tunisian market. The plant materials were dried in an amber environment at 30 ±2°C for 24 h.

The Tunisian Chétoui variety of olive, harvested during the 2023–2024 season at the experimental station of the Centre of Biotechnology of Borj-Cédria was used to obtain olive oil.

Fresh milk for cheese production was sourced from a local farm in Soliman, Tunisia. Its physicochemical parameters determined using a Lactoscan SP milk analyzer (Milkotronic Ltd., Nova Zagora, Bulgaria) were provided by the supplier, including: lipid content (32.46±2.15 g/L), protein content (31.94±1.06 g/L), lactose content (45.80±2.76 g/L), salt content (5.66±0.53 g/L), and density (1.08±0.01 g/mL). All values were reported as mean ± standard deviation ($n$=3).

### Preparation of plant extracts

Each plant was ground to fine powder (20 µm) using a knife mill Grindomix GM 200 (Retsch, Haan, Germany), and then 2 g of the powder was mixed with 20 mL of 80% (*v/v*) methanol solution. The resulting mixtures were subjected to ultrasound-assisted extraction using a sonication bath (Sonoxer DIGIPLUS DL 102 H, Bandelin Electronics GmbH & Co. KG, Berlin, Germany, 40 kHz frequency) for 15 min at 100% ultrasonic power corresponding to 120 W, with the temperature maintained at 40°C [Manai-Djebali *et al*., 2024]. After extraction, the samples were centrifuged at 5,000 rpm (2,795×*g*) for 15 min, and the supernatants were collected. The solvents were subsequently removed from the supernatants using a rotary evaporator (Buchi Rotavapor R-200, Flawil, Switzerland) under reduced pressure (237 mbar) at 40°C until complete evaporation was achieved. Extraction was performed in triplicate.

### Determination of contents of total phenolics, total flavonoids, and condensed tannins of plants

Total phenolic content (TPC) was determined using the spectrophotometric method with the Folin-Ciocalteu reagent according to the procedure described by Habachi *et al*. [2022]. A 125 µL aliquot of dissolved extract (10 µg/mL in methanol 80%, *v/v*) was mixed with 500 µL of distilled water and 125 µL of the Folin-Ciocalteu reagent. After 3 min of agitation at 150 rpm, 1,250 µL of a 7% $Na_2CO_3$ solution was added, and the volume was adjusted to 3 mL with distilled water. The reaction mixture was incubated for 1.5 h in the dark at ambient temperature, and the absorbance was measured at 760 nm. TPC was expressed in mg of gallic acid equivalent (GAE) *per* g of plant dry matter (DM). Measurement was performed in triplicate.

Condensed tannins (CT) were quantified using the vanillin method [Habachi *et al*., 2022]. A volume of 25 µL of extract solution (10 µg/mL in methanol 80%, *v/v*) was mixed with 1,500 µL of a 4% vanillin solution and 750 µL of HCl, and the mixture was incubated for 15 min in the dark. Absorbance was measured at 500 nm. Tannin content was expressed in mg of catechin

equivalent (CE) *per* g of plant DM for each of the three replicates performed.

The total flavonoid content (TFC) was quantified based on the formation of a stable complex between aluminum chloride and flavonoids. Assay was carried out according to the procedure described by Habachi *et al*. [2022] in triplicate. A total of 250 µL of dissolved extract (1 mg/mL) was mixed with 75 µL of a 5% $NaNO_2$ solution and incubated for 6 min in the dark. Following the addition of 150 µL of a 10% $AlCl_3$ solution and 500 µL of a 1 M NaOH solution, the mixture was adjusted to 2.5 mL with distilled water. Absorbance was read at 510 nm, and TFC was expressed in mg of quercetin equivalent *per* g of plant DM.

### Determination of composition of phenolics and other compounds in plants by high-performance liquid chromatography

The content of individual phenolic compounds and some volatile compounds of plant extracts was determined using a high-performance liquid chromatography (HPLC) system (Agilent 1260, Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector (DAD). Chromatographic separation was performed on a Hypersil ODS-C18 column (4.6×100 mm, 0.5 µm; Agilent Technologies), maintained at 25°C. The mobile phase consisted of HPLC water with 0.2% (*v/v*) formic acid (solvent A) and acetonitrile (solvent B), delivered at a flow rate of 0.7 mL/min according to a gradient program: 35% (*v/v*) solvent B for 0–7 min, 60% (*v/v*) solvent B for 7–10 min, 80% (*v/v*) solvent B for 10–15 min, 100% solvent B for 15–26 min, and back to 35% (*v/v*) solvent B for 26–31 min. An injection volume of 10 µL was used. Individual compounds were identified by comparing their retention times and DAD spectral profiles (characteristic absorption maxima) with those of authentic standards. Quantification was performed using calibration curves constructed from authentic standards, with peak areas at 280 nm plotted against known concentrations. Results were expressed in mg *per* g of dry extract (DE).

### Determination of antioxidant activity of plant extracts

Antioxidant activity was evaluated using the assay with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical [Brand-Williams *et al*., 1995]. A solution of 10 mg of DPPH radical in 120 mL of methanol was prepared. The reaction was started by mixing 500 µL of this solution with 500 µL of extract solution in 80% (*v/v*) methanol at various concentrations (10–100 µg/mL) and was continued in the dark for 30 min, followed by absorbance measurement at 517 nm. The percentage inhibition of DPPH radicals was calculated using Equation (1):

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad (1)$$

where: $A_{control}$ is the absorbance of the DPPH radical solution without the extract, and $A_{sample}$ is the absorbance of the DPPH radical solution with the extract. The $IC_{50}$, defined as the concentration of extract (µg/mL) required to inhibit 50% of the DPPH radicals, was determined by interpolation of the plotted curve of % inhibition against extract concentration.

The reducing power was evaluated using the potassium ferricyanide assay according to the procedure described by Yeddes *et al*. [2019]. A volume of 1 mL of each extract solution at concentrations of 50, 100, 200, 400, and 800 µg/mL, prepared in 80% (*v/v*) methanol, was mixed with 0.2 M phosphate buffer (pH 6.6) and a 1% $K_3Fe(CN)_6$ solution in a ratio of 1:1:1 (*v/v/v*), incubated at 50°C for 20 min, and then 1 mL of a 10% trichloroacetic acid solution was added. The absorbance was read at 700 nm, and the concentration of extract producing an absorbance of 0.5 ($A_{0.5}$) was calculated using an absorbance against extract concentration curve.

Measurements were performed in triplicate.

### Preparation of plant-infused oils

To prepare plant-infused oils, each dried plant material (10 g) was macerated in oil (100 mL) and the process of transferring lipid-soluble compounds from plant to oil was assisted by ultrasound using a sonication bath (Sonoxer DIGIPLUS DL 102 H, 40 kHz frequency). The sonication power of 40%, 60%, and 80%, corresponding to 48 W, 72 W, and 96 W, and maceration time of 5 min, 15 min, and 20 min were used. The temperature of the bath was maintained at 70°C [Manai-Djebali *et al*., 2024]. After the sonication process, the mixtures were centrifuged at 4,000 rpm (1,789×*g*) for 15 min to remove plant residues. The supernatant oils were collected and stored at 4°C for further analysis.

### Determination of oil quality indices

Free acidity, expressed as a percentage of oleic acid, was determined by titrating an olive oil sample dissolved in a neutralized ether-ethanol mixture with sodium hydroxide. The peroxide value (PV) was measured by reacting the oil with potassium iodide, followed by titration with sodium thiosulfate. PV was expressed as milliequivalents of active oxygen (meq $O_2$) *per* kg of oil. Specific extinction coefficients, $K_{232}$, $K_{264}$, $K_{268}$, and $K_{272}$, indicating oil oxidation levels, were evaluated by measuring absorbance at 232 nm, 264 nm, 268 nm, and 272 nm, respectively, of a 1% (*w/v*) oil solution in cyclohexane using a 1 cm pathlength cuvette. The variation in specific extinction (ΔK) was calculated using Equation (2):

$$\Delta K = K_{268} - (K_{264} + K_{272})/2 \quad (2)$$

All these indices were determined according to the official methods of the International Olive Council [IOOC, 2024].

The bitterness index ($K_{225}$) was assessed *via* UV spectrophotometry by extracting the polar fraction from 1 g of olive oil dissolved in 5 mL of hexane using 5 mL of 60% (*v/v*) methanol in water. The mixture was vortexed for 2 min and centrifuged at 3,000 rpm (1,370×*g*) for 5 min. The polar fraction was collected, and its absorbance was measured at 225 nm in a 1 cm pathlength cuvette, with $K_{225}$ reported as the absorbance value [Inarejos-Garcia *et al*., 2009]. All measurements were performed in triplicate.

### Determination of pigment content in oils

To determine the content of chlorophylls and carotenoids, a 1.5 g olive oil sample was mixed with 5 mL of cyclohexane, and the absorbance was read at 470 nm ($A_{470}$) and 670 nm ($A_{670}$). The content of chlorophylls and carotenoids (mg/kg) was calculated using Equation (3) and Equation (4), respectively [Jebabli *et al*., 2020].

$$\text{Chlorophyll content} = \frac{A_{670} \times 10^6}{613 \times d \times 100} \quad (3)$$

$$\text{Carotenoid content} = \frac{A_{470} \times 10^6}{2{,}000 \times d \times 100} \quad (4)$$

where: 613 and 2,000 are the specific extinction coefficients (L/(g×cm)) for chlorophylls and carotenoids, respectively; d is the spectrophotometer cell thickness (1 cm); and 100 is a factor accounting for the dilution of the oil (1.5 g in 5 mL cyclohexane, equivalent to 300 g/L) and unit conversion to mg/kg. Both determinations were performed in triplicate.

### Determination of oil phenolic compound content

Oils were extracted following the method of Jebabli *et al*. [2020]. In a 50 mL tube, 2.5 g of olive oil was dissolved in 5 mL of hexane. After agitation, 5 mL of a methanolic solution (60:40 methanol/water, *v/v*) was added, and the mixture was agitated for 2–3 min. The solution was centrifuged at 3,500 rpm (1,370×*g*) for 10 min, and the polar phase was transferred to another tube for analysis of the TPC, TFC and content of CT. The determinations were carried out according to the procedures described above, the same as those used for the analysis of phenolic compounds of plants, and results were expressed in mg GAE/kg oil, mg QE/kg oil, and mg CE/kg oil, respectively.

### Determination of antioxidant activity of oils

The antioxidant activity of the olive oil samples was assessed using the DPPH assay, adapted from the method of Christodouleas *et al*. [2014] with modification. Olive oil (100 mg) was mixed with 2,000 µL of isopropanol, vigorously vortexed for 1 min to ensure homogeneity, and diluted in isopropanol to obtain a stock solution of 50 mg/mL. This stock solution was further diluted in isopropanol to achieve concentrations ranging from 2 to 50 mg/mL. A DPPH radical solution was prepared by dissolving 10 mg of DPPH in 120 mL of isopropanol, freshly prepared to ensure radical stability. Each assay tube contained 1,000 µL of the diluted oil solution and 500 µL of the DPPH solution, mixed thoroughly and incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage inhibition of DPPH radicals was calculated using Equation (1). The $IC_{50}$, defined as the oil concentration (mg/mL) required to inhibit 50% of the DPPH radicals, was determined by plotting % inhibition against the oil concentration curve. Measurement was performed in triplicate.

