Functional Enrichment of Algerian Olive Oils by Black Fig Maceration: Effects on Oxidative Stability, Bioactive Compounds, and Antibacterial Activity

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Received :11/01/2026 | **Accepted:** 27/05/2026 | **Published:** 01/09/2026

Abstract

The present study evaluates the impact of black fig (Ficus carica L.) maceration on the functional quality of Algerian virgin olive oils (cultivars Chemlal and Sigoise) during 45 days of storage. Physicochemical indices, total phenols, flavonoids, chlorophylls, carotenoids, DPPH, and ABTS.+ radical-scavenging activities (including IC50 values), and antibacterial activity against Gram-positive and Gram-negative bacteria were assessed. Results show that storage increased free acidity, peroxide value, and UV extinction coefficients (K232and K270) while reducing phenolic content and antioxidant activity. Black fig maceration significantly mitigated these degradations by enriching the oils with phenols, flavonoids, and pigments, thereby enhancing antioxidant capacity. Filtration stabilized the enriched oils, though a marginal decrease in bioactive levels was observed. The two cultivars exhibited distinct enrichment profiles: Chemlal maintained higher DPPH activity, whereas Sigoise achieved greater phenolic gains. Antibacterial activity was most pronounced against Gram-positive strains and displayed a clear concentration-dependent effect. Multivariate analyses (PCA and HCA) confirmed the integrated effect of maceration and filtration on functional enrichment. This study demonstrates that black fig maceration is a natural and efficient strategy to synergistically enhance the antioxidant and antibacterial properties of olive oils, offering practical applications for functional food development.

Keywords: Virgin olive oil, Ficus carica L. maceration, phenolic compounds, Antioxidant capacity, Antibacterial activity, Chemlal, Sigoise.

1. Introduction

In contemporary food science and ethnopharmacology, the growing interest in functional foods has spurred extensive research into natural strategies to enhance the nutritional and biological properties of edible oils [1]. The Mediterranean diet is widely recognized as a premier nutritional model for promoting human health, largely due to the unique biochemical profiles and synergistic benefits of its staple components [2]. Central to this dietary pattern are the olive tree (Olea europaea L.) and the fig tree (Ficus carica L.) [3]. Virgin olive oil, which serves as the principal lipid source in this region, is valued for its high content of monounsaturated fatty acids and bioactive minor components that provide important antioxidant and anti-inflammatory properties [4].However, virgin olive oil is highly susceptible to oxidative deterioration during storage [5]. This autoxidation process causes a progressive decline in physicochemical quality criteria, including free acidity and peroxide values, which leads to a loss of bioactive phenolics and compromises the health benefits of the oil [6]. Traditional methods to minimize this degradation often rely on adding synthetic antioxidants, but these approaches can alter the sensory profile and reduce the natural authenticity of the product [7]. For this reason, fruit-based maceration has emerged as a practical alternative to promote the solid-liquid mass transfer of phenolic compounds, flavonoids, and natural pigments from plant matrices into the oil without chemical additives [8].Among the potential fruit matrices, the common fig (Ficus carica L.) represents an exceptional dietary source rich in polyphenols, such as gallic and chlorogenic acids, which exhibit strong radical-scavenging protection [9]. In addition to their nutritional value, fruit macerates have a long history as traditional functional foods, especially within North

African and Algerian heritage [10]. Although several investigations have focused on enriching olive oil using various herbs and spices [11], scientific literature regarding the physicochemical interactions and biological kinetics of incorporating dried fruits like Ficus carica into a virgin olive oil matrix remains scarce.

Foundational regional studies have shown that the traditional maceration of dried figs in Algerian olive oils can effectively mitigate quality degradation [12]. This conventional combination creates a protective effect, strengthening both the antimicrobial and radical-scavenging properties compared to the individual food matrices [13]. Recent kinetic evaluations have also highlighted the efficient mass transfer of bioactive compounds during this process [14], suggesting new applications in functional food design and health management [15]. Despite these observations, there is still a lack of systematic studies evaluating the combined effect of black fig maceration on the antioxidant and antibacterial capacities of distinct olive cultivars under controlled storage conditions. Important questions remain regarding how maceration and filtration influence the long-term preservation of bioactive compounds, whether these effects are consistent across cultivars with different initial chemical baselines, and if this strategy can enhance bioactivity while respecting the quality thresholds set by the International Olive Council [16].

To address these gaps, the present study investigates the impact of black fig maceration on the physicochemical, antioxidant, and antibacterial profiles of two prominent Algerian olive cultivars (Olea europaea L. cv. Chemlal and Sigoise) after 45 days of storage. Specifically, this research aims to: (i) evaluate the protective and enriching effects of black fig maceration on oil oxidative stability and bioactive profiles; (ii) compare cultivar-specific responses between Chemlal and Sigoise oils; (iii) explore the biochemical and technological changes associated with subsequent filtration. By applying multivariate statistical analyses, including Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), this work establishes a clear framework to evaluate the feasibility of natural, fruit-based functional enrichment, connecting traditional Algerian knowledge with modern functional food development.

2. MATERIAL AND METHODS

2.1 Plant Materials and Sampling

Dried black figs (Ficus carica L., local variety Aberkane) were collected from the Charef region. Concurrently, virgin olive oil (VOO) samples from two distinct cultivars, Sigoise and Chemlal, were obtained from the Mteiriha farms located in the El Idrissia region. Both sampling locations are situated within the Djelfa province in Algeria. The olives were harvested during the 2022/2023 agricultural season, and the corresponding oils were extracted using a mechanical cold-pressing system. To protect the freshly extracted oils from autoxidation, they were immediately transferred into clean, dry, amber glass bottles with a minimum volume of 250 mL and maintained under refrigeration at 4 °C until laboratory use.

2.2 Preparation and Impregnation Process

The maceration of dried figs in virgin olive oil was performed in November 2022 following a traditional preparation method. The dried figs were sliced into small pieces, and a specific ratio of 50 g of fruit to 120 mL of oil was established. The mixtures were placed in 250 mL hermetically sealed glass jars, ensuring the fig pieces were fully immersed in the liquid phase. These jars were subsequently maintained under standard laboratory lighting conditions at a controlled room temperature of approximately 25 °C. Sampling for subsequent extraction and physicochemical analysis was conducted after a storage period of 45 days. Consequently, eight distinct oil matrices were investigated:

- SO0: Control virgin olive oil of the Sigoise cultivar at Day 0.
- SO45: Stored control Sigoise olive oil at Day 45.
- BF-SO45: Sigoise olive oil macerated with black figs before filtration at Day 45.
- Af-SO45: Sigoise olive oil recovered after black fig filtration at Day 45.
- CO0: Control virgin olive oil of the Chemlal cultivar at Day 0.
- CO45: Stored control Chemlal olive oil at Day 45.
- BF-CO45: Chemlal olive oil macerated with black figs before filtration at Day 45.
- Af-CO45: Chemlal olive oil recovered after black fig filtration at Day 45."

