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Thesis

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By : **SAHLI Samir**

Topic

**Valorization of Biological Activities and Biochemical
Characterization of Olive Oils from Selected Cultivars of
Olive Tree (*Olea europaea L.*).**

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Dedication

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Titre : Valorisation des Activités Biologiques et Caractérisation Biochimique des Huiles d'Olive de Cultivars Sélectionnés de l'Olivier (*Olea europaea L.*).

Résumé :

Cette étude vise à caractériser les propriétés physico-chimiques, biochimiques et biologiques des huiles d'olive extraites de quatre variétés algériennes : Akerma, Bouchouk Guergour, Azeradj et Gordal de Séville, en fonction du stade de maturité des fruits (vert, tacheté et noir). L'objectif est d'évaluer l'impact des facteurs variétaux et de la maturité des fruits sur les paramètres de qualité, la composition lipidique et les activités biologiques, afin d'optimiser la valorisation de ces huiles dans les secteurs agroalimentaire et thérapeutique. Les résultats ont révélé des modifications significatives des paramètres physico-chimiques avec l'évolution de la maturation. L'indice de maturité indique que Bouchouk Guergour atteint un stade avancé plus rapidement qu'Akerma, évoluant de $1,8 \pm 0,09$ au stade vert à $6,2 \pm 0,21$ au stade noir, tandis qu'Akerma progresse plus lentement, de $1,6 \pm 0,08$ à $6,9 \pm 0,17$. Concernant le rendement en huile, Akerma atteint un maximum de $12,71 \pm 0,17$ % au stade tacheté, tandis que Bouchouk Guergour culmine à $19,56 \pm 0,22$ % au stade noir, révélant des stratégies de stockage lipidique distinctes. Les huiles de la variété Azeradj ont montré un rendement modéré atteignant $17,83 \pm 0,19$ % au stade noir, avec une composition équilibrée en acides gras, riche en acide oléique (jusqu'à 68,54 %) et en acide linoléique (15,92 %), traduisant une bonne valeur nutritionnelle et une stabilité oxydative satisfaisante. En revanche, Gordal de Séville, une variété principalement utilisée comme olive de table, a affiché le plus faible rendement en huile ($5,87 \pm 0,11$ %), mais ses huiles ont conservé des paramètres de qualité acceptables, ainsi qu'un potentiel biochimique et biologique intéressant malgré leur faible teneur en matières grasses. L'analyse de la qualité des huiles montre que toutes les variétés respectent les normes du Conseil Oléicole International (COI) pour les huiles vierges extra. L'acidité libre augmente légèrement avec la maturation tout en restant dans les limites acceptables ($<0,8$ %). L'indice de peroxyde augmente de $3,25 \pm 0,2$ à $9,5 \pm 0,3$ meq O₂/kg chez Akerma, et de $9 \pm 0,25$ à $12,5 \pm 0,41$ meq O₂/kg chez Bouchouk Guergour, traduisant une plus grande sensibilité à l'oxydation des fruits plus mûrs. L'indice d'iode, indicateur du degré d'insaturation des acides gras, augmente avec la maturité, atteignant $90,96 \pm 1,71$ chez Akerma et $85,09 \pm 0,9$ chez Bouchouk Guergour au stade noir. Les analyses GC-FID et GC-MS ont révélé des profils en acides gras distincts selon la variété et le stade de maturation. L'acide oléique (C18:1 n-9) est l'acide gras dominant dans toutes les huiles, avec des variations notables : 73,03 % chez Bouchouk Guergour contre 69,30 % chez Akerma au stade vert. L'acide linoléique (C18:2 n-6) augmente avec la maturation, atteignant 16,41 % chez Akerma et 11,62 % chez Bouchouk Guergour au stade noir. L'acide palmitique (C16:0) diminue légèrement chez Akerma (de 17,28 % à 14,22 %) et reste stable chez Bouchouk Guergour (entre 9,24 % et 9,45 %). Les tests biologiques in vivo ont mis en évidence des propriétés anti-inflammatoires et cicatrisantes remarquables des huiles étudiées. L'activité anti-inflammatoire, évaluée par l'inhibition de l'œdème auriculaire induit par le xylène, était maximale pour les huiles issues des fruits au stade noir, avec des taux d'inhibition de $91,09 \pm 0,80$ % pour Akerma et $92,54 \pm 0,88$ % pour Bouchouk Guergour après 4 heures, dépassant l'efficacité du Voltaren ($79,88 \pm 0,50$ %). Les huiles d'Azeradj et de Gordal de Séville ont également montré des réponses modérées, avec des taux d'inhibition dépassant les 80 % au stade noir. L'activité cicatrisante, évaluée chez le rat, a montré que les huiles ont permis une guérison complète des plaies en 28 jours, avec des taux de contraction atteignant 100 % au stade noir pour Akerma et Bouchouk Guergour, et allant jusqu'à 88 à 95 % pour Azeradj et Gordal de Séville, contre 26,24 % pour le groupe témoin traité à la vaseline. Ces résultats confirment le potentiel des huiles d'olive algériennes comme produits de haute qualité, conformes aux standards internationaux, et présentant des perspectives prometteuses dans les domaines agroalimentaire et pharmaceutique. Pour maximiser leur valorisation, les recherches futures devraient s'orienter vers l'optimisation des pratiques culturales et des techniques d'extraction, l'étude approfondie des composés bioactifs, ainsi que le développement de formulations fonctionnelles adaptées aux industries alimentaire, cosmétique et pharmaceutique.

Mots clés : *Olea europaea L.*, huile d'olive, maturation, qualité physico-chimique, acides gras, activité anti-inflammatoire, activité cicatrisante, valorisation, Azeradj, Gordal de Séville, Bouchouk Guergour, Akerma.

Title: Valorization of Biological Activities and Biochemical Characterization of Olive Oils from Selected Cultivars of Olive Tree (*Olea europaea L.*).

Abstract:

This study aims to characterize the physicochemical, biochemical, and biological properties of olive oils extracted from four Algerian varieties: Akerma, Bouchouk Guergour, Azeradj, and Gordal of Sevilla, according to the fruit ripening stage (green, spotted, and black). The objective is to assess the impact of varietal factors and fruit maturity on quality parameters, lipid composition, and biological activities to optimize the valorization of these oils in the agri-food and therapeutic sectors. The results revealed significant changes in physicochemical parameters with fruit ripening. The maturity index indicates that Bouchouk Guergour reaches an advanced ripening stage faster than Akerma, progressing from 1.8 ± 0.09 at the green stage to 6.2 ± 0.21 at the black stage, while Akerma matures more slowly, from 1.6 ± 0.08 to 6.9 ± 0.17 . Regarding oil yield, Akerma reaches a maximum of $12.71 \pm 0.17\%$ at the spotted stage, whereas Bouchouk Guergour peaks at $19.56 \pm 0.22\%$ at the black stage, highlighting different lipid accumulation strategies between varieties. Azeradj oils showed moderate yields of up to $17.83 \pm 0.19\%$ at the black stage, with a balanced fatty acid composition rich in oleic acid (up to 68.54%) and linoleic acid (15.92%), which suggests good nutritional value and oxidative stability. In contrast, Gordal of Sevilla, a variety primarily used for table olives, exhibited the lowest oil yield ($5.87 \pm 0.11\%$), but the oils retained acceptable quality parameters and demonstrated interesting biochemical and biological potential despite their low fat content. Oil quality analysis shows that all varieties comply with the International Olive Council (IOC) standards for extra virgin olive oils. Free acidity slightly increases with maturation while remaining within acceptable limits ($<0.8\%$) for all oils analyzed. The peroxide value increases from 3.25 ± 0.2 meq O_2/kg to 9.5 ± 0.3 meq O_2/kg in Akerma and from 9 ± 0.25 meq O_2/kg to 12.5 ± 0.41 meq O_2/kg in Bouchouk Guergour, indicating greater susceptibility to oxidation in more mature olives. The iodine value, an indicator of fatty acid unsaturation, increases with ripening, reaching 90.96 ± 1.71 in Akerma and 85.09 ± 0.9 in Bouchouk Guergour at the black stage, illustrating an enrichment in unsaturated fatty acids. GC-FID and GC-MS analyses revealed distinct fatty acid profiles depending on the variety and ripening stage. Oleic acid (C18:1 n-9) is the predominant fatty acid in all oils, but with significant variations: 73.03% in Bouchouk Guergour compared to 69.30% in Akerma at the green stage. Linoleic acid (C18:2 n-6) increases with maturation, reaching 16.41% in Akerma and 11.62% in Bouchouk Guergour at the black stage, affecting the oxidative stability of the oils. Palmitic acid (C16:0) slightly decreases with maturation in Akerma (from 17.28% to 14.22%) and remains stable in Bouchouk Guergour (9.24% - 9.45%). In vivo biological tests revealed remarkable anti-inflammatory and wound-healing properties in the studied olive oils. The anti-inflammatory activity, measured by the inhibition of xylene-induced ear edema, was highest for oils extracted from black-stage fruits, with inhibition rates of $91.09 \pm 0.80\%$ for Akerma and $92.54 \pm 0.88\%$ for Bouchouk Guergour after 4 hours, surpassing the effectiveness of Voltaren ($79.88 \pm 0.50\%$). Oils from Azeradj and Gordal of Sevilla also showed moderate anti-inflammatory responses, with inhibition percentages exceeding 80% at the black stage. The wound-healing activity, evaluated using a wound model in rats, demonstrated that olive oils enabled complete wound healing within 28 days, with wound contraction rates reaching 100% at the black stage for Akerma and Bouchouk Guergour, and up to 88–95% for Azeradj and Gordal of Sevilla, compared to 26.24% in the Vaseline-treated control group. These results confirm the potential of Algerian olive oils as high-quality products that meet international standards and offer promising agri-food and pharmaceutical applications. To maximize their valorization, future research should focus on optimizing agronomic practices and extraction techniques, conducting in-depth studies on bioactive compounds, and developing functional formulations suitable for the food, cosmetic, and pharmaceutical industries.

Key words: *Olea europaea L.*, olive oil, ripening, physicochemical quality, fatty acids, anti-inflammatory activity, wound-healing activity, valorization, Azeradj, Gordal of Sevilla, Bouchouk Guergour, Akerma

العنوان: تثمين الأنشطة البيولوجية والتوصيف البيوكيميائي لزيت الزيتون المستخلصة من بعض أصناف الزيتون (*Olea europaea L.*)

الملخص :

تهدف هذه الدراسة إلى تحديد الخصائص الفيزيائية-الكيميائية والبيوكيميائية والبيولوجية لزيت الزيتون المستخلصة من أربع أصناف جزائرية: عكرمة، بوشوك فرفور، أزردج، وغوردال دي إشبيلية، وذلك وفقاً لمرحلة نضج الثمار (أخضر، مرقط، وأسود). الهدف من هذه الدراسة هو تقييم تأثير العوامل الصنفية ودرجة النضج على مؤشرات الجودة، وتركيب الأحماض الدهنية، والأنشطة البيولوجية، من أجل تحسين تثمين هذه الزيوت في القطاعين الغذائي والعلاجي. كشفت النتائج عن تغيرات كبيرة في المؤشرات الفيزيائية-الكيميائية مع تقدم النضج. أظهر مؤشر النضج أن صنف بوشوك فرفور يصل إلى مرحلة النضج المتقدمة بسرعة أكبر من عكرمة، حيث ينتقل من 1.8 ± 0.09 في المرحلة الخضراء إلى 6.2 ± 0.21 في المرحلة السوداء، في حين يتطور صنف عكرمة بوتيرة أبطأ، من 1.6 ± 0.08 إلى 6.9 ± 0.17 . فيما يخص مردود الزيت، بلغ عكرمة أقصى مردود له بنسبة $12.71 \pm 0.17\%$ في المرحلة المرفطة، بينما بلغ بوشوك فرفور نسبة $19.56 \pm 0.22\%$ في المرحلة السوداء، مما يبرز استراتيجيات مختلفة في تراكم الدهون بين الأصناف. أظهرت زيوت أزردج مردوداً متوسطاً بلغ $17.83 \pm 0.19\%$ في المرحلة السوداء، مع تركيب متوازن للأحماض الدهنية، غني بحمض الأوليك (حتى 68.54%) وحمض اللينولييك (15.92%)، مما يشير إلى قيمة غذائية جيدة وثبات أكسدة مقبول. أما صنف غوردال دي إشبيلية، المعروف أساساً كزيتون مائدة، فقد سجل أدنى مردود للزيت ($5.87 \pm 0.11\%$)، ومع ذلك احتفظ بجودة مقبولة وأظهر إمكانات بيوكيميائية وبيولوجية رغم محتواه المنخفض من الدهون. أظهرت تحاليل جودة الزيت أن جميع الأصناف المدروسة تحترم معايير المجلس الدولي للزيتون (IOC) الخاصة بزيت الزيتون البكر الممتاز. ازدادت الحموضة الحرة قليلاً مع النضج، لكنها بقيت ضمن الحدود المقبولة ($0.8\% <$). ارتفعت قيمة البيروكسيد من 3.25 ± 0.2 إلى 9.5 ± 0.3 meq O₂/kg في صنف عكرمة، ومن 9 ± 0.25 إلى 12.5 ± 0.41 meq O₂/kg في بوشوك فرفور، مما يدل على زيادة القابلية للأكسدة في الثمار الناضجة. أما مؤشر اليود، الذي يعكس درجة عدم التشبع في الأحماض الدهنية، فقد ارتفع مع النضج، ليصل إلى 90.96 ± 1.71 في عكرمة و 85.09 ± 0.9 في بوشوك فرفور، مما يعكس زيادة في الأحماض الدهنية غير المشبعة. أظهرت تحاليل GC-FID و GC-MS ملفات مميزة للأحماض الدهنية حسب الصنف ومرحلة النضج. كان حمض الأوليك (C18:1 n-9) الحمض الدهني السائد في جميع الزيوت، لكن بنسب متفاوتة: 73.03% في بوشوك فرفور مقابل 69.30% في عكرمة في المرحلة الخضراء. ازداد تركيز حمض اللينولييك (C18:2 n-6) مع النضج، ليصل إلى 16.41% في عكرمة و 11.62% في بوشوك فرفور في المرحلة السوداء، مما يؤثر على ثبات الأكسدة في الزيوت. انخفض حمض البالميتيك (C16:0) قليلاً مع النضج في عكرمة (من 17.28% إلى 14.22%)، بينما بقي ثابتاً في بوشوك فرفور (من 9.24% إلى 9.45%). أظهرت الاختبارات البيولوجية الحية خصائص مضادة للالتهابات ومساعدة على التئام الجروح ملحوظة في زيوت الزيتون المدروسة. كانت النشاطات المضادة للالتهاب، المقاسة عن طريق تثبيط وذمة الأذن الناتجة عن الزيلين، أعلى ما تكون في الزيوت المستخرجة من الثمار في المرحلة السوداء، حيث بلغت نسبة التثبيط $91.09 \pm 0.80\%$ لعكرمة و $92.54 \pm 0.88\%$ لبوشوك فرفور بعد 4 ساعات، متجاوزة فعالية الفولتارين ($79.88 \pm 0.50\%$). كما أظهرت زيوت أزردج وغوردال دي إشبيلية استجابات معتدلة، تجاوزت فيها نسب التثبيط 80% في المرحلة السوداء. أظهرت فعالية التئام الجروح، التي تم تقييمها باستخدام نموذج حيواني لدى الجرذان، أن زيوت الزيتون ساهمت في شفاء كامل للجروح خلال 28 يوماً، حيث بلغت نسبة تقلص الجروح 100% في المرحلة السوداء لعكرمة وبوشوك فرفور، وبلغت $88-95\%$ لأزردج وغوردال دي إشبيلية، مقابل 26.24% لمجموعة المراقبة المعالجة بالفازلين. تؤكد هذه النتائج الإمكانات الكبيرة لزيوت الزيتون الجزائرية كمكونات عالية الجودة، تحترم المعايير الدولية وتوفر أفاقاً واعدة في المجالات الغذائية والصيدلانية. ومن أجل تعظيم تثمينها، ينبغي توجيه البحوث المستقبلية نحو تحسين الممارسات الزراعية وتقنيات الاستخلاص، ودراسة المركبات النشطة بيولوجياً بعمق، وتطوير تركيبات وظيفية مناسبة للصناعات الغذائية، التجميلية، والدوائية.