### Determination of fatty acid composition of oils

The fatty acid composition of olive oil was analyzed using gas chromatography with flame ionization detection (GC-FID) on an Agilent 7890B GC System (Agilent Technologies). Fatty acid methyl esters (FAMEs) were prepared from 0.1 g of olive oil by adding 1 mL of a methanol solution containing 2 M potassium hydroxide. The mixture was vortexed for 30 s and allowed to react at room temperature for 30 min, as *per* the International Olive Council method [IOOC, 2024]. Subsequently, 1 mL of hexane was added to extract the FAMEs, the mixture was vortexed again, and the phases were separated. The hexane layer containing the FAMEs was collected. For GC-FID analysis, an HP-INNOWax capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Agilent Technologies) was used. The injector was maintained at 250°C, and the detector at 300°C. A temperature program was applied, starting at 140°C (held for 5 min), ramping at 4°C/min to 240°C (held for 10 min). Helium was used as the carrier gas at a flow rate of 1 mL/min. A 1-µL sample of the hexane extract was injected, and the FID response was monitored for peak identification based on retention times compared to standard FAMEs. Results were expressed as a percentage of total fatty acids. Analysis was performed in triplicate.

### Analysis of volatile compounds of oils

Volatile compounds of the oils were extracted using headspace solid-phase micro-extraction (HS-SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS) according to the method of Haddada *et al*. [2007]. A SPME unit with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 1 cm, 50/30 µm fiber (Agilent Technologies) was used to adsorb volatile compounds from the headspace of a 20-mL vial containing 5±0.01 g of olive oil, equilibrated for 2 h at 50±2°C. After equilibrium, the SPME fiber was exposed to the headspace for 30 min to adsorb volatile compounds. The fiber was then transferred to the injection port of an Agilent 7890A/5977 GC-MS system for desorption for over 3 min. Volatile compound analysis was conducted using an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Agilent Technologies), with an injector temperature of 250°C and a transfer line temperature of 220°C. The temperature program ranged from 60°C to 200°C at a rate of 5°C/min. Helium was used as the carrier gas at a flow rate of 1.7 mL/min, and the injection was performed in the splitless mode. Mass spectra of detected compounds were compared to those in the Wiley Registry 9th Edition (Wiley 09) and National Institute of Standards and Technology (NIST 2011) spectral libraries using OpenLab Agilent software (Agilent Technologies). Compounds were identified based on a similarity index greater than 80% with a scan time of 1 s and a mass range of 30–500 *m/z*. Volatile compound contents were expressed as relative percentages (% of total volatile compounds), based on the integrated peak areas from the total ion chromatogram (TIC), without external calibration. Analysis was performed in triplicate.

### Oil oxidative stability analysis

The Rancimat test was used to assess the oxidative stability of olive oils. It was performed using a Rancimat 679 device (Metrohm Co., Basel, Switzerland) according to the procedure approved by the International Organization for Standardization (ISO) No. 6886:2016 [ISO, 2016], with analyses conducted in duplicate. Oil (3 g) was placed into a designated cylindrical vessel. The sample was exposed to an airflow of 10 L/h and heated to 100°C. The volatile compounds released during oxidation were collected in 65 mL of distilled water. Proper glassware cleaning was essential before use: the conductivity cell and electrodes were carefully cleaned, dried at 80°C, and soaked overnight in a detergent solution before being rinsed sequentially with running water, acetone, and distilled water. Induction time was determined based on conductivity measurement and oxidative stability index (day/kg) was calculated using Equation (5):

$$\text{Oxidative stability} = (h \times 1{,}000) / (W \times 24) \quad (5)$$

where: h is induction time (h) and W is oil weight (g).

### Cheese production

The production process began with milk pasteurization at 80°C for 15 min to eliminate pathogenic microorganisms and ensure the microbiological safety of the final product. Following pasteurization, the milk was cooled to 37°C and coagulated using 0.03% rennet (MAFI-MIC 15, Tunis, Tunisia), a natural coagulant, leading to the formation of a homogeneous and smooth curd. The curd was cut into small pieces to facilitate whey drainage. The fragmented curd was transferred into plastic molds and allowed to undergo spontaneous whey expulsion for 24 h at 20°C, contributing to the final texture and consistency of the cheese. Three batches were prepared for each cheese type. After complete drainage, the fresh cheese was cut into cubes of 1×1×1 cm using a stainless-steel knife, and the freshly cut cubes were immersed in olive oil or plant-infused oils. This procedure aimed to enrich the cheese with antioxidants while improving its sensory profile and shelf-life stability. The immersion process was conducted under controlled conditions (24 h at 4°C). Following immersion, the cheese samples were drained and used for analyses.

### Nutritional composition analysis of cheese

The nutritional composition of the cheese was determined by analyzing its water, lipid, protein, and reducing sugar contents, along with its total energy value. Water content was determined by oven drying, where 2 g of cheese was weighed, dried at 105°C for 24 h until constant weight, and the weight loss was calculated as water content (g/100 g cheese). Lipids were determined by homogenizing 1 g of cheese with 10 mL of hexane, followed by centrifugation at 3,500 rpm (1,370×*g*) for 10 min and solvent evaporation from the extract. The lipid residue was weighed to calculate lipid content, expressed in g/100 g cheese. For protein determination, 1 g of cheese was homogenized in 10 mL of 0.1 M phosphate buffer (pH 7.0) and centrifuged at 3,500 rpm (1,370×*g*) for 10 min. Proteins in the supernatant were quantified using the Bradford assay with bovine serum albumin (BSA) as the standard, measuring absorbance at 595 nm [Bradford, 1976]. Protein content was expressed in g/100 g cheese. Reducing sugars, as a proxy for carbohydrate content, were assessed by homogenizing 1 g of cheese in 10 mL of 0.1 M phosphate buffer (pH 7.0), followed by centrifugation at 3,500 rpm (1,370×*g*) for 10 min. A 1 mL aliquot of the supernatant was mixed with 1 mL of 3,5-dinitrosalicylic acid (DNS) reagent, heated at 100°C for 5 min, cooled, and diluted to 10 mL with distilled water [Miller, 1959]. Absorbance was measured at 540 nm, and reducing sugar content was expressed as glucose equivalents in g/100 g cheese. The total energy value (kcal/100 g) was calculated using Equation (6):

$$\text{Energy value} = (\text{Protein} \times 4) + (\text{Lipids} \times 9) + (\text{Carbohydrates} \times 4) \quad (6)$$

### Determination of cheese stability during storage

#### Oxidative stability

Lipid oxidation stability of cheese samples was assessed by measuring content of thiobarbituric acid reactive substances (TBARS) during storage at 4°C for 21 days. Samples (4 g) were collected on days 1, 7, 14, and 21 and mixed with 10 mL of a 10% (*w/v*) trichloroacetic acid (TCA) solution [Antonino *et al.*, 2025]. The mixture was vortexed for 5 min. Subsequently, 5 mL of a 0.02 M thiobarbituric acid (TBA) solution was added, and the mixture was vortexed again. The mixture was centrifuged at 14,000×*g* for 10 min. The supernatant was filtered through 0.45 µm nylon filters and incubated in a thermostat bath at 100°C for 30 min. After cooling, absorbance was measured at 532 nm. The TBARS content was quantified using a calibration curve for malondialdehyde (MDA), and results were expressed as mg MDA *per* kg of cheese. Three samples for each time point were analyzed.

#### Microbiological stability

Microbiological stability of cheese was evaluated over three weeks by monitoring total aerobic mesophilic bacteria, coliforms, lactic acid bacteria, yeasts, and molds. Cheese samples (1 g) were homogenized in physiological saline, serially diluted, and plated on selective media: plate count agar (30°C) for mesophilic bacteria, De Man–Rogosa–Sharpe (MRS) agar (37°C) for lactic acid bacteria, Sabouraud agar (37°C) for yeasts and molds, and violet red bile lactose (VRBL) agar (30°C) for coliforms [Garofalo *et al.*, 2024]. Colony counts were expressed as log CFU/g of cheese after incubation under optimal conditions for each microorganism.

### Statistical analysis

The quantitative data on plants, olive oils and cheeses were expressed as the mean and standard deviation. Their statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) software, version 24 (IBM Corp., Armonk, NY, USA, 2016). The analysis of variance (ANOVA) was performed. The Newman-Keuls post hoc test was employed to determine

significant differences between groups. Statistical significance was established at $p<0.05$.

## RESULTS AND DISCUSSION

### Content of phenolics of different classes in plants

The TPC, TFC and CT content in the oregano, rosemary, basil and thyme are summarized in **Table 1**. The analyses revealed different contents of distinct classes of phenolics of these four plants. Oregano exhibited the highest TPC (9.39 mg GAE/g DM). Both, basil and oregano were the richest in flavonoids (TFC of 9.42–8.46 mg QE/g DM). On the other hand, basil had a significantly ($p<0.05$) lower CT content (1.32 mg CE/g DM) compared to the other plants (1.37–1.40 mg CE/g DM). Oregano stood out for its high phenolic content, which aligned with findings from a previous study that also highlighted a strong antioxidant potential of this plant, making it a valuable ingredient in functional food applications [Mateus *et al*., 2024]. Basil, with its elevated flavonoid levels, may provide significant anti-inflammatory, antioxidant, and antimicrobial properties, as basil flavonoids are well-known for their ability to scavenge free radicals, modulate enzyme function, and inhibit inflammatory mediators [Kamelnia *et al*., 2023]. This makes basil a promising candidate for use in health products targeting chronic inflammation and oxidative stress-related conditions. In turn, the presence of condensed tannins in plants may be responsible for their antioxidant and antimicrobial properties [Fraga-Corral *et al*., 2021]. Tannins are associated with plant defence mechanisms and could be conventionally used for food preservation. The differences in phenolic profiles among these plants underline the importance of plant species selection depending on the desired health benefits or functional properties in product formulation.