3. Physicochemical and Antioxidant Characterization of Olive Oil Samples

3.1. Quality Indices and Pigment Determinations

The physicochemical quality parameters of both control and macerated virgin olive oil samples were evaluated using standard official methods. Acidity and peroxide values were determined following the analytical methods described by the International Union of Pure and Applied Chemistry [17], with the final results expressed as a percentage of oleic acid and milliequivalents of active oxygen per kilogram of oil (meq O2 /kg), respectively. To assist in the physical characterization of the lipid phase, the density was established as the ratio of the mass of a specific volume of oil at 20°C to the mass of an equal volume of distilled water at the same temperature, adhering to the standard criteria outlined within the same analytical framework [17].

Ultraviolet spectrophotometric investigation was conducted to monitor primary and secondary oxidation products. Specifically, the specific extinction coefficients K232 and K270 were determined according to the analytical methods described by the

International Olive Oil Council [18], by measuring the absorbances of a 1% oil solution in cyclohexane at 232 nm and 270 nm. For the pigment profile, total carotenoid and chlorophyll contents in the oil matrices were quantified spectrophotometrically by measuring the ambient absorbance of the lipid phase at 470 nm and 670 nm using cyclohexane as a blank, following the color-pigment correlation protocol described by Isabel Minguez-Mosquera et al. [19].The calculated pigment concentrations were expressed in milligrams per kilogram of oil (mg/kg).

3.2. Liquid–Liquid Extraction of Phenolic Compounds

To isolate the polar phenolic fraction, a liquid–liquid extraction procedure was performed according to the foundational methodology described by Tsimidou et al. [20], with minor modifications. Briefly, a 50 g aliquot of virgin olive oil was dissolved in 50 mL of hexane. The resulting solution was extracted successively three times with 30 mL of a methanol/water mixture (80:20, v/v). The collected methanolic phases were pooled and washed twice with 50 mL of hexane to ensure the complete removal of any residual non-polar lipids. After discarding the upper hexane phase, the methanolic extracts were concentrated to dryness using a rotary evaporator under vacuum at 40 °C. Finally, the obtained dry residue was reconstituted in a specific volume of the methanol/water (80:20, v/v) solvent mixture to serve as the stock extract for subsequent quantitative evaluations.

3.3. Determination of Total Phenolic Content

The total phenolic content of the obtained extracts was quantified using the Folin-Ciocalteu spectrophotometric method. In brief, the colorimetric reaction was monitored at an absorbance of 765 nm, and the final concentrations were calculated using a calibration curve prepared with gallic acid as the standard reference [21].

3.3. Evaluation of Antioxidant Activity

3.3.1. DPPH radical- Scavenging assay

The radical-scavenging capacity of the obtained polar extracts against the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical was evaluated according to the procedure described by Keceli and Gordon [22]. Briefly, a 0.1 mL aliquot of the reconstituted methanolic extract was mixed with 2.9 mL of a freshly prepared methanolic DPPH solution (6.10–5 M). The reaction mixture was shaken vigorously and incubated in the dark at room temperature for 30 minutes. Subsequently, the ambient absorbance was recorded spectrophotometrically at 515 nm against a methanol blank. The DPPH radical-scavenging activity was calculated using the following formula: DPPH(%) = [A0-(A1–AS)]/ A0]100 where A0 is the absorbance of DPPH alone, A1 is the absorbance of DPPH+ extract and AS is the absorbance of the extract alone.

3.3.2. Scavenging activity against the ABTS. + radical cation

The total antioxidant activity of extracts was measured by the ABTS.+ (2,2′-azinobis-(3 ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay of Re et al [23] , with minor modifications. ABTS.+ was generated by the oxidation of ABTS with potassium persulfate. Prior to assay, the ABTS.+ stock solution was diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. Then 990 µL of a diluted ABTS.+ solution was mixed with 10 µL of the test sample, and the absorbance was measured at 734 nm after 30 min. The inhibition percentage of absorbance was calculated using the formula specified in the DPPH method. The antioxidant capacity of test compounds was expressed as IC50, the concentration necessary for 50% reduction of ABTS.+

3.4. Evaluation of Biological Antibacterial Activity

3.4.1. Target Bacterial Strains and Inoculum Preparation

The antibacterial efficacy of the investigated olive oil formulations (SO0, SO45, BF-SO45, Af-SO45, CO0, CO45, BF-CO45, and Af-CO45) was screened against a specific panel of pathogenic and indicator micro-organisms. The bacterial consortium consisted of three Gram-positive strains: Micrococcus luteus, Staphylococcus aureus, and Bacillus subtilis, alongside two Gram-negative strains: Escherichia coli and Salmonella gallinarum.

All strains were maintained and rejuvenated at 37°C using appropriate sterile nutrient broth and nutrient agar media. Before the initiation of the bioassays, isolated colonies from solid stock media were transferred into fresh liquid broth tubes and incubated for 18 hours to guarantee that the microbial cells reached their optimal logarithmic growth phase.

3.4.2. Agar Disk Diffusion Screening

The primary antibacterial screening and zone of inhibition mapping were performed using the agar disk diffusion protocol according to Kappel et al. [24], with slight modifications. The microbial cell density of each overnight culture was adjusted in sterile physiological saline to standard turbidimetric conditions containing approximately 108 CFU.mL-1. A 0.1 mL volume of this adjusted suspension was spread uniformly under aseptic conditions onto sterile Mueller–Hinton agar plates utilizing a sterile swab.

Sterile filter paper disks (6 mm in diameter) were precisely impregnated with 20 µL of the respective extract solutions to yield a decreasing concentration gradient of 1.0, 0.5, 0.25, and 0.125 mg.disc-1. Control disks consisting of the reconstitution solvent (methanol/water, 80:20, v/v) were included as negative controls. After incubating the plates at 37 °C for 18–24 hours, the resulting antibacterial potential was determined by measuring the net diameters of the circular inhibition zones (excluding the

6 mm physical disk diameter) and recorded in millimeters (mm).

3.5. Statistical Analysis

All experimental measurements and bioassays were performed in triplicate (n = 3), and the results were expressed as mean ± standard deviation (SD). Quantitative data were analyzed using one-way analysis of variance (ANOVA) to determine statistically significant differences among the investigated olive oil formulations and the untreated control. When significant differences were detected, mean comparisons were performed using the Newman–Keuls post hoc test. Statistical significance was set at $p < 0.05$.

Furthermore, a Pearson correlation matrix was established to evaluate the linear relationships between bioactive compounds and antioxidant activities. Multiple linear regression analysis was performed to assess the combined contribution of these compounds to antioxidant activity, while multicollinearity among predictors was examined using the variance inflation factor (VIF). In addition, multivariate analyses, including Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and heatmap visualization, were carried out on standardized data to explore sample distribution patterns and variable contributions. All statistical analyses and multivariate modeling were conducted using STATISTICA software, version 5.5.