الكلمات المفتاحية: *Olea europaea L.*، زيت الزيتون، النضج، الجودة الفيزيائية والكيميائية، الأحماض الدهنية، النشاط المضاد للالتهاب، التئام الجروح، التثمين، أزردج، غوردال دي إشبيلية، بوشوك فرفور، عكرمة.

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LIST OF ABBREVIATIONS

ALUM	: Applied Research Laboratory and Research Center
ANOVA	: Analysis of Variance
AOCS	: American Oil Chemists' Society
AV	: Acid Value
COI/IOC	: International Olive Council
DNA	: Deoxyribonucleic Acid
FAME	: Fatty Acid Methyl Esters
FAO	: Food and Agriculture Organization
FID	: Flame Ionization Detector
GC	: Gas Chromatography
GC-FID	: Gas Chromatography-Flame Ionization Detection
GC-MS	: Gas Chromatography–Mass Spectrometry
HFA	: Hydroxy Fatty Acids
HDL	: High-Density Lipoprotein
HPLC	: High-Performance Liquid Chromatography
IM	: Maturity Index
IV	: Iodine Value
LDL	: Low-Density Lipoprotein
MUFA	: Monounsaturated Fatty Acids
NAFB	: National Animal Feed Board
PUFA	: Polyunsaturated Fatty Acids
PV	: Peroxide Value
RSD	: Relative Standard Deviation
SD	: Standard Deviation
SFA	: Saturated Fatty Acids
SPME	: Solid Phase Microextraction
UV	: Ultraviolet
VM	: Volatile Matter
WHO	: World Health Organization

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INTRODUCTION

Introduction

The olive tree (*Olea europaea L.*) is one of the oldest fruit trees cultivated by humans, with a history that dates back over 6,000 years. Originally from the Levant region (Palestine, Syria, Lebanon), it later spread throughout the Mediterranean basin thanks to great ancient civilizations such as the Egyptians, Phoenicians, Greeks, and Romans. This mythical tree quickly acquired symbolic, economic, spiritual, and medicinal value, making it a true pillar of Mediterranean identity (**Tous & Romero, 1994; Zohary et al., 2012**).

In ancient Greece, olive oil was used not only for food, but also in religious ceremonies, medical treatments, cosmetics, and even as an Olympic prize. In Rome, it was considered a staple product, integrated into the fiscal and commercial system. The olive tree was regarded as sacred in many religious traditions: in the Bible, it symbolizes peace (the dove carrying an olive branch after the Flood), while in Islam, it is mentioned as a blessed tree in the Quran (**Surah An-Nur, 24:35**).

In North Africa, particularly in Algeria, olive cultivation dates back to the Carthaginian and Roman periods, as evidenced by the many ancient oil mills found at archaeological sites such as Tipaza, Timgad, and Djemila. The tree has perfectly adapted to the Mediterranean and semi-arid climates of the Maghreb, becoming a symbol of resilience and longevity. Even today, some centuries-old olive trees continue to produce fruit, reinforcing their image as a living heritage.

Culturally, the olive tree is deeply rooted in Algerian popular traditions. It often appears in proverbs, traditional songs, and agricultural practices passed down through generations. Locally produced olive oil is not only a staple food but also an ancestral remedy used to treat burns, joint pain, digestive disorders, and even for hair and skin care (**Cherfaoui et al., 2020**).

The olive tree (*Olea europaea L.*) is one of the oldest and most iconic fruit crops in the Mediterranean region. It plays a fundamental role not only in the economy but also in biodiversity and cultural heritage. Extra-virgin olive oil, extracted directly from the fruit without chemical refining, is widely recognized for its numerous nutritional and therapeutic benefits (**Boskou, 2006; Covas, 2007**). It is a key component of the Mediterranean diet, extensively studied for its health benefits, primarily due to its high content of monounsaturated fatty acids and polyphenols with antioxidant properties (**Montedoro et al., 1992**).

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The global olive oil market has experienced considerable growth in recent years, largely due to rising consumer awareness of its nutritional and health benefits. Olive oil is known for its high levels of monounsaturated fatty acids and bioactive compounds, including phenols and tocopherols, which contribute to its therapeutic properties. These compounds offer various health benefits, such as antioxidant activity, anti-inflammatory effects, and cardiovascular protection (**López-Miranda et al., 2020; Guerreiro et al., 2021**). Additionally, recent studies highlight the importance of olive oil in promoting metabolic health and reducing the risk of chronic diseases (**Gomez et al., 2023; Romero et al., 2022**).

The quality and characteristics of olive oil are influenced by several factors, including the ripening stage, geographic origin, and extraction methods. The olive oil quality is usually measured via well-established parameters, such as free acidity, peroxide value, and iodine value. Pre- and post-harvest factors, such as cultivar, environmental stress, harvesting methods, and fruit storage conditions, have been shown to significantly affect oil quality parameters and phenolic content (**Ben-Ayed et al., 2018**). In addition to cultivar and geographic origin, the harvest year also plays a critical role in modulating oil quality and wax composition. Seasonal climatic variability can lead to interannual differences in phenolic content, fatty acid profiles, and waxes (**Lukić et al., 2013**).

In recent years, scientific interest in the biological potential of olive oil has significantly increased. Numerous studies have explored the mechanisms through which olive oil exerts anti-inflammatory, antioxidant, and regenerative effects, particularly in skin tissue and chronic disease models (**Covas et al., 2022; López-Yerena et al., 2021**). However, there is a growing need for in vivo evaluations of these effects across different olive cultivars and ripening stages, particularly those cultivated in underrepresented regions such as North Africa. To date, comparative investigations into the therapeutic efficacy of olive oils from specific Algerian varieties remain limited.

Globally, olive oil production is dominated by Mediterranean countries, particularly Spain, Italy, Greece, and Tunisia. Algeria holds a significant position in this sector, with an estimated olive-growing area of approximately 500 000 hectares (**FAO, 2022**). However, despite its potential, Algerian production remains underutilized due to various challenges related to the variability of agro-climatic conditions, cultivation methods, and post-harvest processing practices (**Ben Temime et al., 2006**).

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Olive cultivation is practiced in more than 47 countries worldwide, with an annual global olive oil production of approximately 3.2 million tons (**FAO, 2022**). Spain is the world's leading producer, followed by Italy, Greece, and Tunisia. Mediterranean countries account for about 98% of global olive oil production, benefiting from favorable climatic and soil conditions for olive tree cultivation (**IOC, 2021**).