### Composition of phenolics and other compounds in plant extracts determined by high-performance liquid chromatography

The studied plants exhibited distinct contents of individual phenolics and some volatile compounds (**Table 2**). Among phenolics, oregano, rosemary and basil were particularly rich in rosmarinic acid with contents of 20.25, 15.30, and 12.11 mg/g dry extract (DE), respectively. The findings were consisted with literature data that indicates rosmarinic acid and its methyl ester as main phenolic compounds of these plants [Mateus *et al*., 2024; Popescu *et al*., 2023; Yeddes *et al*., 2019]. Additionally, the rosemary extract had a high content of carnosic acid (24.53 mg/g DE) and carnosol (5.05 mg/g DE), which were absent in the other plant extracts. These volatile compounds, widely studied for their antioxidant properties, contribute to the efficacy of rosemary in various therapeutic and food applications [Habtemariam, 2023]. Caffeic acid was identified in all extracts, with the highest content determined in oregano (5.02 mg/g DE). Ferulic acid and *p*-coumaric acid were also abundant in the oregano extract, at 4.21 and 3.11 mg/g DE, respectively. Flavonoids, such as luteolin and apigenin, were detected in all plant extracts. The content of the former was significantly ($p<0.05$) higher in thyme and rosemary extracts (2.20–2.56 mg/g DE) than in the remaining plants, while the content of the latter in oregano, rosemary and basil extracts (0.79–0.94 mg/g DE) significantly ($p<0.05$) exceeded its content in the thyme extract. The thyme extract stood out due to its high content of thymol (25.51 mg/g DE) and carvacrol (15.21 mg/g DE), two its well-known volatile compounds with antimicrobial and antioxidant activities [Nieto, 2020]. Rosemary and thyme extracts were also rich in chlorogenic acid, whereas the basil extract lacked this compound. The basil extract contained significant amounts of chicoric acid (7.17 mg/g DE), which was absent in the other plants analyzed. In contrast, a previous study by Popescu *et al*. [2023] reported a lower chicoric acid content of 1.30 mg/g in basil extract dry weight, possibly due to differences in extraction methods, basil cultivars, or growing conditions.

### Antioxidant activity of plant extracts

The results in **Table 1** highlight the antioxidant activity of rosemary, thyme, basil, and oregano extracts, evaluated as DPPH radical scavenging activity and reducing power. Rosemary and thyme extracts demonstrated the highest DPPH radical scavenging activity with an $IC_{50}$ value of 15.2–15.6 µg/mL, followed by basil (20.3 µg/mL) and oregano (25.0 µg/mL). The rosemary extract had also the highest reducing power with an $A_{0.5}$ value

**Table 1.** Content of phenolics of different classes in plants and antioxidant activity of plant extracts.

| Content/antioxidant activity | Oregano | Rosemary | Basil | Thyme |
|---|---|---|---|---|
| Total phenolic content (mg GAE/g DM) | $9.39\pm1.63^{a}$ | $6.78\pm0.04^{c}$ | $6.00\pm1.47^{d}$ | $7.34\pm1.92^{b}$ |
| Total flavonoid content (mg QE/g DM) | $8.46\pm1.27^{a}$ | $6.15\pm0.30^{b}$ | $9.42\pm2.09^{a}$ | $6.22\pm1.83^{b}$ |
| Condensed tannin content (mg CE/g DM) | $1.38\pm0.05^{a}$ | $1.40\pm0.17^{a}$ | $1.32\pm0.10^{b}$ | $1.37\pm0.03^{a}$ |
| DPPH assay ($IC_{50}$, µg/mL) | $25.0\pm1.2^{a}$ | $15.2\pm1.3^{c}$ | $20.3\pm1.5^{b}$ | $15.6\pm1.8^{c}$ |
| Reducing power ($A_{0.5}$, µg/mL) | $415\pm11^{a}$ | $301\pm12^{c}$ | $341\pm22^{b}$ | $328\pm10^{b}$ |

Values are expressed as mean ± standard deviation from three replicates. Significant differences between plants are indicated by different superscripts ($p<0.05$). GAE, gallic acid equivalent; QE, quercetin equivalent; CE, catechin equivalent; DM, dry matter; $IC_{50}$, concentration of extract required to inhibit 50% of DPPH radicals; $A_{0.5}$, concentration of extract required to achieve an absorbance of 0.5 in the reducing power assay.

**Table 2.** Content of individual phenolic compounds and some volatile compounds in plants (mg/g dry extract) determined by high-performance liquid chromatography.

| Compound | Oregano | Rosemary | Basil | Thyme |
|---|---|---|---|---|
| Rosmarinic acid | 20.25±1.50$^{a}$ | 15.30±0.41$^{b}$ | 12.11±0.30$^{c}$ | 0.51±0.08$^{d}$ |
| Carnosic acid | – | 24.53±1.99 | – | – |
| Carnosol | – | 5.05±1.03 | – | – |
| Caffeic acid | 5.02±0.25$^{a}$ | 4.10±0.38$^{b}$ | 3.22±0.20$^{c}$ | 3.12±0.20$^{c}$ |
| Ferulic acid | 4.21±0.31$^{a}$ | 2.55±0.26$^{b}$ | 2.29±0.55$^{b}$ | 1.56±0.10$^{c}$ |
| *p*-Coumaric acid | 3.11±0.25$^{a}$ | 1.22±0.10$^{b}$ | 1.01±0.09$^{b}$ | 1.33±0.05$^{b}$ |
| Chlorogenic acid | 1.51±0.19$^{b}$ | 2.10±0.30$^{a}$ | – | 2.31±0.96$^{a}$ |
| Luteolin | 1.33±0.10$^{b}$ | 2.20±0.20$^{a}$ | 1.54±0.19$^{b}$ | 2.56±0.22$^{a}$ |
| Apigenin | 0.79±0.11$^{a}$ | 0.94±0.01$^{a}$ | 0.81±0.15$^{a}$ | 0.63±0.01$^{b}$ |
| Vanillic acid | 0.94±0.01$^{a}$ | 0.83±0.01$^{a}$ | 0.55±0.11$^{b}$ | – |
| Protocatechuic acid | 0.87±0.01$^{a}$ | – | 1.01±0.09$^{a}$ | – |
| Thymol | – | – | – | 25.51±0.72 |
| Carvacrol | – | – | – | 15.21±0.41 |
| Chicoric acid | – | – | 7.17±0.37 | – |

Values are expressed as mean ± standard deviation from three replicates. Significant differences between plants are indicated by different superscripts ($p$<0.05).

of 301 µg/mL. Reducing power of thyme and basil extracts did not differ significantly ($p$≥0.05) from each other (328–341 µg/mL), but was higher than that of the oregano extract (415 µg/mL). These results are consistent with other reports showing rosemary's exceptional antioxidant properties, which have been linked to its high phenolic content, particularly rosmarinic acid as well as phenolic diterpenes [Yeddes *et al.*, 2019]. Moreover, antioxidant efficacy of thyme extract is supported by its high content of thymol and carvacrol, compounds that, in addition to phenolics, contribute significantly to its reducing power and antiradical activity [Nieto, 2020].

## Optimization of olive oil enrichment

The preliminary study aimed to evaluate the impact of sonication power and maceration time on the enrichment of oils with various plant materials. The study was conducted by varying the sonication power at 40%, 60%, and 80%, and applying different maceration times (5, 15, and 20 min), with a fixed plant-to-oil ratio of 1:10 (*w/v*). The results, expressed as $IC_{50}$ values of DPPH assay, are presented in **Table 3**. Optimal antioxidant activity was consistently achieved at 40% sonication power and 15 min of maceration, as evidenced by the lowest $IC_{50}$ values across all plant-infused oils. Specifically, rosemary-infused oil exhibited the highest antioxidant capacity with an $IC_{50}$ of 0.42 mg/mL, followed by thyme (1.31 mg/mL), basil (1.51 mg/mL), and oregano (2.72 mg/mL) at these conditions. These values were significantly

**Table 3.** DPPH radical scavenging activity ($IC_{50}$, mg/mL) of olive oils enriched with oregano, rosemary, basil, and thyme *via* ultrasound-assisted extraction at different sonication powers and process times.

| Oil/Maceration time | 40% Power | 60% Power | 80% Power |
|---|---|---|---|
| Olive oil + oregano, 5 min | 6.71±0.34$^{aB}$ | 4.31±0.22$^{bB}$ | 6.65±0.33$^{aB}$ |
| Olive oil + oregano, 15 min | 2.72±0.14$^{bC}$ | 7.70±0.29$^{aA}$ | 7.54±0.38$^{aA}$ |
| Olive oil + oregano, 20 min | 8.03±0.40$^{aA}$ | 7.62±0.38$^{bA}$ | 6.23±0.31$^{cB}$ |
| Olive oil + rosemary, 5 min | 2.42±0.12$^{aA}$ | 1.91±0.10$^{bB}$ | 1.87±0.09$^{bB}$ |
| Olive oil + rosemary, 15 min | 0.42±0.02$^{cC}$ | 1.63±0.08$^{bC}$ | 2.16±0.11$^{aA}$ |
| Olive oil + rosemary, 20 min | 1.86±0.11$^{bB}$ | 4.84±0.24$^{aA}$ | 1.89±0.09$^{bB}$ |
| Olive oil + basil, 5 min | 7.12±0.16$^{cA}$ | 9.59±0.48$^{aA}$ | 7.66±0.38$^{bA}$ |
| Olive oil + basil, 15 min | 1.51±0.08$^{cC}$ | 7.53±0.48$^{aB}$ | 6.37±0.32$^{bB}$ |
| Olive oil + basil, 20 min | 5.01±0.55$^{bB}$ | 5.50±0.28$^{bC}$ | 6.43±0.62$^{aB}$ |
| Olive oil + thyme, 5 min | 3.47±0.17$^{bB}$ | 1.61±0.08$^{cB}$ | 4.32±0.22$^{aA}$ |
| Olive oil + thyme, 15 min | 1.31±0.07$^{cC}$ | 2.08±0.10$^{bA}$ | 3.33±0.19$^{aB}$ |
| Olive oil + thyme, 20 min | 3.60±0.18$^{bA}$ | 2.16±0.11$^{cA}$ | 4.42±0.12$^{aA}$ |

Values are expressed as mean ± standard deviation from three replicates. Lowercase letters (a–c) indicate significant differences between sonication powers for the same plant and time ($p$<0.05). Uppercase letters (A–C) indicate significant differences between times for the same plant and sonication power ($p$<0.05). $IC_{50}$, oil concentration required to inhibit 50% of the DPPH radicals.

lower ($p$<0.05) than those at 60% and 80% power for the same plants and time, suggesting that moderate sonication power enhances the extraction of bioactive compounds.

These results align with the findings of Ben Hamouda *et al*. [2025], who demonstrated that UAE conditions, such as sonication power, duration, and solute/solvent ratio, significantly affected the antioxidant activity of carob extracts. Sonication power, for instance, enhances extraction efficiency by increasing cavitation, which disrupts cell walls and releases bioactive compounds. A power of 320 W yielded a total phenolic content of 16.68 mg GAE/g from *Rubus alceifolius* leaves [Tran *et al*., 2023]. The duration of sonication also played a critical role; longer times, such as 37.20 min for *Corchorus olitorius* leaves, yielded 13.92 mg GAE/g [Biswas *et al*., 2023].