4.Results

4.1. Physicochemical quality and oxidative status

In both olive oils, the refractive index remained highly stable and fell within the range established by the International Olive Council (IOC, 2019), indicating that the overall lipid structure of the oils was not significantly altered by storage or by maceration with black fig.

Free acidity increased markedly after 45 days of storage. In the Chemlal oil, it rose from 0.38% to 1.20%, and then reached 1.41% before filtration. In the Sigoise oil, it increased from 0.45% to 1.15%, and then to 1.29% before filtration. This increase reflects the partial hydrolysis of triacylglycerols and the release of free fatty acids. Nevertheless, all values remained below the 2% limit, indicating that the oils remained compliant from an acidity standpoint. Filtration partially reduced acidity, especially in the Sigoise oil, suggesting the removal of the aqueous fraction or polar compounds originating from the fig. This trend is visible in panels A and B of Figure 1.

The peroxide value, a marker of primary oxidation, increased significantly during storage. The stored control reached 18.20 and 16.50 in the Chemlal and Sigoise oils, respectively. After maceration with the black fig, the values decreased relative to the stored control, reaching 15.01 and 12.44 before filtration. This indicates that the black fig exerted a partial protective effect against primary oxidation, probably through the contribution of phenolic and antioxidant compounds. All values remained below the legal international limit of 20 meq O2/kg.

The K232 and K270 coefficients increased after storage, reflecting the formation of conjugated dienes and secondary oxidation products. The stored controls displayed the highest values, particularly for K270, which reached 0.290 and 0.285 in the Chemlal and Sigoise oils, respectively. After maceration, K270 decreased to values close to or below the International Olive Council (IOC) limit, confirming a reduction of the secondary oxidation products in the fig-enriched oils.

Moisture increased before filtration, particularly in the Sigoise oil, where it reached 0.194%, probably as a consequence of water transfer from the black fig to the oil phase. After filtration, moisture dropped sharply to 0.063% and 0.042% in the Chemlal and Sigoise oils, respectively. Filtration therefore appears to be a crucial step for stabilizing the oil after maceration.
4.2. Phenolic compounds, flavonoids and pigments Storage alone reduced the level of bioactive compounds. Total phenols decreased from 180.50 to 148.10 mg /kg in the Chemlal oil, and from 165.20 to 135.20 mg /kg in the Sigoise oil. This decrease is consistent with the progressive oxidation of phenolic compounds during storage.

Maceration with the black fig significantly increased the total phenol content, which reached 186.34 and 199.14 mg /kg in the Chemlal and Sigoise oils, respectively, before filtration. This demonstrates the transfer of antioxidant compounds from the fig into the oil phase. After filtration, a portion of these compounds decreased, but the values remained higher than those of the stored controls, especially in the Sigoise oil, which retained 184.53 mg /kg. Panels C and D of Figure 1 show the corresponding evolution of these bioactive compounds, while panel I confirms the inter-varietal comparison of total phenols.
Total flavonoids followed a similar pattern: a decrease after storage, an enrichment after maceration, and a partial reduction after filtration. This suggests that certain flavonoids may be associated with the particles or the fractions removed during the filtration process.

Chlorophylls and carotenoids increased markedly before filtration. In the Chemlal oil, chlorophylls increased from 5.30 to 10.45 mg/kg after maceration, while in the Sigoise oil, they increased from 4.10 to 9.75 mg/kg. Carotenoids also showed a substantial increase following the same trend. These pigments may contribute to the color, nutritional value, and antioxidant activity of the enriched oils.

4.3. Antioxidant activity

The results of DPPH radical-scavenging activities showed significant differences after storage. The Chemlal oil exhibited a decrease in DPPH inhibition from 75.10% to 55.35%, while the Sigoise oil recorded a reduction from 71.60% to 52.50%. A similar trend was observed for the ABTS.+ radical cation scavenging activity, confirming that storage induced a significant loss of radical-scavenging capacity in both olive oil varieties.

Following maceration with the black fig, the antioxidant activity increased strongly. The Chemlal oil reached 82.50% and 78.90% for DPPH inhibition and ABTS.+ radical cation scavenging activity, respectively, before filtration, while the Sigoise oil reached 76.50% and 73.20% for the corresponding parameters. This confirms that the enrichment in phenolic compounds, flavonoids, and pigments improved the radical-scavenging capacity of the oils. These trends are summarized in panels E and F of Figure 1, whereas the inter-varietal comparison of DPPH activity is presented in panel J.

The results of the ABTS.+ assay expressed as IC50 confirmed this pattern, representing the concentration needed to decrease the initial radical concentration by 50%. As a smaller IC50 value corresponds to a higher antioxidant activity, storage led to an increase in IC50 values, therefore reducing the antioxidant efficiency. Conversely, maceration strongly reduced the IC50. parameters, which reached 0.255 and 0.285 mg.mL-1 in the Chemlal and Sigoise oils, respectively, before filtration. After filtration, the values remained better than those of the stored controls, as shown in panels G and H of Figure 1.

4.4. Inter-varietal comparison

The response to maceration was not identical between the two cultivars. The Chemlal oil exhibited higher initial total phenols and DPPH radical-scavenging activity, indicating a significant functional richness at baseline. However, the Sigoise oil reached the highest phenolic content after maceration and retained a high value after filtration, suggesting a greater enrichment capacity for phenolic compounds.

Conversely, the DPPH inhibition activity remained higher in the Chemlal oil under the main experimental conditions, particularly after maceration. This difference demonstrates that the total phenolic content alone does not fully explain the antioxidant activity; the chemical nature of the transferred compounds, their polarity, their bioavailability in the oil phase, and the contribution of pigments may also be involved.

4.5. Integrated presentation of the physicochemical and antioxidant figures

Figure 1 gathers all experimental curves and graphical comparisons on a single page. Specifically, panels A and B present the quality and oxidation indices, while panels C and D display the bioactive compounds and pigments. The antioxidant capacities are illustrated in panels E and F for DPPH and ABTS.+ radical cation scavenging activities, and in panels G and H for ABTS.+ IC50 values. Finally, panels I and J present the inter-varietal comparisons of total phenolic content and DPPH activity, respectively.