The export of olive oil is a valuable source of foreign exchange for many producing countries. However, to remain competitive in the international market, Algerian olive oil must meet the quality standards established by the International Olive Council (IOC), which requires a better understanding of the physicochemical and bioactive parameters of local oils (**Benrabah et al., 2020**).

The olive tree is a hardy species, particularly well adapted to arid and semi-arid climatic conditions. It plays a crucial role in soil conservation by preventing erosion and contributes to climate change mitigation through its ability to sequester atmospheric carbon. Its genetic diversity is a key asset for varietal improvement and resilience to diseases and abiotic stresses (**Gutiérrez et al., 2020**).

Different olive varieties exhibit specific morphological and biochemical characteristics that influence the quality of the oil produced. The content of phenolic compounds, fatty acid profiles, and antioxidant properties vary depending on the variety, terroir, and cultivation practices. A better characterization of local varieties would help optimize their use and enhance the quality of the oils produced.

Extra-virgin olive oil is particularly valued for its health benefits, largely attributed to its richness in bioactive compounds such as polyphenols, tocopherols, and monounsaturated fatty acids. These compounds are known for their role in preventing numerous chronic diseases, including cardiovascular diseases, metabolic disorders, and certain types of cancer (**Covas, 2007**).

The polyphenols found in olive oil, such as oleuropein and hydroxytyrosol, exhibit well-documented antioxidant and anti-inflammatory properties. Several clinical studies have shown that regular consumption of olive oil reduces oxidative stress and improves cardiovascular health by lowering LDL ("bad cholesterol") levels and increasing HDL ("good cholesterol") levels (**Montedoro et al., 1992; Gutiérrez et al., 2020**). Additionally, olive oil contributes to blood sugar regulation and may play a protective role against type 2 diabetes (**Boskou, 2006**).

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From an economic perspective, the valorization of polyphenol-rich olive oils represents a development opportunity for producers. Olive oils certified for their health benefits are increasingly in demand in the international market, highlighting the growing importance of product certification and traceability in the olive oil sector (FAO, 2022). Moreover, the development of olive oil-derived products, such as cosmetics and dietary supplements, represents a rapidly expanding market.

Algeria possesses a rich varietal diversity well-adapted to local climatic conditions. Some of the most well-known local olive varieties include Akerma, Bouchouk Guergour, Azeradj, Chemlal, and Sigoise. These varieties are distinguished by their morphological characteristics, fatty acid profiles, and bioactive properties, offering significant potential for producing high-quality olive oil.

However, despite this rich genetic heritage, the Algerian olive sector faces several challenges that hinder its competitiveness in the international market (Benrabah et al., 2020):

- **Lack of scientific characterization:** Few in-depth studies have been conducted to analyze the physicochemical and biochemical quality of local oils. A better characterization would help identify the strengths of each variety and optimize their valorization.
- **Traditional farming techniques and extraction methods:** The use of rudimentary agricultural practices and suboptimal extraction processes limits both the yield and quality of olive oil.
- **Insufficient processing and storage infrastructure:** Many producers lack modern equipment for proper oil pressing and preservation, which can alter its organoleptic and nutritional properties.
- **Absence of certification and quality labels:** Unlike major olive-producing nations, Algeria has yet to establish an effective system for Protected Geographical Indication (PGI) or Controlled Designation of Origin (AOC) for its local olive oils, limiting their recognition and competitiveness in international markets.
- **Yield variability due to climatic conditions:** The semi-arid climate of many Algerian olive-growing regions leads to irregular production cycles, impacting supply stability and pricing.
- **Limited integration into the international market:** Algeria still exports relatively little olive oil compared to other producing countries like Tunisia or Spain. A better-structured sector and an adapted export strategy could allow Algerian olive oils to access broader markets.

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Improving agricultural techniques, modernizing processing infrastructures, and establishing quality certifications could significantly enhance the competitiveness of Algerian olive oil, positioning it as a premium product in the international market.

Algeria boasts a rich diversity of olive varieties, well adapted to local climatic conditions. Among these, Akerma, Bouchouk Guergour, and Azeradj are well established in the northern regions and are recognized for their specific agronomic and biochemical characteristics. Additionally, some introduced varieties, such as Gordal of Sevilla, are also cultivated due to their dual-purpose use for both table olives and olive oil production (**Djerrou et al., 2022; Boudjemaa et al., 2019; Rahmani et al., 2017**).

The valorization of local olive oils largely depends on a better understanding of their physicochemical and biological properties. Several studies have shown that oil quality is influenced not only by the variety but also by cultivation conditions, the ripening stage of the olives, and the extraction techniques used (**Gutiérrez et al., 2020**). In Algeria, the lack of in-depth studies on the biochemical composition and biological activities of olive oils remains a major obstacle to their optimal promotion in national and international markets (**Benrabah et al., 2020**).

The olive tree is deeply rooted in the history and culture of Mediterranean civilizations. Mentioned in numerous ancient texts, it has long been associated with peace, wisdom, and prosperity. In Algeria, olive cultivation dates back to antiquity, with some centuries-old olive groves serving as a testament to the importance of this sector in the country's heritage (**Tous & Romero, 1994; FAO, 2022**).

Despite its rich historical heritage and strong potential for olive cultivation, the olive oil industry in Algeria remains underexploited. Although the country possesses an abundance of local varieties with promising agronomic characteristics, scientific knowledge about their oils remains fragmented, particularly regarding their biochemical quality and biological activities. Most studies conducted to date have focused only on traditional quality parameters (acidity index, moisture content, peroxide value, etc.), while their bioactive properties — such as anti-inflammatory, antioxidant, or wound-healing effects — remain insufficiently documented (**Benrabah et al., 2020; Gutiérrez et al., 2020; Bouarroudj et al., 2016**).

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This scientific gap hinders the valorization of these oils both industrially and in the development of innovative therapeutic or cosmetic applications. Therefore, it is essential to conduct in-depth research to thoroughly characterize the biochemical composition of these oils and highlight their functional and nutritional potential.

Unlike major olive oil-producing nations such as Spain, Italy, and Tunisia, Algeria has yet to implement effective strategies for certifying and promoting its local olive oils (**FAO, 2022**). This raises a fundamental question: Do Algerian varieties have a competitive potential in terms of physicochemical, biochemical, and bioactive quality? What impact does the ripening stage have on these properties?

The main objective of this thesis is to study the physicochemical quality, biochemical composition, and biological activities of olive oils extracted from three Algerian local varieties (Akerma, Bouchouk Guergour, and Azeradj) as well as from an introduced variety (Gordal of Sevilla), at different stages of fruit maturation. To achieve this general objective, several specific aims were defined:

- To characterize the physicochemical and biochemical properties of oils extracted from the four selected varieties according to the fruit maturity stage;
- To evaluate *in vivo* their biological properties, particularly anti-inflammatory and wound-healing activities;
- To compare the results obtained according to the variety and maturity stage, in order to identify the most interesting profiles from a nutritional and therapeutic perspective;
- To explore the possibilities for valorizing these oils in the fields of health, cosmetics, and agri-food, with a view toward local and sustainable development.

In this study, it is assumed that the composition and properties of olive oils are strongly influenced by the olive variety as well as by the fruit maturity stage at harvest. Based on this general assumption, two specific hypotheses were formulated:

- Hypothesis 1:

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Olive oils extracted from the three local Algerian varieties (Akerma, Bouchouk Guergour, and Azeradj) present specific physicochemical profiles (acidity, peroxide value, moisture content, etc.) and biochemical profiles (fatty acid composition, phenolic compound content, flavonoid content, etc.) that are significantly different from one another, and these characteristics also vary depending on the fruit maturation stages (green, spotted, black).