## Pigment content and quality indices of olive oils

The analysis of the chemical composition of pure olive oil and olive oils infused with various plants revealed notable differences in carotenoid and chlorophyll contents (**Table 4**). Carotenoid content was significantly ($p$<0.05) higher in thyme and oregano oils (5.30–5.46 mg/kg) than in the other oils, potentially enhancing their antioxidant properties, while olive oil showed the lowest carotenoid content (2.58 mg/kg). Chlorophyll content was significant in oregano, basil and thyme oils (17.04–17.64 mg/kg), which may contribute to their vibrant green color and perceived freshness. Olive oil contained more than three times less chlorophylls (5.19 mg/kg). This transfer of carotenoids and chlorophylls from herbs to oil during maceration is consistent with the findings of Karacabey *et al*. [2016], who reported that it was dependent on process conditions, particularly temperature; as at higher temperatures, oil viscosity decreases and mass diffusion increases.

Among oil quality indices, the specific extinction coefficient ($K_{232}$) varied according to the plant added (**Table 4**); with the rosemary oil exhibiting the highest value (3.28). The bitterness index was significantly ($p$<0.05) lower in basil, rosemary and thyme oils (0.10–0.13) than oregano oil (0.17), indicating a milder flavor profile, while olive oil displayed a higher level of bitterness (0.23). Moisture content remained consistently low across all samples, ensuring oil stability. The peroxide values indicate the extent of lipid oxidation, reflecting the level of primary oxidation products in oils [Zhang *et al*., 2021]. All plant-infused oils had lower peroxide values than olive oil, with rosemary oil showing a favorable level (11.00 meq $O_2$/kg oil), suggesting good preservation potential of plant antioxidants. Free acidity levels vary among the oils, with values within the limit of the IOOC (0.8) [IOOC, 2019], which could impact flavor and shelf life. Overall, these findings highlight the importance of plant selection and enrichment methods in maximizing the beneficial properties of infused oils, contributing to their potential applications in food and health industries.

## Phenolic contents and antioxidant capacity of olive oils

The total phenolic content of olive oil was significantly enhanced through infusion with oregano, rosemary, basil, and thyme (**Table 4**). In particular, the rosemary-infused oil showed the highest TPC (556.1 mg GAE/kg), followed by oregano and thyme (529.8–534.4 mg GAE/kg), and basil oil (511.7 mg GAE/kg). This

**Table 4.** Chemical composition and quality parameters of olive oil and plant-infused olive oils.

| Parameter | Olive oil + oregano | Olive oil + rosemary | Olive oil + basil | Olive oil + thyme | Olive oil |
|---|---|---|---|---|---|
| $K_{232}$ | 2.77±0.08$^{bc}$ | 3.28±0.17$^{a}$ | 2.83±0.08$^{b}$ | 3.07±0.09$^{b}$ | 2.38±0.08$^{c}$ |
| ΔK | 0.0012±0.0008$^{c}$ | 0.0025±0.0005$^{b}$ | 0.0014±0.0001$^{c}$ | 0.0015±0.0006$^{c}$ | 0.0037±0.0007$^{a}$ |
| Carotenoid content (mg/kg oil) | 5.30±0.18$^{a}$ | 4.59±0.06$^{b}$ | 3.82±0.07$^{c}$ | 5.46±0.15$^{a}$ | 2.58±0.05$^{d}$ |
| Chlorophyll content (mg/kg oil) | 17.31±0.60$^{a}$ | 10.15±0.19$^{b}$ | 17.64±0.28$^{a}$ | 17.04±0.40$^{a}$ | 5.19±0.10$^{c}$ |
| Bitterness index | 0.17±0.01$^{a}$ | 0.11±0.01$^{b}$ | 0.10±0.01$^{b}$ | 0.13±0.01$^{b}$ | 0.23±0.01$^{a}$ |
| Moisture (%) | 0.30±0.01$^{a}$ | 0.30±0.01$^{a}$ | 0.24±0.01$^{b}$ | 0.24±0.01$^{b}$ | 0.18±0.01$^{c}$ |
| Peroxide value (meq $O_2$/kg oil) | 11.50±0.10$^{c}$ | 11.00±0.10$^{d}$ | 13.75±0.10$^{b}$ | 12.00±0.10$^{c}$ | 14.50±0.01$^{a}$ |
| Free acidity (%) | 0.43±0.01$^{b}$ | 0.69±0.01$^{a}$ | 0.44±0.01$^{b}$ | 0.65±0.01$^{a}$ | 0.34±0.01$^{b}$ |
| Total phenolic content (mg GAE/kg oil) | 534.4±2.8$^{b}$ | 556.1±20.4$^{a}$ | 511.7±5.1$^{c}$ | 529.8±5.3$^{b}$ | 318.6±12.0$^{d}$ |
| Total flavonoid content (mg QE/kg oil) | 464.7±2.1$^{b}$ | 484.9±6.6$^{a}$ | 337.2±8.7$^{c}$ | 337.9±3.0$^{c}$ | 233.6±6.4$^{d}$ |
| Condensed tannin content (mg CE/kg oil) | 53.83±1.33$^{b}$ | 56.80±1.18$^{a}$ | 57.05±1.73$^{a}$ | 52.22±1.09$^{b}$ | 52.54±2.01$^{b}$ |
| DPPH assay ($IC_{50}$, mg/mL) | 2.36±0.18$^{d}$ | 3.18±0.11$^{b}$ | 2.38±0.14$^{d}$ | 2.58±0.09$^{c}$ | 7.85±0.19$^{a}$ |

Values are expressed as mean ± standard deviation from three replicates. Significant differences between oils are indicated by different superscripts ($p$<0.05). $K_{232}$, specific extinction coefficient; ΔK, variation in specific extinction coefficients, GAE, gallic acid equivalent; QE, quercetin equivalent; CE, catechin equivalent; $IC_{50}$, oil concentration required to inhibit 50% of the DPPH radicals.

was a substantial increase compared to the control olive oil (318.6 mg GAE/kg), highlighting the effective transfer of phenolics from the herbs into the oil. The total flavonoid content followed a similar trend, with the rosemary- and oregano-infused oils showing the highest values (484.9 and 464.7 mg QE/g kg, respectively). The higher TPC and TFC in these infused oils aligns with previous studies that emphasize the role of phenolic compounds from herbs like rosemary, oregano, sage, and wild nettle in enhancing oil antioxidant properties [Karacabey *et al*., 2016; Manai-Djebali *et al*., 2024 ; Yfanti *et al*., 2024]. Condensed tannin content of rosemary and basil oils was also elevated compared to olive oil, contributing to the bitterness and oxidative stability of the oils, although the increase was less pronounced than TPC and TFC (**Table 4**). In the case of oregano and thyme oils, CT content did not even differ significantly ($p \geq 0.05$) from that in olive oil.

The DPPH radical scavenging activity results highlighted significant differences in antioxidant capacity between the herb-infused olive oils and the control (**Table 4**). Oregano- and basil-infused oils exhibited the most potent antioxidant effect with an $IC_{50}$ of 2.36–2.38 mg/mL, followed closely by thyme (2.58 mg/mL). This indicated that these herbs substantially enhance the radical-scavenging capacity of the olive oil, likely due to the high content of phenolic compounds known for their ability to donate hydrogen atoms and neutralize free radicals. Interestingly, the rosemary-infused oil, despite having the highest total phenolic content (556.1 mg GAE/Kg), exhibited a higher $IC_{50}$ (3.18 mg/mL) compared to the other herb-infused oils. This apparent discrepancy may be attributed to the nature of the phenolic compounds present in rosemary. While rosemary is known to contain potent antioxidants, such as rosmarinic acid and carnosic acid [Yeddes *et al*., 2019], the efficiency of these compounds in DPPH assays might vary due to their specific molecular structure, particularly their steric hindrance and redox potential [Chiorcea-Paquim *et al*., 2020]. Moreover, the mechanism of action of rosemary compounds as antioxidants may involve both radical scavenging and metal chelation, which might not be fully captured by the DPPH assay alone, as noted in previous reports [Liu *et al*., 2024]. In contrast, the virgin olive oil exhibited a significantly higher $IC_{50}$ (7.85 mg/mL), underscoring the limited radical-scavenging capacity of olive oil on its own, despite its natural phenolic content, such as hydroxytyrosol, oleuropein and its derivatives [Jukić Špika *et al*., 2022]. This reinforces the efficacy of using herb infusion to enhance the antioxidant properties of olive oil.

## Fatty acid composition of olive oils

The fatty acid composition of the pure olive oil and plant-infused olive oils is shown in **Table 5**. For the content of most fatty acids, no significant ($p \geq 0.05$) differences were found between the oils (except C18:2, C20:0 and C22:1). The predominant fatty acid across all samples was oleic acid (C18:1), with values ranging from 68.51 to 69.03% total fatty acids. This aligns well with the typical composition of extra virgin olive oil, where oleic acid generally accounts for 55–83% of the total fatty acids [IOOC, 2021]. The presence of this monounsaturated fatty acid is a key factor contributing to olive oil's health benefits, including cardiovascular protection and anti-inflammatory effects [Marcelino *et al*., 2019]. Palmitic acid (C16:0), the major saturated fatty acid in olive oil, showed a similar content across the samples (12.56 to 12.79% total fatty acids), which was in line with the established values of 7.5–20% total fatty acids in olive oils [IOOC, 2021]. Linoleic acid (C18:2), a polyunsaturated fatty acid, was another critical

**Table 5.** Fatty acid composition (% total fatty acids) of olive oil and plant-infused olive oils.

| Fatty acid | Olive oil + oregano | Olive oil + rosemary | Olive oil + basil | Olive oil + thyme | Olive oil |
|---|---|---|---|---|---|
| C14:0 | 0.09±0.10$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.02±0.01$^{a}$ |
| C16:0 | 12.64±0.01$^{a}$ | 12.56±0.01$^{a}$ | 12.79±0.04$^{a}$ | 12.69±0.01$^{a}$ | 12.66±0.03$^{a}$ |
| C16:1 | 0.59±0.01$^{a}$ | 0.58±0.01$^{a}$ | 0.55±0.01$^{a}$ | 0.57±0.03$^{a}$ | 0.57±0.02$^{a}$ |
| C18:0 | 0.00±0.00$^{a}$ | 0.01±0.01$^{a}$ | 0.01±0.01$^{a}$ | 0.00±0.00$^{a}$ | 0.01±0.01$^{a}$ |
| C18:1 | 68.77±0.05$^{a}$ | 69.03±0.01$^{a}$ | 68.51±0.02$^{a}$ | 68.85±0.05$^{a}$ | 68.86±0.11$^{a}$ |
| C18:2 | 15.89±0.01$^{b}$ | 15.85±0.01$^{b}$ | 16.17±0.01$^{a}$ | 15.94±0.01$^{b}$ | 15.87±0.02$^{b}$ |
| C18:3 | 0.01±0.05$^{a}$ | 0.01±0.01$^{a}$ | 0.02±0.01$^{a}$ | 0.00±0.01$^{a}$ | 0.02±0.01$^{a}$ |
| C20:0 | 0.81±0.06$^{a}$ | 0.69±0.01$^{b}$ | 0.76±0.03$^{a}$ | 0.70±0.01$^{ab}$ | 0.74±0.05$^{a}$ |
| C20:1 | 0.95±0.03$^{a}$ | 0.98±0.01$^{a}$ | 0.90±0.02$^{a}$ | 0.94±0.02$^{a}$ | 0.96±0.03$^{a}$ |
| C22:0 | 0.01±0.01$^{a}$ | 0.01±0.01$^{a}$ | 0.01±0.01$^{a}$ | 0.01±0.01$^{a}$ | 0.01±0.01$^{a}$ |
| C22:1 | 0.14±0.01$^{a}$ | 0.01±0.01$^{b}$ | 0.01±0.02$^{b}$ | 0.01±0.01$^{b}$ | 0.02±0.01$^{b}$ |
| C24:0 | 0.01±0.01$^{a}$ | 0.01±0.01$^{a}$ | 0.02±0.01$^{a}$ | 0.01±0.01$^{a}$ | 0.02±0.01$^{a}$ |