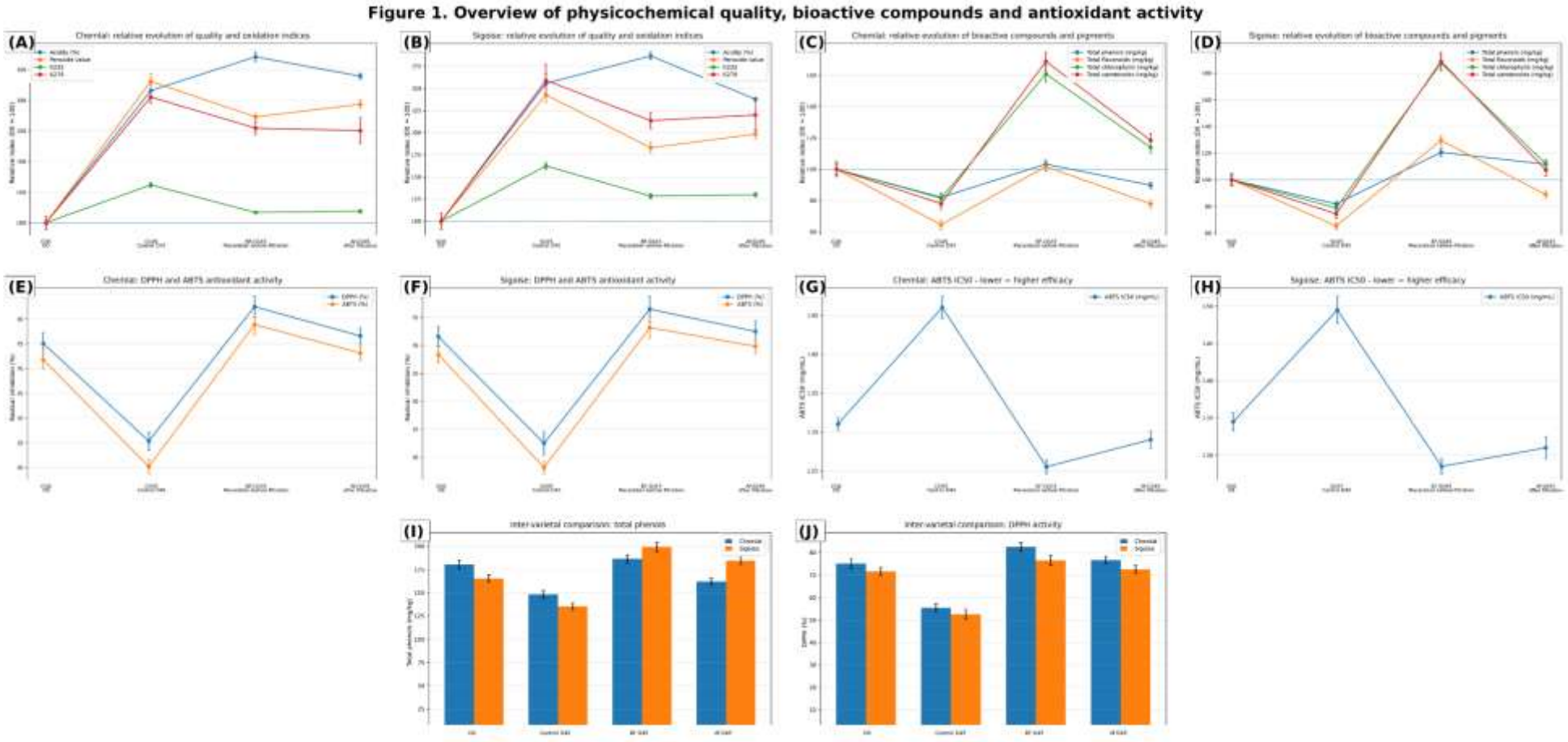

Figure 1. Overview of physicochemical quality, bioactive compounds and antioxidant activity in Chemlal and Sigoise oils after storage, black fig maceration and filtration. A: Chemlal quality and oxidation indices; B: Sigoise quality and oxidation indices; C-D: bioactive compounds and pigments in Chemlal and Sigoise; E-F: DPPH and ABTS antioxidant activity; G-H: ABTS IC50; I-J: inter-varietal comparison of total phenols and DPPH activity.

4.6. Data and statistical approach

The compared treatments evaluated in this study were J0 (initial control), the control stored for 45 days (J45), the macerated oil before filtration (BF-J45), and the macerated oil after filtration (AF-J45). The studied factors included the cultivar (Chemlal versus Sigoise), the treatment, and their corresponding interaction. Furthermore, complementary statistical analyses included targeted contrasts, Pearson/Spearman correlations, multiple regression, principal component analysis (PCA), and hierarchical clustering.

4.7. Integrated presentation of the statistical figures

Figure 2 gathers all statistical graphical outputs on a single page. Specifically, panel (a) presents the statistical importance of the two-way ANOVA factors and their interaction, while panel (b) shows the Pearson correlation matrix. Furthermore, panels (c) and (d) display the hierarchical clustering of samples and the global hierarchical heatmap profile, respectively. Regarding the principal component analysis (PCA), the contribution of variables to the separation is presented in panel (e), whereas the projection of individuals in the PC1–PC2 space is illustrated in panel (f).

4.8. Statistical results and interpretation

4.8.1. Descriptive statistics

Descriptive statistics included the mean, standard deviation, coefficient of variation, and the 95% confidence interval. Most parameters exhibited a low coefficient of variation, indicating a high homogeneity of the experimental replicates and satisfactory analytical stability. Therefore, the differences observed among the treatments were not primarily due to experimental variability, but rather reflected the biological and technological modifications associated with storage, maceration, and filtration.

For example, in the Chemlal oil, the total phenolic content decreased from 180.50 to 148.10 mg /kg after storage, whereas a higher value of 186.34 mg /kg was recorded in the macerated sample. The magnitude of this variation largely exceeded the experimental standard deviation, thereby suggesting a biologically marked and statistically significant treatment effect.

4.8.2. One-way ANOVA and post-hoc comparison

One-way ANOVA applied separately to each cultivar showed that most parameters differed significantly among the four experimental states. Specifically, acidity, peroxide value, total phenolic content, DPPH and ABTS.+ radical scavenging activities, alongside ABTS.+ IC50, showed very low p-values, thereby confirming that storage and maceration strongly modified the oil profiles.

Table 1. One-way ANOVA results for the effect of treatment on physicochemical, bioactive and antioxidant parameters in Chemlal and Sigoise oils.

Parameter	Chemlal F / p	Sigoise F / p	Interpretation
Acidity	876; 2.09e-10	486.6; 2.17e-09	Storage and maceration strongly modified acidity.
Peroxide value	474.6; 2.39e-09	269.2; 2.27e-08	Primary oxidation varied according to treatment.
Total phenols	47.0; 2.00e-05	127.4; 4.31e-07	Maceration with black fig increased phenolic content.
DPPH	111.6; 7.23e-07	85.7; 2.01e-06	Antioxidant activity varied significantly.
$ABTS^{\cdot+}$	153.7; 2.06e-07	172.6; 1.31e-07	ABTS confirmed the antioxidant gain.
$ABTS^{\cdot+}$ IC_{50},	212.3; 5.80e-08	134.0; 3.54e-07	A lower IC50 after maceration indicated higher antioxidant efficiency.

Tukey's HSD post-hoc testing clarified the structure of the significant differences revealed by the ANOVA. Stored controls generally exhibited the least favorable profiles for oxidation markers and antioxidant activity. By contrast, BF-J45 often corresponded to the best functional profile before filtration. Furthermore, AF-J45 retained a clear advantage over the stored control, although it sometimes showed slightly lower values than BF-J45, which reflects the partial removal of bioactive or particulate compounds during the filtration process.