➤ Hypothesis 2:

Thanks to their richness in bioactive compounds (unsaturated fatty acids, polyphenols, natural antioxidants), these olive oils possess high biological potential, particularly anti-inflammatory and wound-healing effects, which could justify their use in nutritional, pharmaceutical, or cosmetic applications. This potential is expected to vary according to variety and maturity stage, thus allowing the identification of optimal varietal profiles for targeted therapeutic uses.

This work presents notable scientific originality by combining physicochemical, biochemical, and biological approaches on local varieties that have been little studied, in an Algerian context. It is also one of the few studies to evaluate the effect of fruit maturation stage on the *in vivo* bioactivity of olive oils, particularly in animal models for wound-healing and anti-inflammatory activities. Thus, this research is part of a multidisciplinary approach at the intersection of agronomy, biochemistry, and experimental pharmacology, aiming to provide a better understanding of the relationship between oil composition and its biological effects.

This thesis follows the **IMRAD** methodology and consists of the following sections:

- **Introduction:** Presentation of the scientific context, research problem, objectives, and hypotheses.
- **Materials and Methods:** Description of the olive varieties, study conditions, and analytical protocols applied to the oils (physicochemical, biochemical, and biological analyses).
- **Results:** Presentation and analysis of the findings on olive oil quality.
- **Discussion:** Interpretation of the results by comparing them with existing scientific literature.
- **Conclusion and Perspectives:** Summary of the results, implications, and recommendations for the valorization of the studied oils.



MATERIALS AND METHODS

1.1. Plant Material

This study focused on four olive tree varieties cultivated in the Sétif region, located in northeastern Algeria: Akerma and Bouchouk Guergour (originating from Draâ Kebila), as well as Azeradj and Gordal de Sevilla (originating from the northwestern part of Setif).

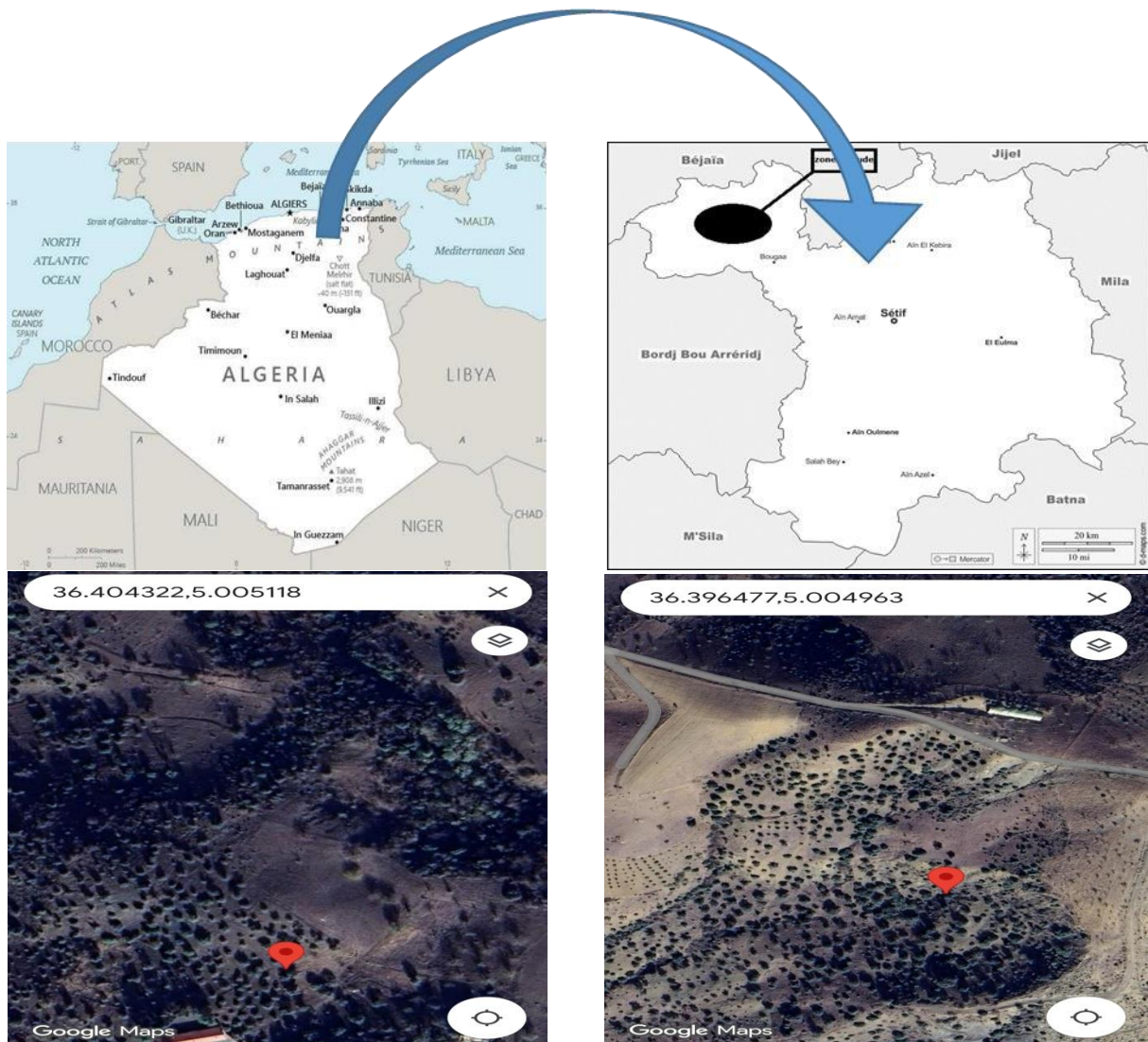


Figure 1. Geographical location of the study region.

The study area is located in the High Plateaus of northeastern Algeria, between 35°40' and 36°5' north latitude and 5° to 6° east longitude, within the Tell Atlas mountain range (Figure 1). It is characterized by a semi-arid Mediterranean climate, with hot and dry summers, and cold and rainy winters extending from September to May. The altitude ranges between 1100 and 1200 meters, with annual precipitation between 500 and 800 mm.

The olive plots sampled in this study are precisely located at the following geographic coordinates:

- **Plot 1**, corresponding to Akerma and Bouchouk Guergour varieties (Draâ Kebila area): 36.404322° N, 5.005118° E.
- **Plot 2**, corresponding to Azeradj and Gordal of Sevilla varieties (northwestern Sétif region): 36.396477° N, 5.004963° E.

The olive trees were cultivated in non-irrigated fields under traditional agronomic practices, in calcareous-clay soils typical of the region. Organic fertilization was performed once per year using decomposed livestock manure applied in autumn. The trees were approximately 25 years old and were planted at a spacing of 6 × 6 meters. For each variety, fruits were collected from 10 randomly selected healthy trees, with an average yield of 15 to 20 kg per tree. These standardized growing conditions were implemented to minimize environmental variability and ensure reliable comparison of results.

The region receives moderate annual rainfall, averaging approximately 480 mm, and relative humidity fluctuates between 38% and 52%. The calcareous-clay soil, combined with limited water availability, is known to affect the biochemical profile of olive fruits, influencing both oil yield and composition (**Rouabhi et al., 2018**).