Values are expressed as mean ± standard deviation from three replicates. Significant differences between oils are indicated by different superscripts ($p<0.05$).

compound, with levels ranging from 15.85 to 16.17% total fatty acids in the infused oils in our study, again within the typical range (3.5–21%) for olive oils [Tsimihodimos & Psoma, 2024]. The minor fatty acids, such as palmitoleic acid (C16:1) and stearic acid (C18:0), remained consistent with the known fatty acid profiles of extra virgin olive oils [IOOC, 2021], further supporting the conclusion that the infusion process with oregano, rosemary, basil, and thyme does not significantly alter the fatty acid composition. This stability in fatty acid profile indicates that the enrichment with aromatic herbs mainly affects the phenolic composition and antioxidant properties of the oils, rather than altering their lipid structure. This observation is critical, as it suggests that the health-promoting properties associated with the fatty acids in olive oil are preserved, while additional benefits may arise from the enhanced phenolic content contributed by the herbs.

## Volatile compounds in olive oils

The volatile compound profiles of olive oils infused with various aromatic plants (rosemary, oregano, basil, thyme) and non-infused olive oil are shown in **Table 6**. Each infused oil exhibited a unique composition, underscoring the predominance of specific volatile compounds. The rosemary-infused oil was dominated by camphor (28.11% of total volatile compounds), 1,8-cineole (32.44%), and α-pinene (17.00%), reflecting its terpene-rich profile. The oregano-infused oil was characterized by high levels of *p*-cymene (32.12%), thymol (18.45%), α-pinene (15.45%), and carvacrol (14.85%). The basil-infused oil stood out with exceptional concentrations of methyl chavicol (52.34%), linalool (17.35%), and limonene (13.98%), indicating its sweet and aromatic profile. The thyme-infused oil showed a high content of carvacrol (74.01%), along with notable levels of terpinene (5.34%) and thymol (5.33%), emphasizing its intense aromatic properties. In contrast, the non-infused olive oil was dominated by hexanal (62.35%) and octanal (13.28%), which are characteristic of non-infused vegetable oils, giving it a simpler, herbaceous profile.

The analysis of volatile compounds in plant-infused olive oils highlights distinct chemical profiles, crucial for their biological activities. Each infused oil contained specific bioactive compounds, including terpenes and volatile phenols, which may contribute to their antioxidant, antimicrobial, and anti-inflammatory properties. Rosemary essential oil, rich in camphor, 1,8-cineole, and α-pinene, exhibited strong radical-scavenging

**Table 6.** Volatile compound contents (% total volatile compounds) in olive oil and plant-infused olive oils.

| Volatile compound | Olive oil + rosemary | Olive oil + oregano | Olive oil + basil | Olive oil + thyme | Olive oil |
|---|---|---|---|---|---|
| α-Pinene | 17.00±0.51$^{a}$ | 15.45±0.07$^{b}$ | 5.02±0.03$^{c}$ | 1.85±0.06$^{d}$ | 0.82±0.02$^{e}$ |
| *p*-Cymene | 1.35±0.22$^{b}$ | 32.12±0.36$^{a}$ | – | 1.15±0.24$^{b}$ | 0.35±0.22$^{c}$ |
| Limonene | 0.35±0.03$^{d}$ | 1.40±0.55$^{b}$ | 13.98±2.99$^{a}$ | 0.98±0.03$^{c}$ | – |
| Terpinene | 0.25±0.01$^{c}$ | 12.89±0.18$^{a}$ | 0.18±0.01$^{c}$ | 5.34±0.16$^{b}$ | – |
| Hexanal | – | – | – | – | 62.35±0.01$^{a}$ |
| Octanal | – | – | – | – | 13.28±0.01$^{a}$ |
| 1,8-Cineole | 32.44±0.42$^{a}$ | 5.98±0.03$^{b}$ | 1.54±0.05$^{d}$ | 2.35±0.07$^{c}$ | -– |
| Linalool | 3.52±0.02$^{b}$ | 0.24±0.01$^{c}$ | 17.35±0.52$^{a}$ | 0.30±0.01$^{c}$ | 0.08±0.01$^{d}$ |
| Citral | – | 0.61±0.01$^{b}$ | 0.96±0.02$^{a}$ | 0.00±0.01$^{c}$ | 0.00±0.01$^{c}$ |
| (*E*)-2-Hexenal | – | – | – | – | 15.12±0.01$^{a}$ |
| 2-Pentylfuran | – | – | – | – | 6.01±0.01$^{a}$ |
| Borneol | 7.23±1.02$^{a}$ | 2.36±0.17$^{b}$ | 0.30±0.01$^{c}$ | 0.12±0.01$^{d}$ | – |
| Bornyl acetate | 4.35±0.13$^{a}$ | 1.45±0.04$^{b}$ | – | 0.98±0.03$^{c}$ | – |
| Camphor | 28.11±0.45$^{a}$ | – | 0.13±0.01$^{bc}$ | – | – |
| Methyl chavicol | – | – | 52.34±0.37$^{a}$ | – | – |
| Eugenol | – | – | 3.45±0.31$^{a}$ | – | – |
| Thymol | 0.12±0.01$^{c}$ | 18.45±0.55$^{b}$ | 0.07±0.01$^{c}$ | 5.33±0.01$^{b}$ | – |
| Carvacrol | – | 14.85±0.45$^{b}$ | 0.01±0.01$^{c}$ | 74.01±2.22$^{a}$ | – |

Values are expressed as mean ± standard deviation from three replicates. Significant differences between oils are indicated by different superscripts ($p$<0.05).

**Table 7.** Oxidative stability of olive oil and plant-infused olive oils in the Rancimat test.

| Oil | Induction time (h) | Oxidative stability index (day/kg) |
|---|---|---|
| Olive oil + oregano | 34.42±0.02$^{c}$ | 409.8±0.3$^{c}$ |
| Olive oil + rosemary | 82.25±1.23$^{a}$ | 978.8±15.4$^{a}$ |
| Olive oil + basil | 30.89±0.50$^{d}$ | 367.8±6.0$^{d}$ |
| Olive oil + thyme | 51.48±0.17$^{b}$ | 612.9±2.1$^{b}$ |
| Olive oil | 34.44±0.59$^{c}$ | 410.0±7.1$^{c}$ |

Values are expressed as mean ± standard deviation from three replicates. Significant differences between oils are indicated by different superscripts ($p$<0.05).

activity and anti-inflammatory potential [Becer *et al*., 2023]. Thymol and carvacrol, the main volatile compounds of the thyme-infused oil in our study, demonstrate remarkable antimicrobial activity, effectively inhibiting pathogenic microorganisms [Mączka *et al*., 2023]. In turn, methyl chavicol and linalool, the main compounds of basil essential oil, are primarily associated with antioxidant and antimicrobial effects, respectively [Zhakipbekov *et al*., 2024]. Finally, the oregano-infused oil was rich in *p*-cymene, a compound also known for its superior antimicrobial and antioxidant properties [Balahbib *et al*., 2021].

## Oxidative stability of olive oils in Rancimat test

The induction times of oxidation of pure olive oil and plant-infused olive oils measured in the Rancimat test are presented in **Table 7**. The infusion with aromatic plants significantly influenced olive oil oxidative stability. The rosemary-infused oil exhibited the best oxidation stability, with an induction time of 82.25 h, which was 2.4 times higher than for the control oil (34.44 h). The thyme-infused oil also showed a notable improvement, with an induction time of 51.48 h. Although the stability of the thyme-infused oil was significantly ($p$<0.05) lower than that of the rosemary-infused oil, it was still superior to the control oil. In terms of oxidative stability index (expressed in day/kg), the rosemary-infused oil recorded a value of 978.8 day/kg compared to 410.0 day/kg for the control oil, indicating a significant reduction in oxidation. In contrast, infusion of olive oils with basil and oregano did not yield significant benefits, with induction times of 30.89 h and 34.42 h, respectively, both close to the control oil. The oxidative stability index for these oils was also low, with 367.8 day/kg for basil and 409.8 day/kg for oregano. In a previous study, essential oils from plants such as sage, oregano, rosemary, and thyme were reported to effectively protect the oleic acid of olive oil oxidized by irradiation at 360 nm for 32 h [Barreca *et al*., 2021]. In turn, Özcan *et al*. [2022] studied the effect of thyme, rosemary, and sage essential oils and extracts from these plants on the oxidative stability of olive oil during storage and found that the rosemary extract contributed to maintaining oil quality by stabilizing free fatty acid level.

## Nutritional composition of cheeses

The nutritional composition of fresh cheeses stored in different infused olive oils (rosemary, oregano, basil, thyme), as well as in pure olive oil, compared to a control fresh cheese not immersed in oil is shown in **Table 8**. The cheeses stored in olive oil exhibited relatively homogeneous protein content, around 10 g/100 g, compared to 22.56 g/100 g determined in the control cheese, suggesting a dilution effect due to oil absorption. In contrast, the lipid content was significantly higher in the cheeses stored in oils, reaching approximately 48–49 g/100 g, compared to 21.63 g/100 g determined in the control cheese, indicating substantial lipid uptake from the oil. This lipid absorption results in a higher energy value for the cheeses preserved in oils (approximately 479–496 kcal/100 g), while the control cheese, with its lower lipid content, had a lower caloric value (293.2 kcal/100 g). The carbohydrate content remained low across all samples (1.41–2.06 g/100 g), contributing negligibly to total energy. Additionally, the water content was lower in the cheeses immersed in oil (approximately 37–40 g/100 g) compared to the control cheese (52.15 g/100 g).