4.8.3. Two-way ANOVA: cultivar effect, treatment effect and interaction

Two-way ANOVA revealed three sources of variation: the cultivar, the treatment, and their corresponding interaction (cultivar x treatment). As shown in Figure 2(a), the treatment effect was the dominant factor for most parameters, including acidity, peroxide value, K232, K270, moisture content, bioactive compounds, antioxidant activity, and ABTS. + IC50. This dominance confirms that the transition from J0 to the stored control, followed by BF-J45 and AF-J45, was the main driver of the observed modifications.

The cultivar x treatment interaction was significant for several parameters, notably acidity, peroxide value, K232, moisture

content, as well as total phenolic, flavonoid, and carotenoid contents. This indicates that the Chemlal and Sigoise oils did not respond in a strictly identical manner to storage and maceration processes. Specifically, the Sigoise cultivar appeared to benefit more from maceration in terms of phenolic enrichment, whereas the Chemlal oil maintained an overall higher DPPH radical scavenging activity.

4.8.4. Targeted contrasts: storage, maceration, filtration, and final benefit

Targeted contrasts were performed to quantify the effect of each technological step. Storage induced a clear degradation of the oil profiles: acidity increased by 215.8% in the Chemlal oil and by 155.6% in the Sigoise oil, while the peroxide value increased by 230.9% and 142.6%, respectively. Concurrently, total phenolic content decreased by approximately 18% in both cultivars, whereas DPPH radical scavenging activity decreased by more than 26%.

Maceration with the black fig partially reversed this degradative trend. Between the J45 control and the BF-J45 samples, the peroxide value decreased by 17.5% in the Chemlal oil and by 24.6% in the Sigoise oil. Concurrently, total phenolic content increased by 25.8% and 47.3%, respectively, while DPPH radical scavenging activity increased by 49.1% and 45.7%. Furthermore, ABTS.+ activity increased by 57.2% and 52.0%, whereas the ABTS.+ IC50values decreased by 44.6% and 42.4%. These variations confirm the protective and enriching effects of the black fig maceration.

Filtration strongly reduced the moisture content, especially in the Sigoise oil (-78.4%), and concurrently diminished the acidity. Although a partial loss of bioactive compounds and antioxidant activities was observed, the filtered oil remained globally more functional than the stored control. Consequently, the final benefit from the J45 control to the AF-J45 samples remained highly marked: total phenolic content increased by 9.4% in the Chemlal oil and by 36.5% in the Sigoise oil, while DPPH radical scavenging activity increased by about 38%, and ABTS activity by about 45%. In tandem, the ABTS.+ IC50 values decreased by approximately 37%, accompanied by a substantial decrease in the peroxide value.

4.8.5. Correlations between bioactive compounds and antioxidant activity

The Pearson correlation matrix presented in Figure 2(b) showed strong positive relationships between the bioactive compounds and antioxidant responses. Total phenolic content was strongly correlated with DPPH ($r = +0.85$) and ABTS.+ ($r = +0.85$) activities, while showing a strong negative correlation with the ABTS.+ IC50 values ($r = -0.87$). Flavonoids, chlorophylls, and carotenoids also showed positive correlations with both the DPPH and ABTS.+assqys.

These results indicate that the improvement in antioxidant activities after maceration was closely linked to the enrichment in phenolic compounds, flavonoids, and pigments. The negative correlation with the ABTS.+ IC50 values confirms the biological coherence of the model: as the concentration of antioxidant compounds increases, the concentration required to achieve 50% inhibition consequently decreases.

4.8.6. Multiple linear regression

Multiple linear regression models explained a significant proportion of the variance in antioxidant responses, with R2 values ranging from approximately 0.79 to 0.80 for the DPPH, ABTS.+ and ABTS.+ IC50 models. However, the global p-values were not statistically significant, primarily due to the limited sample size ($n = 8$). The multicollinearity observed between the chlorophylls and carotenoids, as reflected by a very high variance inflation factor (VIF), also limited the independent interpretation of each individual predictor.

These models should therefore be considered strictly exploratory. While they strongly support the idea that bioactive compounds contribute substantially to the antioxidant activity, they do not allow for a definitive ranking of the specific contribution of each molecular family without a larger dataset.

4.8.7. PCA: profile separation and variable contribution

Principal Component Analysis (PCA) efficiently summarized the global structure of the data. The first two principal components (PC1 and PC2) explained 83.2 % of the total variance, with 57.6% assigned to PC1 and 25.6% to PC2. As illustrated in Figure 2(e), the variables related to bioactive compounds and antioxidant activities contributed positively to PC1, whereas the lipid oxidation markers and ABTS.+ IC50 values clearly opposed this profile along the same axis.

The sample projection illustrated in Figure 2(f) clearly separated the stored controls from the macerated samples. Specifically, the BF-J45 samples were positioned on the side associated with total phenolic content, flavonoids, and DPPH and ABTS.+ radical scavenging activities, confirming their functional enrichment. Conversely, the J45 controls were strongly associated with the peroxide value, K232, K270, and ABTS.+ IC50 values, reflecting an oxidized and less efficient profile. Interestingly, the AF-J45 samples occupied an intermediate position, which is highly consistent with a compromise between antioxidant enrichment and stabilization induced by filtration.

4.8.8. Hierarchical clustering and global heatmap

The hierarchical clustering analysis presented in Figure 2(c) confirmed the proximity of the oil profiles according to their technological stages. The two stored controls were clustered together, indicating that storage produced a globally similar profile in both cultivars. Concurrently, the BF-J45 samples were grouped with the enriched profiles, while the AF-J45 samples remained closely aligned with the BF-J45 cluster, albeit with slightly reduced intensities for some bioactive compounds.

The hierarchical heatmap illustrated in Figure 2(d) completed this interpretation by showing the standardized values for all measured parameters. Specifically, the stored controls were characterized by high oxidation markers and high ABTS.+ IC50 values, whereas the BF-J45 samples displayed a distinctive profile rich in phenolic compounds, pigments, and antioxidant

activities. Interestingly, the AF-J45 samples retained a functional structure close to that of the BF-J45 cluster, while reflecting the corrective effect of filtration on reducing the moisture content and some polar fractions.