1.2. Variety Description

- **Azeradj:** This local variety, known for its drought resistance, originates from the Kabylie region (Béjaïa) and accounts for approximately 10% of Algeria's olive-growing area (Figure 2). It is characterized by early flowering and an alternating bearing habit. The fruits are medium-sized, elongated in shape, with an oil yield ranging between 24% and 28% (**TIFAV, 2023**).
- **Gordal of Sevilla:** Introduced from Spain, this table olive variety is mainly cultivated in western Algeria (Figure 3). It is characterized by its large, fleshy fruits (1.2–6.2 g) and relatively large pit, making it ideal for table consumption. However, its oil content is low, not exceeding 6% (**TIFAV, 2023**).
- **Akerma:** This variety originates from the Sétif region in Algeria. It is primarily cultivated for olive oil production (Figure 4). Akerma olives are medium-sized, ovoid in shape, with an estimated oil yield ranging from 20% to 25% (**TIFAV, 2023**).
- **Bouchouk Guergour:** Native to the Guergour region, also located in the Sétif province, this variety is known for its hardiness and adaptation to local climatic conditions (Figure 5). Bouchouk

Guergour olives are medium to large in size, elliptical in shape, and have an oil yield ranging from 18% to 22% (TIFAV, 2023).

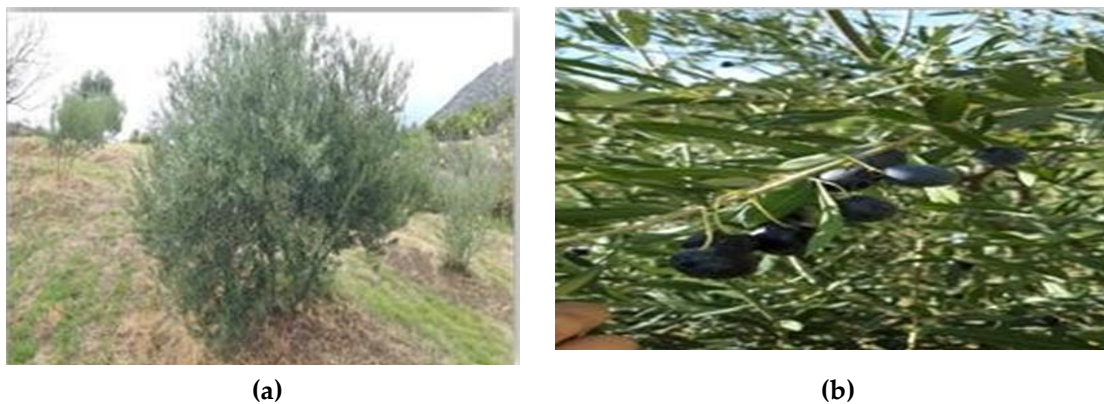


Figure 2. (a) *Azeradj* olive tree and (b) its olives.



Figure 3. (a) *Gordal of Sevilla* olive tree and (b) its olives



Figure 4. (a) *Bouchouk Guergour* olive tree and (b) its olives.



Figure 5. (a) *Akerma* olive tree and (b) its olives

1.3. Sampling

Olive samples were collected following a rigorous protocol:

- **Akerma** and **Bouchouk Guergour** were harvested in the rural commune of Draâ Kebila from randomly selected trees under homogeneous soil and climatic conditions.
- **Azeradj** and **Gordal of Sevilla** were sampled from extraction units located in the northwestern region of Sétif.

The collection process is summarized in Table 1:

Table 1. Collection process of the 4 varieties used in the study

Steps	Akerma	Bouchouk Guergour	Azeradj	Gordal of Sevilla
Harvest date	October 2022	December 2022	November 2022	November 2022
Number of trees sampled	3	3	3	3
Quantity of olives collected (kg)	5	5	5	5
Harvest method	Manual	Manual	Manual	Manual
Storage method	Ventilated crates	Ventilated crates	Ventilated crates	Ventilated crates
Oil extraction method	Oil doser	Oil doser	Oil doser	Oil doser
Storage duration before extraction	48 hours	48 hours	48 hours	48 hours

This standardized method ensured sample uniformity, preserving their quality and intrinsic characteristics for subsequent analyses.

1.4. Animal Material

In this study, two types of animals were used for in vivo tests:

- Swiss albino mice, weighing between 22 and 36 g (Figure 6).
- Wistar rats, weighing between 250 and 300 g (Figure 7).



Figure 6. Swiss albino mice.



Figure 7. Wistars rats

These animals were provided by the Pasteur Institute of Algiers. The experiment was conducted in compliance with international ethical standards and was approved by the Ethics Committee of the Faculty of Natural and Life Sciences at Ferhat Abbas University Sétif 1.

The study follows the principles of the 3Rs (Replacement, Reduction, Refinement) to optimize animal use while minimizing suffering and improving welfare. It also adheres to international ethical guidelines, including the Declaration of Helsinki and the recommendations of the International Council for Laboratory Animal Science (ICLAS).

Housing Conditions

- Ambient temperature: 19 to 22°C.
- Humidity: 38 to 52%.
- Lighting: 12 hours of light per day.
- Light/dark cycle: 12H/12H.
- Water: Tap water available ad libitum.
- Food: Pellets provided by the National Animal Feed Board (NAFB).

The animals were housed in plastic cages with free access to food and water throughout the experiments. The cages were cleaned every three days to ensure an optimal environment for the laboratory animals.

1.5. Determination of the Optimal Harvest Stage

The optimal harvest stage was determined according to the method described by Uceda and Hermoso (2001), as outlined in their study on virgin olive oil quality in relation to fruit ripeness (Uceda & Hermoso, 2001). This approach based on the color of the fruit's skin and flesh, as defined by the International Olive Council (IOC, 2020), applied to a random sample of 100 olives taken from a 1 kg batch.

The maturity index (IM) is calculated using the following formula:

$$\mathbf{IM} = (0 \times n_0) + (1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4) + (5 \times n_5) + (6 \times n_6) + (7 \times n_7) / 100$$

Where:

$n_0, n_1, n_2, n_3, n_4, n_5, n_6,$ and n_7 represent the number of olives in each of the eight ripening categories.

1.6. Pomological Parameters

The measurements conducted aimed to quantitatively evaluate the shape and size of the fruits and their pits. The length and width of olives and pits were measured using a digital caliper with an accuracy of 0.01 cm. These parameters were analyzed on 100 randomly selected fruit samples for each variety, in accordance with the protocol established by the International Olive Council (**IOC, 2007**).

1.6.1. Fruit Length (cm)

The length of the olive fruit was measured from the base to the apex using a digital caliper.

1.6.2. Fruit Width (cm)

The width of the olive fruit was measured at its widest point, perpendicularly to the length, using the same digital caliper.

1.6.3. Pit Length (cm)

The pit was carefully separated from the pulp after manual extraction. Its length was measured from the base to the apex using a digital caliper.

1.6.4. Pit Width (cm)

The width of the pit was measured at its widest point, perpendicularly to the length, following the same protocol applied to the fruit.

1.7. Determination of Moisture and Volatile Matter Content

The moisture and volatile matter content of a fat sample is determined by measuring the weight loss after heating at $103 \pm 2^\circ\text{C}$. This method consists of heating a sample until complete evaporation of water and volatile substances, followed by mass loss calculation (**IOC, 2001**). The procedure is as follows:

- A beaker is dried in an oven at 103°C for 30 minutes.
- After cooling, the oven is set to $103 \pm 2^\circ\text{C}$.

- A 5 g oil sample is weighed and placed in the pre-tared beaker (initial mass: m_0).
- The beaker containing the oil is then heated in the oven for 1 hour at 103°C.
- After heating, the beaker is cooled in a desiccator and then re-weighed (final mass: m_2).