## Oxidative stability of cheeses during storage

The TBARS values of cheeses demonstrated that their immersion in plant-infused olive oils significantly affected their oxidative stability over time (**Table 9**). On day 1, the TBARS values

**Table 8.** Nutritional composition of the untreated fresh cheese and cheeses treated with olive oils.

| Cheese | Proteins (g/100 g) | Lipids (g/100 g) | Carbohydrates (g/100 g) | Water (g/100 g) | Energy value (kcal/100 g) |
|---|---|---|---|---|---|
| Cheese + rosemary-infused oil | 10.51±0.53$^{b}$ | 48.55±2.43$^{a}$ | 1.99±0.10$^{a}$ | 38.36±1.92$^{b}$ | 487.2±12.7$^{a}$ |
| Cheese + oregano-infused oil | 10.56±0.53$^{b}$ | 48.23±2.41$^{a}$ | 2.01±0.10$^{a}$ | 38.66±1.93$^{b}$ | 484.4±12.6$^{a}$ |
| Cheese + basil-infused oil | 10.76±0.54$^{b}$ | 49.55±2.48$^{a}$ | 1.86±0.09$^{a}$ | 37.19±1.86$^{b}$ | 496.4±12.9$^{a}$ |
| Cheese + thyme-infused oil | 9.99±0.50$^{b}$ | 48.09±2.40$^{a}$ | 1.55±0.08$^{ab}$ | 40.12±2.01$^{b}$ | 479.0±12.5$^{a}$ |
| Cheese + pure olive oil | 10.64±0.53$^{b}$ | 49.06±2.45$^{a}$ | 1.41±0.07$^{b}$ | 38.69±1.93$^{b}$ | 489.7±12.7$^{a}$ |
| Untreated fresh cheese | 22.56±1.13$^{a}$ | 21.63±1.08$^{b}$ | 2.06±0.10$^{a}$ | 52.15±2.61$^{a}$ | 293.2±7.6$^{b}$ |

Values are expressed as mean ± standard deviation from three replicates. Significant differences between cheeses are indicated by different superscripts ($p$<0.05).

**Table 9.** Content of thiobarbituric acid reactive substances of the untreated fresh cheese and cheeses treated with olive oils (mg MDA/kg cheese) over storage time.

| Cheese | Day 1 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| Cheese + rosemary-infused oil | $0.41\pm0.04^{bA}$ | $0.40\pm0.02^{bC}$ | $0.42\pm0.02^{bE}$ | $0.53\pm0.02^{aE}$ |
| Cheese + oregano-infused oil | $0.41\pm0.02^{cA}$ | $0.41\pm0.01^{cC}$ | $0.63\pm0.03^{bC}$ | $0.86\pm0.09^{aC}$ |
| Cheese + basil-infused oil | $0.42\pm0.02^{cA}$ | $0.43\pm0.03^{cC}$ | $0.66\pm0.02^{bC}$ | $0.86\pm0.07^{aC}$ |
| Cheese + thyme-infused oil | $0.44\pm0.03^{cA}$ | $0.41\pm0.01^{cC}$ | $0.52\pm0.01^{bD}$ | $0.66\pm0.05^{aD}$ |
| Cheese + pure olive oil | $0.40\pm0.03^{dA}$ | $0.49\pm0.02^{cB}$ | $0.83\pm0.03^{bB}$ | $1.06\pm0.02^{aB}$ |
| Untreated fresh cheese | $0.45\pm0.02^{dA}$ | $0.65\pm0.05^{cA}$ | $1.97\pm0.05^{bA}$ | $2.98\pm0.12^{aA}$ |

Values are expressed as mean ± standard deviation ($n$=3). Lowercase letters (a–d) indicate significant differences between time points within the same sample. Uppercase letters (A–E) indicate significant differences between treatments at the same time point ($p$<0.05). MDA, malondialdehyde equivalent.

for all cheeses ranged between 0.40 and 0.45 mg MDA/kg and did not differ significantly ($p$≥0.05) from each other. After 7 days, TBARS values increased significantly ($p$<0.05) only for the untreated control cheese to 0.65 mg MDA/kg and for the cheese preserved in non-enriched oil to 0.49 mg MDA/kg. For the cheeses treated with the plant-infused oils, TBARS values did not differ significantly ($p$≥0.05) and were in the range of 0.40–0.43 mg MDA/kg. By day 14, the cheese immersed in the rosemary-infused oil showed the best oxidative stability, with a TBARS value of 0.42 mg MDA/kg. The thyme-infused oil yield a slightly higher result (0.52 mg MDA/kg cheese) followed by oregano- and basil-infused oils with a similar effect (0.63–0.66 mg MDA/kg cheese). The untreated cheese and the cheese treated with pure olive oil exhibited much higher TBARS values (1.97 and 0.83 mg MDA/kg, respectively), reaffirming the protective effect of plant-infused oils. On day 21, the rosemary-infused oil maintained its superior effectiveness yielding a TBARS value of 0.53 mg MDA/kg cheese, closely followed by the thyme-infused oil (0.66 mg MDA/kg cheese). In contrast, the cheeses treated with basil- and oregano-infused oils displayed higher TBARS values (0.86 mg MDA/kg), suggesting that rosemary and thyme ensured more stable preservation. The cheese immersed in non-infused olive oil (1.06 mg MDA/kg) and the untreated cheese (2.98 mg MDA/kg) showed significantly higher oxidation levels, confirming the notable protective effect of plant-infused oils.

The application of herb-infused oils for cheese preservation, as explored in this study, addresses challenges observed in previous research on oil-cheese interactions. Klisović *et al.* [2022] found that the immersion of semi-hard, hard, and soft whey cheeses in extra virgin olive oil (EVOO) for two months accelerated hydrolytic and oxidative degradation, with phenolic content reductions of 85.0–93.5% and increases in oxidation indicators (*e.g.*, $K_{232}$) and trans-oleic fatty acid beyond EVOO quality limits, driven by fat migration and cheese composition (moisture, proteins). Similarly, Popescu *et al.* [2023] demonstrated that microencapsulated basil extract at 0.6–0.9% (*w/w*) in cream cheese inhibited post-fermentation, improved water retention, and enhanced textural properties, extending shelf life by 7 days at 4°C, despite the challenges of direct plant extract use due to sensory impacts.

## Microbiological stability of cheeses

The microbiological stability study revealed significant differences in bacterial, fungal, and coliform counts across various cheese samples over 21 days (**Figure 1**). Lactic acid bacteria decreased significantly in cheeses treated with the plant-infused oils (oregano, rosemary, basil, and thyme), while the untreated fresh cheese showed no significant variation. This suggests that the plant-infused oils alter the microbial environment. The total aerobic mesophilic flora increased in the untreated cheese during storage from 5.27 to 7.94 log CFU/g. In turn, the cheeses treated with herb-infused oils, particularly rosemary and basil, showed significantly reduced microbial proliferation ($p$<0.05), with the total aerobic mesophilic flora counts of 4.64 log CFU/g (rosemary) and 4.58 log CFU/g (basil) at day 7, and 4.53 log CFU/g (rosemary) and 4.49 log CFU/g (basil) at day 14 (**Figure 1B**). Yeast and mold counts (**Figure 1C**) followed a similar trend, with the untreated cheese displaying continuous fungal growth, reaching 7.75 log CFU/g on day 21. In contrast, the cheeses treated with the herb-infused oils showed reduced counts, with oregano at 5.27 log CFU/g (day 7) and 5.14 log CFU/g (day 14), rosemary at 5.43 log CFU/g (day 7) and 5.32 log CFU/g (day 14), and basil at 5.06 log CFU/g (day 7) and 4.49 log CFU/g (day 14), compared to the untreated cheese. Total and thermotolerant coliform counts (**Figure 1D**) in the untreated fresh cheese increased from 5.15 log CFU/g on day 1 to 6.86 log CFU/g on day 21 at 4°C, while in the cheese stored in the non-enriched olive oil their count reached 6.07 log CFU/g by day 21. In contrast, the cheeses treated with the herb-infused oils showed significantly lower coliform counts ($p$<0.05), with basil (4.73 log CFU/g on day 7, 4.90 log CFU/g on day 21), oregano (4.85 log CFU/g on day 7, 4.92 log CFU/g on day 21), thyme (4.90 log CFU/g on day 7, 5.04 log CFU/g on day 21), and rosemary (4.88 log CFU/g on day 7, 5.04 log CFU/g on day 21) demonstrating pronounced reductions, particularly on days 7 and 14. These findings highlight

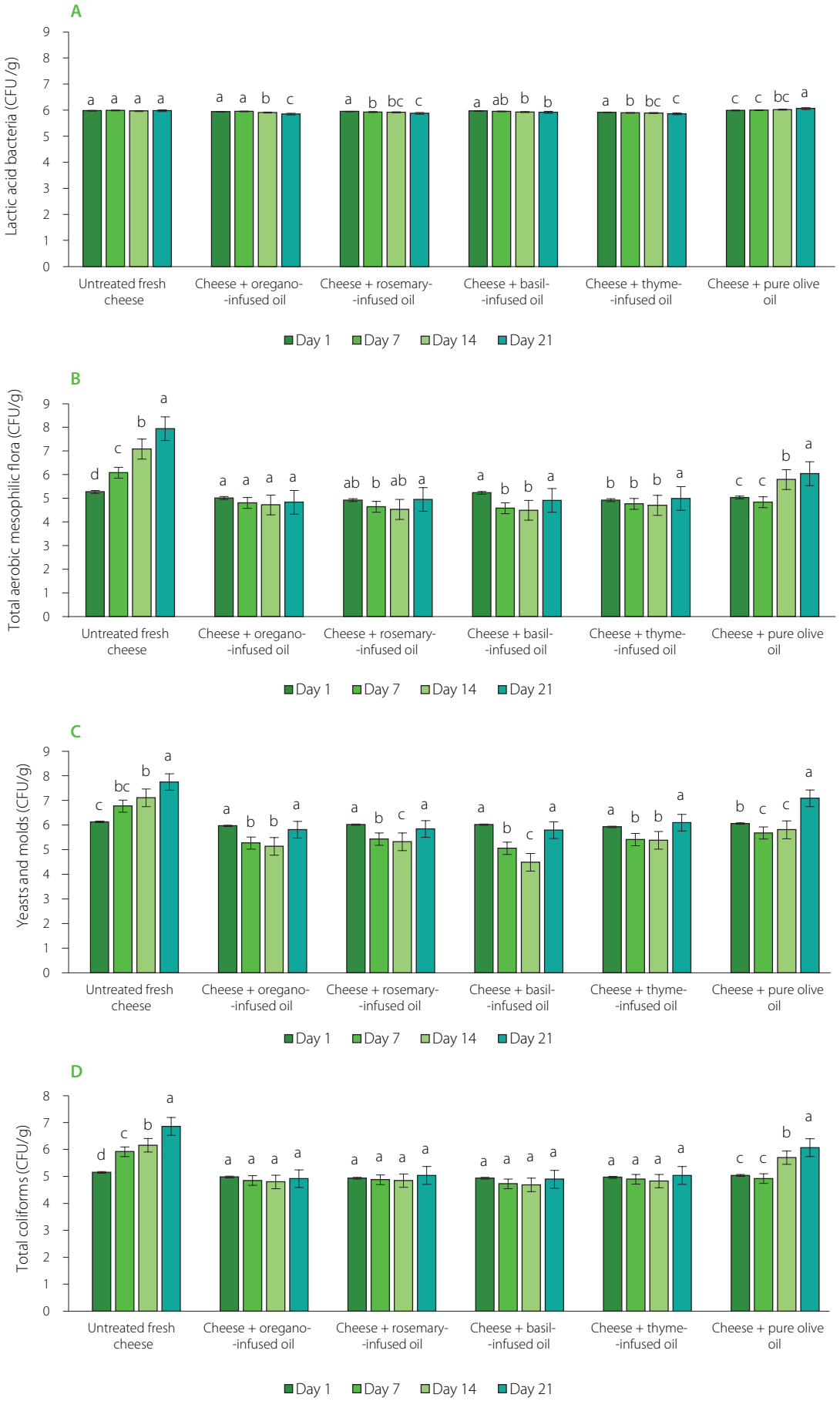


**Figure 1.** Counts of lactic acid bacteria (**A**), total aerobic mesophilic flora (**B**), yeasts and molds (**C**), and total coliforms (**D**) of the untreated cheese and cheeses treated with plant-infused oils during 21 days of storage. Results are presented as mean and standard deviation following three repetitions. For each cheese, significant differences between time points are indicated by different letters ($p<0.05$).

the antimicrobial and antifungal potential of the plant-infused oils, particularly oregano and basil, in improving cheese microbiological stability and shelf life.