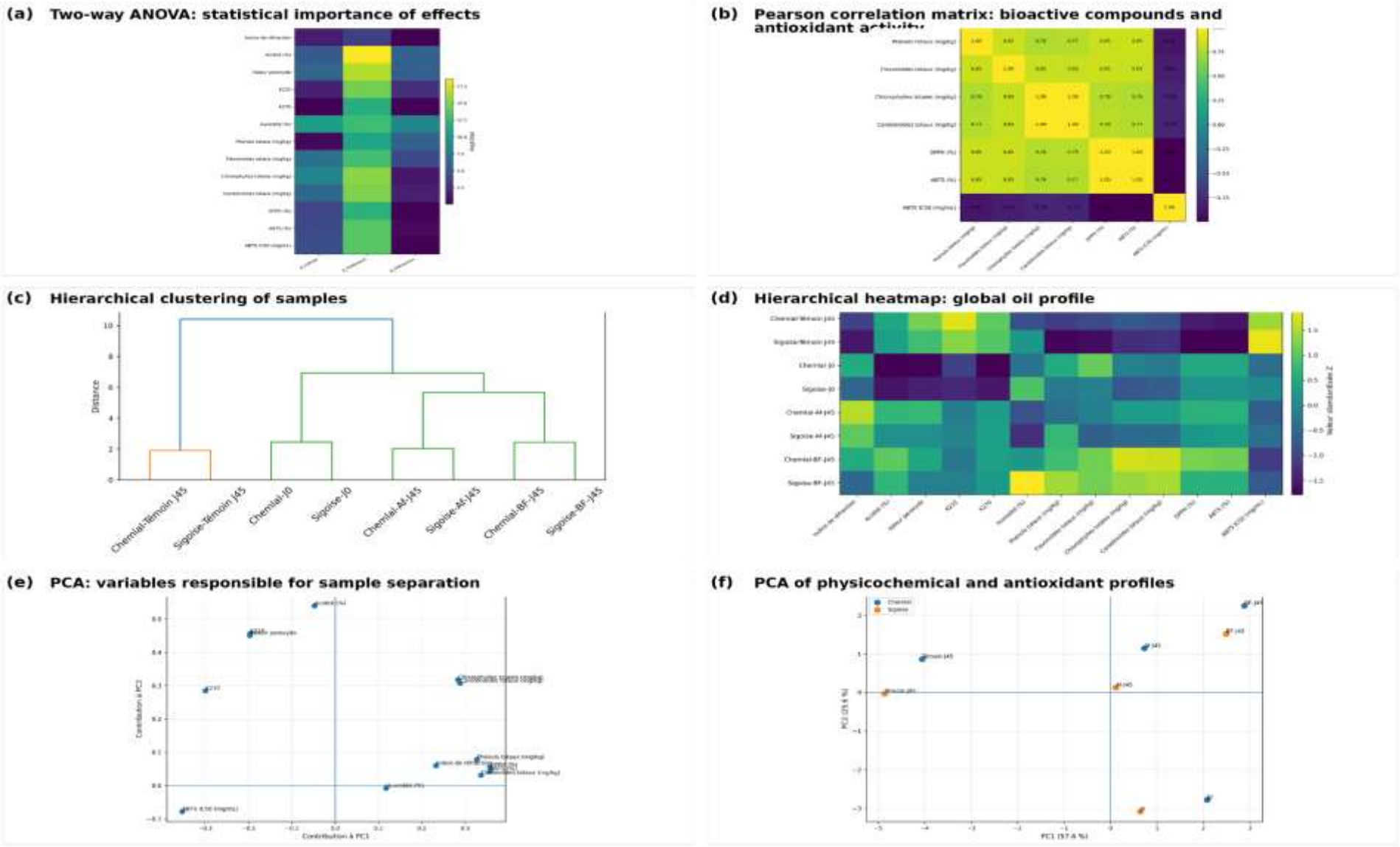

Figure 2. Integrated statistical analysis of physicochemical, bioactive and antioxidant profiles in Chemlal and Sigoise oils. (a) Two-way ANOVA effect importance; (b) Pearson correlation matrix of bioactive compounds and antioxidant activity; (c) hierarchical clustering of samples; (d) hierarchical heatmap of global oil profiles; (e) PCA variables responsible for sample separation; (f) PCA projection of physicochemical and antioxidant profiles.

4.9. Antibacterial activity

The different olive oil formulations (Sigoise and Chemlal cultivars) at varying continuous concentrations (1.0, 0.5, 0.25, and 0.125 mg.disc-1.) were screened for their antibacterial activity against M. luteus, S. aureus, and B. subtilis (Gram-positive), as well as E. coli and S. gallinarum (Gram-negative) following the agar disk diffusion method (Table 2).

Table 2 . Antibacterial activity (inhibition zone diameters, mm) of Chemlal and Sigoise olive oil formulations at different disc concentrations.

Varieties	Concentrations (mg·disc $^{-1}$)	*Micro-organism Inhibition zone diameter (mm·)*				
		Gram-positive(+)			*Gram-negative(-)*	
		M.luteus	*S. aureus*	*B. subtilis*	*E. coli*	*S.gallinarum*
SO_0	1	15,75 ± 0,44	14,20 ± 0,45	3,10 ± 0,30	---	01,95 ± 0,25
	0,5	13,50 ± 0,25	12,15 ± 0,35	1,80 ± 0,15	---	---
	0,25	11,10 ± 0,55	10,10 ± 0,50	---	---	---
	0,125	8,35 ± 0,20	----	----	---	---
SO_{45}	1	12,15 ± 0,38	11,10 ± 0,30	1,50 ± 0,20	---	----
	0,5	10,10 ± 0,50	9,30 ± 0,25	----	---	----
	0,25	7,20 ± 0,40	----	----	---	----
	0,125	---	----	----	---	----
BF-SO_{45}	1	17,40 ± 0,50	16,35 ± 1,97	4,95 ± 0,45	01,35 ± 1,61	04,85 ± 0,40
	0,5	15,25 ± 0,45	14,70 ± 0,25	3,40 ± 0,30	----	03,10 ± 0,20
	0,25	13,10 ± 0,30	12,40 ± 0,05	1,90 ± 0,10	----	01,45 ± 0,15
	0,125	10,50 ± 0,25	10,15 ± 0,18	----	----	----
Af-SO_{45}	1	15,90 ± 0,40	15,89 ± 1,01	4,10 ± 0,35	00,95 ± 0,10	03,90 ± 0,35
	0,5	14,14 ± 1,22	13,80 ± 0,35	2,80 ± 0,20	----	02,20 ± 0,15
	0,25	11,84 ± 1,67	11,60 ± 0,25	1,40 ± 0,15	----	----
	0,125	09,72 ± 1,22	09,20 ± 0,15	----	----	----
CO_0	1	16,10 ± 0,55	15,37 ± 1,88	4,20 ± 0,40	----	2,50±,30
	0,5	13,80 ± 0,40	13,15 ± 0,45	2,50 ± 0,30	----	1,10 ± 0,15
	0,25	11,45 ± 0,25	11,20 ± 0,30	1,20 ± 0,10	----	----
	0,125	09,15 ± 0,30	9,10 ± 0,15	----	----	----
CO_{45}	1	12,42 ± 0,09	12,30 ± 0,40	2,10 ± 0,25	----	----
	0,5	10,47 ± 0,48	10,15 ± 0,35	1,10 ± 0,15	----	----
	0,25	08,40 ± 0,20	8,20 ± 0,20	----	----	----
	0,125	----	----	----	----	----
BF-CO_{45}	1	18,50 ± 0,60	17,90 ± 1,44	5,40 ± 0,50	2,10 ± 0,20	05,15 ± 0,45
	0,5	16,40 ± 0,55	15,89 ± 1,01	3,90 ± 0,25	1,50 ± 0,15	03,40 ± 030
	0,25	14,15 ± 0,30	13,28 ± 0,46	2,10 ± 0,15	----	01,85 ± 0,20
	0,125	11,80 ± 0,45	11,15 ± 0,30	----	----	----
Af-CO_{45}	1	17,38 ± 1,97	17,10 ± 0,50	4,80 ± 0,35	1,50 ± 0,15	04,20 ± 0,35
	0,5	15,42 ± 0,37	15,20 ± 0,40	3,20 ± 0,20	----	02,65 ± 0,25
	0,25	13,05 ± 0,35	12,60 ± 0,60	1,50 ± 0,10	----	01,10 ± 0,15
	0,125	10,45 ± 0,25	10,25 ± 0,20	----	----	----