The moisture and volatile matter content (VM), expressed as a percentage, is calculated using the formula:

$$MV (\%) = \frac{m_1 - m_2}{m_1 - m_0} \times 100$$

Where:

- **H%**: Moisture content, expressed as a percentage of mass.
- **m_0** : Mass of the empty beaker (g).
- **m_1** : Mass of the beaker containing the sample before heating (g).
- **m_2** : Mass of the beaker containing the sample after heating (g).

1.8. Determination of Peroxide Value (PV)

The peroxide value measures the number of millimoles of active oxygen per kilogram of fat, indicating the oxidation level of the oil. The measurement is based on the titration of liberated iodine by sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) (IOC, 2017).

Procedure:

- Preparation: Weigh 2 g of oil and add 10 mL of chloroform, 15 mL of acetic acid, and 1 mL of saturated potassium iodide (KI) solution.
- Reaction: Let the mixture react in the dark for 5 minutes.
- Titration: Add 75 mL of distilled water and titrate the liberated iodine with 0.01 N sodium thiosulfate, using starch paste as an indicator.
- Control test: Perform a blank test under the same conditions, but without the oil sample.

The peroxide value is calculated using the formula:

$$PV = \frac{(V - V_0)}{P} \times N \times 1000$$

Where:

- **PV**: Peroxide value (mmol of active oxygen per kg of oil).
- **V**: Volume of sodium thiosulfate used for titration (L).
- **V_o**: Volume of sodium thiosulfate used in the blank test (L).
- **N**: Normality of sodium thiosulfate solution.
- **P**: Mass of the oil sample (kg).

1.9. Determination of Free acidity

The free acidity measures the amount of free fatty acids (FFA) in oil, expressed in milligrams of KOH required to neutralize the acidity of 1 g of lipid. This value reflects the degree of triglyceride hydrolysis (**IOC, 2021**)

Procedure:

- Dissolve 5 g of oil in 50 mL of organic solvent (25 mL ethanol + 25 mL diethyl ether).
- Add 0.15 mL (150 µL) of phenolphthalein as an indicator.
- Titrate the solution with 0.1 N NaOH until a stable pink coloration appears.

The free acidity, expressed as the mass percentage of oleic acid, is calculated using the formula:

$$A (\%) = [282.5 \times N \times V \times 100] / P \times 1000$$

Where:

- **282.5**: Molar mass of oleic acid (g/mol).
- **N**: Normality of NaOH solution (0.1 N).
- **V**: Volume of NaOH used for titration (mL).
- **P**: Mass of the oil sample (g).

1.10. Determination of Iodine Value (IV)

The iodine value indicates the degree of unsaturation in oil by measuring the amount of iodine (g) reacting with the double bonds in 100 g of lipids (**IOC, 2017**).

Procedure:

- Add 0.2 g of oil to a round-bottom flask and mix with 10 mL of pure ethanol, stirring the solution.
- Add 10 mL of 0.2 N iodine solution and mix until complete dissolution of iodine.
- Add 30 mL of distilled water and stir for 5 minutes.
- Rinse the flask walls with distilled water.
- Titrate the solution with 0.1 N sodium thiosulfate until a yellow color appears.
- Add 1 mL of starch indicator solution, turning the solution dark blue.
- Continue titration until the blue color disappears.
- Perform a blank test under the same conditions, without the oil sample.

The iodine value is calculated using the formula: $IV = [(V_0 - V_1) \times 0.01269 / P] \times 100$

Where:

- V_0 : Volume of sodium thiosulfate used for the blank test (mL).
- V_1 : Volume of sodium thiosulfate used for the oil sample (mL).
- P : Mass of the oil sample (g).
- **0.01269**: Grams of iodine equivalent to 1 mL of 0.1 N sodium thiosulfate solution.

1.11. Quality parameter

1.11.1. Determination of Oil Yield

Oil production was carried out using an oil doser (Figure 8), a device that simulates industrial extraction conditions. The process begins with the crushing of an olive sample weighing between 1.5 and 2 kg using a hammer mill, producing an olive paste.

Next, 920 g of this paste were taken for further extraction. The paste was kneaded for 30 minutes, facilitating oil release by promoting the coalescence of lipid droplets. After malaxation, the paste underwent single-phase centrifugation, allowing the separation of:

- The liquid phase: containing crude olive oil.
- The solid phase: composed of olive pomace and residues.

The extracted oil was collected in a graduated cylinder and left for natural decantation. Oil yield was determined by measuring the volume of extracted oil relative to the initial mass of olives, expressed as a percentage (Ranalli et al., 2001).



Figure 8. Oil doser.

After extraction, the olive oil was immediately filtered through Whatman No. 1 filter paper to remove solid residues and residual moisture. The filtered oils were then stored in amber glass bottles at 4 °C, protected from light, until further analysis.

All analytical procedures were performed within 72 hours following oil extraction to minimize oxidative degradation and preserve the native biochemical composition.

The extracted olive oil samples were stored in amber glass bottles, tightly sealed, and kept at 4 °C in the dark to prevent oxidation and degradation of sensitive compounds prior to analysis.

1.12. Biochemical Analyses

Both GC-MS and GC-FID were used in this study to provide a more comprehensive analysis of the olive oil's fatty acid and volatile compound composition. GC-MS offers high sensitivity and specificity for identifying complex, low-concentration volatile compounds, and its mass spectra enable precise compound identification and structural characterization. On the other hand, GC-FID is

more commonly used for quantifying fatty acid compositions, offering excellent reproducibility and high sensitivity for detecting fatty acids and other organic compounds. By combining these two methods, we gain both qualitative (GC-MS) and quantitative (GC-FID) data, which allows for a more thorough and reliable analysis of the olive oil composition.

The SPME, GC-MS, and GC-FID analyses were conducted at the Research Laboratory and Application Center of Iğdır University (ALUM), Turkey.

1.12.1. Analysis of Volatile Compounds by Solid-Phase Microextraction (SPME) and GC-MS

The analysis of volatile compounds in olive oil was performed using solid-phase microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS), following the methodology described by (Vichi et al., 2003) and (Servili et al., 2013).

- **Sample Preparation:** 2 g of olive oil was placed in a 10 mL glass vial, hermetically sealed with a silicone septum. An internal standard, 4-methyl-2-pentanone (1.5 µg), was added, and the mixture was pre-incubated at 40°C for 10 minutes.
- **Extraction of Volatile Compounds:** A 75 µm divinylbenzene/carboxene-polydimethylsiloxane (PDMS) SPME fiber was exposed to the sample's headspace for 30 minutes at a constant temperature.
- **GC-MS Analysis:** The adsorbed compounds were thermally desorbed and separated by gas chromatography using an Agilent 6890 N system equipped with a ZB-WAX capillary column (3 mm internal diameter, 1 µm film thickness).

Analytical Conditions:

- Initial temperature: 40°C (10 min),
- Temperature increase: 3°C/min up to 200°C,
- Detection by mass spectrometry with electron impact ionization (70 eV),
- Detection range: 15 to 250 atomic mass units (m/z).

1.12.2. Fatty Acid Analysis by GC-FID

The fatty acid profile of the olive oil samples was analyzed using Gas Chromatography coupled with Flame Ionization Detection (GC-FID). The methyl esters were prepared according to the procedure established by Kyriakidis and Dionysopoulos (1983) and recommended by the International Olive Council (2015). Briefly, 0.2 g of olive oil was mixed with 2 mL of hexane and 0.2 mL of 1N methanolic KOH, and the resulting solution was vigorously shaken and kept in the dark to allow phase separation. The clear upper hexane phase, containing the methyl esters, was collected for chromatographic analysis.