Factors controlling microbial growth in cheese include water activity, salt concentration, redox potential, pH, temperature, and possibly bacteriocin production by certain microorganisms. These "hurdles" may not be significant individually, but together they effectively inhibit microbial growth [Hayaloglu, 2016]. Microbiological monitoring over four time points revealed significant differences between control and cheeses treated with plant-infused oils, they inhibited coliforms, molds, and yeasts during the first two weeks, extending shelf life. The control cheese developed surface mold after two weeks, whereas the flavored cheeses remained stable beyond this period. Inhibition of undesirable microbes, including yeasts and molds, was observed as early as in the first week. The total aerobic mesophilic flora is a key hygiene indicator in cheese. The cheeses stored in the plant-infused oils had microbial loads below the French regulatory limit of 5 log CFU/mL [Benyahia *et al*., 2021]. The total aerobic mesophilic flora levels decreased during the first two weeks, likely due to salting and water loss, limiting microbial growth [Hamama, 1989]. Unlike our findings, Rhiat *et al*. [2011] reported the total absence of coliforms, though their presence does not always indicate fecal contamination, as some originate from dairy equipment residues. Enriched olive oil helped preserve cheese organoleptic properties and extended shelf life. According to Mason & Wasserman [1987], bioactive compounds in the oil interfere with microbial membrane proteins, such as adenosine triphosphatase, either by direct interaction with hydrophobic protein regions or by disrupting proton translocation across the membrane, thereby preventing adenosine diphosphate (ADP) phosphorylation.

## CONCLUSIONS

The findings of this study underscore the efficacy of ultrasound-assisted extraction in enhancing the functional properties of olive oil through the infusion of phenolic-rich herbs such as oregano, rosemary, basil, and thyme. The plant-infused olive oils exhibited significantly improved antioxidant capacity, and the basil-infused oil showed the strongest DPPH radical scavenging potential. The most effective improvement in the oxidative stability of olive oil was achieved by rosemary. When applied for fresh cheese immersion, these plant-infused oils significantly reduced lipid oxidation and microbial proliferation during storage. Among the formulations, rosemary- and thyme-infused oils provided superior protection against oxidation, while oregano- and basil-infused oils exhibited strong antimicrobial effects, leading to an extended shelf life. These results position bioactive-infused olive oils as promising alternatives to synthetic preservatives in the dairy industry. Beyond their preservative role, these enriched oils contribute to the development of innovative functional foods that align with consumer demands for natural, health-promoting dietary options. Their ability to enhance the nutritional profile of cheese while improving storage stability offers potential applications in artisanal and industrial dairy production. Future research should explore consumer sensory acceptance, large-scale processing feasibility, and the potential synergistic effects of combining different bioactive compounds. This study lays the groundwork for further innovations in the field of functional foods, with implications for both food preservation and human health.

## ACKNOWLEDGEMENTS

The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work (project number TU-DSPP-2024-115).

## RESEARCH FUNDING

This research was funded by Taif University, Saudi Arabia, Project No. TU-DSPP-2024-115 and by Tunisian Ministry of Higher Education and Scientific Research (LR15CBBC06).

## CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

## ORCID IDs

N. Ben Youssef https://orcid.org/0000-0001-8706-2244
N. Farhat https://orcid.org/0000-0001-7769-0095
H. Gadhoumi https://orcid.org/0000-0002-9293-9191
K. Hessini https://orcid.org/0000-0002-8929-9234
H. Manai-Djebali https://orcid.org/0000-0001-5703-0460
A. Mejri https://orcid.org/0000-0002-8172-5163
S. Nait-Mohamed https://orcid.org/0000-0002-2996-1724
M. Saidani Tounsi https://orcid.org/0000-0003-1548-1655

## REFERENCES

1. Antonino, C., Natrella, G., Caponio, G.R., Zago, L., Difonzo, G., Faccia, M. (2025). Reuse of UHT milk close to expiring and almond okara in the production of fresh cheese. *Food Frontiers*, *6*(4), 2052-2064. https://doi.org/10.1002/fft2.70045
2. Araujo, H.C.S., de Jesus, M.S., Sandes, R.D.D., Leite Neta, M.T.S., Narain, N. (2024). Functional cheeses: Updates on probiotic preservation methods. *Fermentation*, *10*(1), art. no. 8. https://doi.org/10.3390/fermentation10010008
3. Balahbib, A., El Omari, N., El Hachlafi, N., Lakhdar, F., El Menyiy, N., Salhi, N., Mrabti, H.N., Bakrim, S., Zengin, G., Bouyahya, A. (2021). Health beneficial and pharmacological properties of p-cymene. *Food and Chemical Toxicology*, *153*, art. no. 112259. https://doi.org/10.1016/j.fct.2021.112259
4. Barreca, S., La Bella, S., Maggio, A., Licata, M., Buscemi, S., Leto, C., Pace, A., Tuttolomondo, T. (2021). Flavouring extra-virgin olive oil with aromatic and medicinal plants essential oils stabilizes oleic acid composition during photo-oxidative stress. *Agriculture*, *11*(3), art. no. 266. https://doi.org/10.3390/agriculture11030266
5. Becer, E., Altundağ, E.M., Güran, M., Seda Vatansever, H., Ustürk, S., Hanoğlu, D.Y., Hüsnü Can Başer, K. (2023). Composition and antibacterial, anti-inflammatory, antioxidant, and anticancer activities of *Rosmarinus officinalis* L. essential oil. *South African Journal of Botany, 160*, 437-445. https://doi.org/10.1016/j.sajb.2023.07.028
6. Ben Hamouda, G., Bettaieb Rebey, I., Zar Kalai, F., Hammami, M., Ksouri, R. (2025). Enhancing antioxidant yield from carob (*Ceratonia siliqua* L.): evaluating the efficacy of maceration and ultrasound-assisted methods. *International Journal of Environmental Health Research*, *35*(7), 1793–1805. https://doi.org/10.1080/09603123.2024.2407456
7. Benyahia, F.A., Zitoun, O.A., Meghzili, B., Foufou, E., Zidoune, M.N. (2021). Use of *Pergularia tomentosa* plant enzymatic coagulant system in fresh cheese-making. *Food and Nutrition Sciences*, *12*(11), 1028–1040. https://doi.org/10.4236/fns.2021.1211076
8. Biswas, A., Dey, S., Xiao, A., Deng, Y., Birhanie, Z.M., Roy, R., Akhter, D., Liu, L., Li, D. (2023). Ultrasound-assisted extraction (UAE) of antioxidant phenolics from *Corchorus olitorius* leaves: a response surface optimization. *Chemical and Biological Technologies in Agriculture*, *10*(1), art. no. 64. https://doi.org/10.1186/s40538-023-00443-2
9. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