Means (mean±SD, n=3) with the same letter in the same column are not significantly different. Diameter of zone of inhibition (mm) not including diameter of 6 mm disc;. –: No inhibitory effects

4.9.1. Baseline Trends and Differential Phenotypic Susceptibility

The agar disc diffusion assays revealed a clear, concentration-dependent relationship across all investigated oil formulations. As the applied concentration on the disc decreased systematically from 1.0 to 0.125 mg·disc−1, the diameters of the inhibition zones contracted proportionally, establishing a typical dose-response profile. The consistently low standard deviations

(frequently ≤1.0 mm) across these measurements confirm the notable repeatability and analytical reliability of the screening framework.

A highly significant variance in phenotypic susceptibility was recorded between the two primary microbial groups. Gram-positive bacteria displayed pronounced vulnerability to the oil treatments; Micrococcus luteus emerged as the most sensitive strain, followed closely by Staphylococcus aureus and Bacillus subtilis.

In sharp contrast, the Gram-negative pathogens (Escherichia coli and Salmonella gallinarum) exhibited remarkable intrinsic resistance, characterized by minor or entirely absent inhibition halos under standard baseline conditions. This group-specific resistance is fundamentally dictated by the structural layout of the Gram-negative cell envelope. The presence of a lipid-rich outer lipopolysaccharide membrane works as an effective chemical barrier, severely restricting the transmembrane diffusion of hydrophobic or bulky amphiphilic phenolic compounds present in the lipid phase. This structural barrier effect is strongly supported by Bouafia et al. [25]

4.9.2. Influence of Storage Period and Fig Maceration

The technical and functional lifecycle of the oil matrices heavily influenced their final anti-infective performance. Over the 45-day storage period, spontaneous oxidative and thermal degradation significantly compromised the quality of the non-macerated control samples SO45 and CO45. Their inhibition zones underwent a marked downward shift compared to fresh baseline oils SO0 and CO0, showing specific activity losses that exceeded 30% to 40%. This sharp decline underscores the progressive loss of native secoiridoids and unstable antioxidant pigments during storage.

Significantly, enrichment via dried black fig maceration efficiently reversed this degenerative trend. The enriched formulations BF-SO45 and BF-CO45 consistently exhibited larger inhibition zones for all tested micro-organisms, outperforming the stored controls and occasionally surpassing the fresh oil baselines. The absolute quantitative peak was reached by the unfiltered macerated Chemlal formulation BF-CO45 at a dose of 1.0 mg.disc-1, which yielded maximum inhibition zones of 18.50 ± 0.60 mm against M. luteus and 17.90 ± 1.44 mm against S. aureus. Crucially, this intense enrichment successfully overwhelmed Gram-negative defenses, establishing measurable inhibition zones of 2.10 ±0.20 mm against E. coli (for BF-CO45) and 4.85 ± 0.40 mm against S. gallinarum (for BF-SO45).

Subsequent filtration processing (Af-SO45 and Af-CO45) led to a minor, statistically visible contraction of the inhibition zones compared to the unfiltered (BF) formulations. This slight decrease indicates a partial loss of active components, likely due to the mechanical trapping or cold-adsorption of suspended polyphenol-lipid complexes onto the filter membranes. Nevertheless, the post-filtration bioactivity scores remained significantly higher than those of the stored controls, confirming that the functional benefits brought by black fig maceration persist strongly after clarification.

4.9.3. Cultivar Variations and Phytochemical Mechanisms

Intra-varietal analysis indicated unique baseline characteristics and enrichment efficiencies between the two Algerian cultivars. The native Chemlal matrix (CO0) exhibited a higher initial baseline against M. luteus and S. aureus than Sigoise (SO0), indicating a structurally richer background of indigenous polyphenols and higher intrinsic bioactivity. Conversely, the Sigoise series demonstrated a steeper relative vertical gain in bioactivity following the maceration step (BF-SO45). This behavior proves that the Sigoise matrix offers an excellent thermodynamic medium and high chemical affinity for extracting and dissolving exogenous bioactive molecules from dried fig tissue.

From a mechanistic standpoint, the clear synergy observed between the phenolic profiles, antioxidant values, and expanded antibacterial halos proves that mass transfer from the dried black fig successfully enriched the oil phase with low-molecular-weight, amphiphilic polyphenols and flavonoids. These mobile compounds easily partition into the bacterial cell wall, causing structural damage to the plasma membrane, dissipating cellular proton motive forces, and triggering intracellular enzyme inactivation.

Consequently, these results strongly support the industrial application of dried black fig maceration before filtration as a sustainable technological method to produce high-value, functional olive oils. This process effectively upgrades the nutritional and chemical properties of the matrices while serving as an organic preservation barrier against dangerous foodborne microorganisms.

5. General Discussion

The present results provide an integrated demonstration that black fig maceration can simultaneously improve the chemical, antioxidant, and antibacterial profiles of olive oil while maintaining the main quality indices within acceptable limits. The stability of the refractive index suggests that the bulk triacylglycerol matrix was preserved, whereas the changes in free acidity, peroxide value, K232, and K270 reflect the expected sensitivity of olive oil to hydrolytic and oxidative phenomena during storage. The fact that these indices remained within the International Olive Council (IOC) quality framework is important because functional enrichment is technologically valuable only if it does not compromise basic quality compliance [16].

The increase in free acidity after storage and prior to filtration is consistent with the partial hydrolysis of triacylglycerols and the subsequent release of free fatty acids, while the elevation in peroxide values and UV absorbances reflects primary and secondary oxidation phenomena. Such trajectories agree with the classical lipid oxidation framework, in which peroxide

formation, conjugated diene generation, and subsequent secondary oxidation products progressively alter edible oils during storage [26, 5]. The recent shelf-life literature on virgin olive oil also confirms that peroxide value, K232, K270, and phenolic depletion are sensitive indicators of oxidative deterioration under storage conditions [6].

The most notable finding is that black fig maceration partially reversed the oxidative deterioration caused by storage. The decrease in peroxide values and K270 absorbances after maceration suggests that the phenolic and pigment fractions transferred from the fig tissue either limited ongoing oxidation or improved the antioxidant balance of the oil matrix. This interpretation is consistent with historical and modern evidence showing that olive oil polyphenols are strongly associated with resistance to oxidation [27], and that the phenolic fraction contributes to oxidative stability, sensory properties, and biological value [28, 29].