10. 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**
11. Chiorcea-Paquim, A.-M., Enache, T.A., De Souza Gil, E., Oliveira-Brett, A.M. (2020). Natural phenolic antioxidants electrochemistry: Towards a new food science methodology. *Comprehensive Reviews in Food Science and Food Safety*, *19*(4), 1680–1726. **https://doi.org/10.1111/1541-4337.12566**
12. Christodouleas, D.C., Fotakis, C., Nikokavoura, A., Papadopoulos, K., Calokerinos, A.C. (2014). Modified DPPH and ABTS assays to assess the antioxidant profile of untreated oils. *Food Analytical Methods*, *8*(5), 1294–1302. **https://doi.org/10.1007/s12161-014-0005-6**
13. Delgado, A., Gonçalves, S., Romano, A. (2023). Mediterranean diet: The role of phenolic compounds from aromatic plant foods. *Foods*, *12*(4), art. no. 840. **https://doi.org/10.3390/foods12040840**
14. Deshmukh, V., Gutte, R.K. (2024). Sustainable Health and Functional Foods. In W. Leal Filho, T. Dibbern, S.R. de Maya, M.-C. Alarcón-del-Amo, L.M. Rives (Eds.), *The Contribution of Universities Towards Education for Sustainable Development* (1st ed.), Springer Cham, pp. 439–451. **https://doi.org/10.1007/978-3-031-49853-4_25**
15. Fraga-Corral, M., Otero, P., Echave, J., Garcia-Oliveira, P., Carpena, M., Jarboui, A., Nuñez-Estevez, B., Simal-Gandara, J., Prieto, M.A. (2021). By-products of agri-food industry as tannin-rich sources: A review of tannins' biological activities and their potential for valorization. *Foods*, *10*(1), art. no. 137. **https://doi.org/10.3390/foods10010137**
16. Gabbia, D. (2024). Beneficial effects of tyrosol and oleocanthal from extra virgin olive oil on liver health: Insights into their mechanisms of action. *Biology*, *13*(10), art. no. 760. **https://doi.org/10.3390/biology13100760**
17. Garofalo, G., Taspinar, T., Busetta, G., Mastrangelo, S., Portolano, B., Sardina, M.T., Gaglio, R., Erten, H., Settanni, L. (2024). Description of Ewiss cheese, a new ewe milk cheese processed by Swiss cheese manufacturing techniques: Microbiological, physicochemical, and sensory aspects. *Journal of Dairy Science*, *107*(9), 6614–6628. **https://doi.org/10.3168/jds.2024-24711**
18. Habachi, E., Rebey, I.B., Dakhlaoui, S., Hammami, M., Sawsen, S., Msaada, K., Merah, O., Bourgou, S. (2022). *Arbutus unedo*: Innovative source of antioxidant, anti-inflammatory and anti-tyrosinase phenolics for novel cosmeceuticals. *Cosmetics*, *9*(6), art. no. 143. **https://doi.org/10.3390/cosmetics9060143**
19. Habtemariam, S. (2023). Anti-inflammatory therapeutic mechanisms of natural products: insight from rosemary diterpenes, carnosic acid and carnosol. *Biomedicines*, *11*(2), art. no. 545. **https://doi.org/10.3390/biomedicines11020545**
20. Haddada, F.M., Manai, H., Daoud, D., Fernandez, X., Lizzani-Cuvelier, L., Zarrouk, M. (2007). Profiles of volatile compounds from some monovarietal Tunisian virgin olive oils. Comparison with French PDO. *Food Chemistry*, *103*(2), 467–476. **https://doi.org/10.1016/j.foodchem.2006.08.023**
21. Hamama, A. (1989). Bacteriological quality of Moroccan fresh cheeses. In J.-L. Tisserand (Ed.), *Le lait dans la région méditerranéenne*, CIHEAM, Paris, France, pp. 223–227, (in French, English abstract).
22. Hayaloglu, A.A. (2016). Cheese: Microbiology of cheese. In *Reference Module in Food Science*. Elsevier Inc., Amsterdam, Netherlands, p. 9. **https://doi.org/10.1016/B978-0-08-100596-5.00675-2**
23. Inarejos-Garcia, A.M., Androulaki, A., Salvador, M.D., Fregapane, G., Tsimidou, M.Z. (2009). Discussion on the objective evaluation of virgin olive oil bitterness. *Food Research International*, *42*(2), 279–284. **https://doi.org/10.1016/j.foodres.2008.11.009**
24. IOOC (2021). *Trade standard applying to olive oils and olive-pomace oils.* No. COI/T.15/NC No. 3; Version 17. International Olive Council, Madrid, Spain.
25. IOOC (2019). *Trade standard applying to olive oils and olive pomace oils*. No. COI/T.15/NC No 3/Rev. 14, November 2019. International Olive Council, Madrid, Spain.
26. IOOC (2024). *Standards and methods – Chemistry and standardisation unit*. International Olive Council, Madrid, Spain.
27. ISO (2016). ISO Standard No. 6886:2016: *Animal and vegetable fats and oils — Determination of oxidative stability (accelerated oxidation test)*, International Organization for Standardization.
28. Ioannis, M., Alexandros, S., Sotirios, E., Stamatia, C., Erminta, T. (2024). Chapter 5 – Antioxidants from aromatic herbs in food preservation. In M. Pateiro (Ed.), *Natural Antioxidants to Enhance the Shelf-Life of Food*, Academic Press, pp. 103–146. **https://doi.org/10.1016/B978-0-443-15386-0.00005-9**
29. Jebabli, H., Nsir, H., Taamalli, A., Abu-Reidah, I., Álvarez-Martínez, F.J., Losada-Echeberria, M., Barrajón Catalán, E., Mhamdi, R. (2020). Industrial-scale study of the chemical composition of olive oil process-derived matrices. *Processes*, *8*(6), art. no. 701. **https://doi.org/10.3390/pr8060701**
30. Jukić Špika, M., Liber, Z., Montemurro, C., Miazzi, M.M., Ljubenkov, I., Soldo, B., Žanetić, M., Vitanović, E., Politeo, O., Škevin, D. (2022). Quantitatively unraveling hierarchy of factors impacting virgin olive oil phenolic profile and oxidative stability. *Antioxidants*, *11*(3), art. no. 594. **https://doi.org/10.3390/antiox11030594**
31. Kamelnia, E., Mohebbati, R., Kamelnia, R., El-Seedi, H.R., Boskabady, M.H. (2023). Anti-inflammatory, immunomodulatory and anti-oxidant effects of *Ocimum basilicum* L. and its main constituents: A review. *Iranian Journal of Basic Medical Sciences*, *26*(6), 617-627. **https://doi.org/10.22038/IJBMS.2023.67466.14783**
32. Karacabey, E., Özkan, G., Dalgıç, L., Sermet, S.O. (2016). Rosemary aromatization of extra virgin olive oil and process optimization including antioxidant potential and yield. *Turkish Journal of Agriculture - Food Science and Technology*, *4*(8), 628–635. **https://doi.org/10.24925/turjaf.v4i8.628-635.724**
33. Klisović, D., Koprivnjak, O., Novoselić, A., Pleadin, J., Lešić, T., Brkić Bubola, K. (2022). Compositional changes in the extra virgin olive oil used as a medium for cheese preservation. *Foods*, *11*(15), art. no. 2329. **https://doi.org/10.3390/foods11152329**
34. Liu, X.-Y., Wang, W.-Z., Yao, S.-P., Li, X.-Y., Han, R.-M., Zhang, D., Zhao, Z., Wang, Y., Zhang, J.-P. (2024). Antioxidation activity enhancement by intramolecular hydrogen bond and non-browning mechanism of active ingredients in rosemary: Carnosic acid and carnosol. *The Journal of Physical Chemistry B*, *128*(31), 7627–7638. **https://doi.org/10.1021/acs.jpcb.4c02949**
35. Mączka, W., Twardawska, M., Grabarczyk, M., Wińska, K. (2023). Carvacrol – A natural phenolic compound with antimicrobial properties. *Antibiotics*, *12*(5), art. no. 824. **https://doi.org/10.3390/antibiotics12050824**
36. Manai-Djebali, H., Yeddes, W., Hammami, M., Nait-Mohamed, S., Habachi, E., Msaada, K., Ben Youssef, N. (2024). Exploring the synergistic potential of wild nettle and olive oil: bioactive compounds, antioxidant capacity, and antibacterial properties. *International Journal of Environmental Health Research*, *34*(8), 3046–3055. **https://doi.org/10.1080/09603123.2023.2287589**
37. Marcelino, G., Hiane, P.A., Freitas, K. de C., Santana, L.F., Pott, A., Donadon, J.R., Guimarães, R. de C.A. (2019). Effects of olive oil and its minor components on cardiovascular diseases, inflammation, and gut microbiota. *Nutrients*, *11*(8), art. no. 1826. **https://doi.org/10.3390/nu11081826**
38. Mason, T.L., Wasserman, B.P. (1987). Inactivation of red beet β-glucan synthase by native and oxidized phenolic compounds. *Phytochemistry*, *26*(8), 2197–2202. **https://doi.org/10.1016/S0031-9422(00)84683-X**
39. Mateus, A.R.S., Serrano, C., Almeida, C., Soares, A., Rolim Lopes, V., Sanches-Silva, A. (2024). Beyond thymol and carvacrol: characterizing the phenolic profiles and antioxidant capacity of Portuguese oregano and thyme for food applications. *Applied Sciences*, *14*(19), art. no. 8924. **https://doi.org/10.3390/app14198924**
40. Miller, G.L. (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426–428. **https://doi.org/10.1021/ac60147a030**
41. Navarro, M. del C., Gálvez, I., Hinchado, M.D., Otero, E., Torres-Piles, S., Francisco-Morcillo, J., de La Fuente, M., Martín-Cordero, L., Ortega, E. (2024). Immunoneuroendocrine, stress, metabolic, and behavioural responses in high-fat diet-induced obesity. *Nutrients*, *16*(14), art. no. 2209. **https://doi.org/10.3390/nu16142209**
42. Nieto, G. (2020). A review on applications and uses of *Thymus* in the food industry. *Plants*, *9*(8), art. no. 961. **https://doi.org/10.3390/plants9080961**
43. Özcan, M., AL Juhaimi, F., Uslu, N., Ghafoor, K., Babiker, E., Mohamed Ahmed, I. (2022). Use of herbal essential oil and extracts as antioxidant sources in quality stabilization of extra virgin olive oil stored in different time and packages. *Journal of Food Measurement and Characterization*, *16*, 700–713. **https://doi.org/10.1007/s11694-021-01195-z**
44. Popescu, L., Cojocari, D., Lung, I., Kacso, I., Ciorîță, A., Ghendov-Mosanu, A., Balan, G., Pintea, A., Sturza, R. (2023). Effect of microencapsulated basil extract on cream cheese quality and stability. *Molecules*, *28*(8), art. no. 3305. **https://doi.org/10.3390/molecules28083305**
45. Rhiat, M., Labioui, H., Driouich, A., Aouane, M., Chbab, Y., Driouich, A., Mennane, Z., Ouhssine, M. (2011). Comparative study of bacteriological home costs Moroccans marketed (Mahlabats) and the cheeses manufactured in the laboratory. *Afrique Science: Revue Internationale Des Sciences et Technologie*, *7*(3), 108-112 (in French, English abstract).
46. Tran, C.H., Nghiem, M.T., Dinh, A.M.T., Dang, T.T.N., Van Do, T.T., Chu, T.N., Mai, T.H., Phan, V.M. (2023). Optimization conditions of ultrasound-assisted extraction for phenolic compounds and antioxidant activity from *Rubus alceifolius* Poir leaves. *International Journal of Food Science*, *2023*(1), art. no. 7576179. **https://doi.org/10.1155/2023/7576179**

47. Tsimihodimos, V., Psoma, O. (2024). Extra virgin olive oil and metabolic diseases. *International Journal of Molecular Sciences*, *25*(15), art. no. 8117. **https://doi.org/10.3390/ijms25158117**
48. Yeddes, W., Chalghoum, A., Aidi-Wannes, W., Ksouri, R.,Saidani Tounsi, M. (2019). Effect of bioclimatic area and season on phenolics and antioxidant activities of rosemary (*Rosmarinus officinalis* L.) leaves. *Journal of Essential Oil Research*, *31*(5), 432–443. **https://doi.org/10.1080/10412905.2019.1577305**
49. Yfanti, P., Lazaridou, P., Boti, V., Douma, D., Lekka, M.E. (2024). Enrichment of olive oils with natural bioactive compounds from aromatic and medicinal herbs: Phytochemical analysis and antioxidant potential. *Molecules*, *29*(5), art. no. 1141. **https://doi.org/10.3390/molecules29051141**
50. Zhakipbekov, K., Turgumbayeva, A., Akhelova, S., Bekmuratova, K., Blinova, O., Utegenova, G., Shertaeva, K., Sadykov, N., Tastambek, K., Saginbazarova, A., Urazgaliyev, K., Tulegenova, G., Zhalimova, Z., Karasova, Z. (2024). Antimicrobial and other pharmacological properties of *Ocimum basilicum*, Lamiaceae. *Molecules*, *29*(2), art. no. 388. **https://doi.org/10.3390/molecules29020388**
51. Zhang, N., Li, Y., Wen, S., Sun, Y., Chen, J., Gao, Y., Sagymbek, A., Yu, X. (2021). Analytical methods for determining the peroxide value of edible oils: A mini-review. *Food Chemistry*, 358, art. no. 129834. **https://doi.org/10.1016/j.foodchem.2021.129834**