The bioactive enrichment observed in both cultivars, especially the increase in total phenolic content, total flavonoids, chlorophylls, and carotenoids, is chemically coherent with the composition of dark fig fruits. Black and dark-colored figs are known to contain high levels of polyphenols, flavonoids, and anthocyanins, and their antioxidant capacity is closely related to these compounds [30]. In addition, fig fruits contain phenolic acids and flavonoids whose levels vary with the cultivar, tissue, and processing state [31, 9]. The stronger phenolic gain observed in the Sigoise cultivar may therefore reflect a more favorable extraction-partitioning behavior between the fig matrix and this specific oil phase.

The antioxidant results reinforce this interpretation. The parallel increase in DPPH and ABTS.+ radical-scavenging activity after maceration, together with the decrease in the ABTS.+IC50 values, indicates that the enriched oils gained a higher capacity to neutralize free radicals. The methodological coherence of the response is important because the DPPH assay reflects the hydrogen- or electron-donating capacity of the matrix in a radical system [32], whereas the ABTS.+ method is applicable to both hydrophilic and lipophilic antioxidant systems [23]. The consistent direction of both assays suggests that the observed enhancement is not an artifact of a single analytical method.

The correlation analysis provides additional mechanistic support. The positive correlations observed between total phenols and the DPPH/ ABTS.+ radical-scavenging capacities, and the negative correlation linking total phenols to the ABTS.+IC50 values, confirm that phenolic enrichment is a major driver of antioxidant improvement. This is consistent with the role of olive oil phenols as hydrophilic antioxidants capable of protecting the lipid matrix and contributing to biological activity [33, 4]. The inclusion of total phenolic content based on the Folin-Ciocalteu principle is also appropriate for global screening, even if this method estimates total reducing capacity rather than isolating a single molecular family [34].

However, the results also show that total phenolic content alone does not fully determine the final antioxidant activity. The Chemlal cultivar maintained higher DPPH radical-scavenging activity under several conditions, whereas Sigoise reached a higher total phenolic content after maceration. This discrepancy indicates that the specific chemical structure of the transferred phenolics, the balance between simple phenols, flavonoids, and secoiridoid derivatives, the contribution of pigments, and their distribution within the oil phase are likely to control the final antioxidant response. This point agrees with the broader literature showing that phenolic activity depends not only on concentration, but also on molecular structure, polarity, and matrix interactions [28, 29, 4].

Filtration produced a dual technological effect on the matrix. On the one hand, it markedly reduced moisture content and partially decreased acidity, which is favorable for stability because residual water can facilitate hydrolysis, microbial persistence, and phase instability. On the other hand, filtration reduced a portion of the phenolic, flavonoid, and antioxidant gains, suggesting the removal of suspended particles, aqueous microdroplets, or polar fractions carrying these bioactive compounds. Therefore, filtration should be considered not merely as a clarification step, but as a stabilization-bioactivity trade-off that must be optimized according to the intended product: maximum bioactivity prior to filtration or greater final stability after filtration.

The antibacterial results broaden the functional significance of the enriched oils. The largest zones of inhibition were observed after maceration, especially against Gram-positive bacteria, while the stored controls were less active. This trend is consistent with the antimicrobial potential of olive phenolic compounds, including hydroxytyrosol, tyrosol derivatives, and oleuropein-related structures, which have been associated with bactericidal or growth-inhibitory effects [35, 36]. Studies on extra virgin olive oils have also shown that antimicrobial activity is primarily related to phenolic content and composition, rather than to the lipid fraction alone [37, 38].

The weaker response of Gram-negative bacteria is biologically expected because their outer membrane acts as an additional permeability barrier, reducing the access of hydrophobic and amphipathic antimicrobial compounds to intracellular targets [39]. The stronger inhibition of Micrococcus luteus, Staphylococcus aureus, and Bacillus subtilis therefore supports a mechanism in which the transferred phenolic compounds and other phytochemicals interact more efficiently with Gram-positive cell envelopes. The measurable, although weaker, activity recorded against Escherichia coli and Salmonella gallinarum indicates that the fig-enriched oils retain a broad antimicrobial potential, but should not be interpreted as broad-spectrum preservatives without additional minimum inhibitory concentration (MIC) and challenge-test validation.

The multivariate analyses strengthen the global interpretation of the dataset. Principal Component Analysis (PCA) and hierarchical clustering separated the stored controls from the macerated oils, showing that storage moved samples toward an oxidized and less functional profile, whereas black fig maceration shifted them toward a phenol-rich and antioxidant-rich profile. The intermediate spatial position of the filtered oils confirms that filtration preserved part of the enrichment while improving physicochemical stabilization. This multivariate coherence is important because it shows that the observed effects are not isolated parameter changes, but part of an integrated transformation of the oil matrix.

6. General Conclusion

This study demonstrates that the maceration of Chemlal and Sigoise olive oils with black fig constitutes an effective natural strategy for enhancing the functional profile of stored oils. Although 45 days of storage increased free acidity, peroxide values, and UV absorbances (K232 and K270) while reducing the phenolic content and antioxidant activity, maceration partially reversed these deteriorative effects. The enriched oils showed higher total phenolic content, total flavonoids, chlorophylls, and carotenoids, accompanied by improved DPPH and ABTS.+ radical-scavenging activities and lower ABTS.+ IC50 values.

The two cultivars responded differently to the enrichment process. Chemlal showed a stronger initial antioxidant behavior and maintained higher DPPH radical-scavenging activity under several conditions, whereas Sigoise displayed a greater capacity for phenolic enrichment after maceration. This confirms that the response to fruit-based enrichment depends not only on the added plant matrix, but also on the intrinsic physicochemical and compositional properties of the oil cultivar.

Filtration was confirmed as a critical technological step. It substantially reduced moisture content and contributed to oil stabilization, but it also caused a partial reduction in bioactive fractions and antioxidant activity. Nevertheless, the final filtered oils remained more functional than the stored controls, demonstrating that filtration provides a practical trade-off between product stability and the preservation of the enrichment effect.

The antibacterial results further support the functional potential of the black fig-enriched olive oils. The macerated samples exhibited larger zones of inhibition, particularly against Gram-positive bacteria, and the response was concentration-dependent. These findings suggest that the transferred phenolic and flavonoid compounds contribute not only to antioxidant protection but also to antibacterial activity. However, further microbiological validation using minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and food model assays is required before industrial preservation claims can be made.

Overall, black fig maceration appears to be a promising, natural, and technologically simple approach for producing olive oils with enhanced antioxidant and antibacterial properties. Future studies should focus on the detailed molecular characterization of the transferred compounds using advanced chromatographic techniques like HPLC-DAD and LC-MS/MS. This will provide deeper insights into the precise causal relationships between specific phytochemical structures and their resulting bioactivities, while optimizing the balance between enrichment intensity, oxidative stability, and sensory acceptability.

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