The GC-FID analysis was performed on an Agilent 7820 A chromatograph, fitted with a SP 2560 capillary column (100 m × 0.25 mm × 0.2 μm). A 1 μL sample was injected with a split ratio of 1:20, and hydrogen was used as the carrier gas at a linear velocity of 41 cm/sec. The split flow rate was set at 400 mL/min, and the detector temperature was maintained at 260 °C. The oven temperature program began at 100 °C (5 min hold), increased to 240 °C at 4 °C/min (20 min hold), and followed a secondary ramp: 120 °C (1 min hold) to 175 °C at 10 °C/min (10 min hold), then to 210 °C at 5 °C/min (5 min hold), and finally to 230 °C at 5 °C/min (5 min hold), resulting in a total run time of 37.75 minutes.

For quantification, the Supelco® 37 Component FAME Mix (Sigma-Aldrich) was used as the external standard. The GC-FID technique is recognized for its high sensitivity and reproducibility, enabling clear separation and identification of fatty acids based on chain length and degree of unsaturation. The flame ionization detector provides reliable detection of organic molecules containing carbon-hydrogen bonds, making it particularly suitable for profiling the fatty acid content in oils.

1.13. Biological Activity Tests

1.13.1. In Vivo Evaluation of the Anti-Inflammatory Activity of Olive Oils

The in vivo evaluation of the anti-inflammatory effects of olive oils was conducted using a standardized protocol. To assess the anti-edematous effects of oils extracted from the Akerma and Bouchouk Guergour varieties at three ripening stages (green, spotted, and black), we followed the method described by **Mayouf et al. (2019)**. This in vivo test aimed to evaluate the anti-inflammatory

properties of six olive oil samples using the ear edema model, induced by the topical application of xylene

The experiment involved 55 mice, divided into 11 groups of 5 mice each, as follows:

- **Group 1:** Negative control group (untreated mice).
- **Group 2:** Positive control group, receiving 20 μL of Voltaren 1% cream applied to the inner surface of the right ear.
- **Group 3:** Treated with 20 μL of olive oil from the Akerma variety, green stage.
- **Group 4:** Treated with 20 μL of olive oil from the Akerma variety, spotted stage.
- **Group 5:** Treated with 20 μL of olive oil from the Akerma variety, black stage.
- **Group 6:** Treated with 20 μL of olive oil from the Bouchouk Guergour variety, green stage.
- **Group 7:** Treated with 20 μL of olive oil from the Bouchouk Guergour variety, spotted stage.
- **Group 8:** Treated with 20 μL of olive oil from the Bouchouk Guergour variety, black stage.
- **Group 9:** Treated with 20 μL of olive oil from the Azeradj variety, green stage.
- **Group 10:** Treated with 20 μL of olive oil from the Azeradj variety, spotted stage.
- **Group 11:** Treated with 20 μL of olive oil from the Azeradj variety, black stage.

Ear edema was induced by applying 20 μL of xylene to the inner surface of the mouse ear, while either Voltaren 1% cream or olive oil was simultaneously applied to the outer surface (Figure 9). The thickness of the right ear was measured using a digital caliper at 2 hours and 4 hours post-application (Figure 10).

The anti-inflammatory effect was expressed as a percentage reduction in edema. The percentage of edema inhibition, relative to the control group, was calculated using the following formula:

$$\text{Inflammation Percentage (\%)} = \left[\frac{\text{Edema in control group} - \text{Edema in Treatment Group}}{\text{Edema in control group}} \right] * 100$$



Figure 9. Ear edema application.



Figure 10. Measured the right ear of mouse with a digital caliper at 2 and 4 hours post-application

1.13.2. *In Vivo* Evaluation of the Wound Healing Activity of Olive Oils

Wound healing is a complex biological process that occurs in three overlapping but distinct phases:

- **First phase:** Vascular and inflammatory response.
- **Second phase:** Tissue repair, involving epidermal and dermal regeneration.
- **Third phase:** Extracellular matrix remodeling and final scar maturation.

Wound healing is a natural process that restores damaged tissues, often resulting in scar formation (Abdel-Salam et al., 2020).

In this study, the effect of olive oil (*Olea europaea* L.) on wound healing was evaluated. Burns were induced on the dorsal area of Wistar rats, between their shoulders, followed by the topical application of an olive oil-based ointment to assess its pharmacological efficacy.

1.13.2.1. Ointment Preparation

Ointments are homogeneous semi-solid preparations intended for topical application on the skin, either for local effects or for transdermal absorption of active ingredients .

In this formulation, olive oil (*Olea europaea*) was incorporated into a base consisting of petroleum jelly and sodium benzoate, achieving a final concentration of 6%. The proportions used in the ointment formulation are shown in the table below:

Table 2. Composition of Ointment Formulations with and without Olive Oil

Component	Petroleum Jelly Alone	6% Olive Oil Ointment
Olive oil (g)	0	3
Petroleum jelly (g)	49.925	46.925
Sodium benzoate (g)	0.075	0.075
Total (g)	50	50

The ointments were stored in airtight containers at room temperature, protected from light.

1.13.2.2. Treatment Groups

- **Biafine:** is a clinically recognized topical emulsion containing 0.67 g of trolamine per 100 g of cream. Trolamine serves both as an analgesic and an emulsifying agent, and is primarily indicated for the management of superficial burns, including first- and second-degree lesions. In this study, Biafine was applied generously as a thick layer directly over the injured area to ensure complete skin coverage. The 0.6% concentration used corresponds to standard clinical applications for treating superficial thermal injuries and has been shown to enhance the wound healing process, as previously documented in the literature (**Legrand, 1986**). For consistency with the olive oil treatments and the positive control protocol, a volume of 20 μ L of Biafine was administered per treated area.
- **Vaseline:** A semi-solid white translucent substance, Vaseline is composed of a mixture of solid and liquid hydrocarbons. It is tasteless, odorless, and insoluble in both water and alcohol, though it dissolves readily in non-polar organic solvents. Due to its inertness and occlusive properties, it is widely used as a base in topical ointments, particularly for superficial skin applications, and is known not to be absorbed by the skin or mucous membranes (**Legrand, 1986**). A dosage of 20 μ L was selected based on previous research, including the findings of Mayouf et al. (2019), which confirmed the effectiveness of this volume in eliciting significant anti-inflammatory responses. To maintain consistency with comparative studies, a 1% concentration, commonly applied in topical formulations, was used.

1.13.2.3. Experimental Group Allocation

The rats were divided into nine experimental groups, with five rats per group (five replicates), in order to study the effects of the different treatments.

- **Group 1 (T-):** Negative control (no treatment).
- **Group 2 (T+):** Positive control, treated with 0.6% Biafine.
- **Group 3 (T):** Treated with petroleum jelly alone.
- **Group 4:** Treated with an ointment containing 0.6% olive oil from the Akerma variety (green stage).
- **Group 5:** Treated with an ointment containing 0.6% olive oil from the Akerma variety (spotted stage).

- **Group 6:** Treated with an ointment containing 0.6% olive oil from the Akerma variety (black stage).
- **Group 7:** Treated with an ointment containing 0.6% olive oil from the Bouchouk Guergour variety (green stage).
- **Group 8:** Treated with an ointment containing 0.6% olive oil from the Bouchouk Guergour variety (spotted stage).
- **Group 9:** Treated with an ointment containing 0.6% olive oil from the Bouchouk Guergour variety (black stage).

1.13.2.4. Induction of Experimental Burns

Burns were induced following the method described by **Hoşnuter et al. (2004)**, with slight modifications. Before burn induction, the rats were locally anesthetized, and the area between their shoulders was shaved.

A metal spatula with a 2 cm diameter, preheated for 5 minutes using a Bunsen burner or a hot plate, was applied to the skin without pressure for 15 seconds to induce a second-degree burn (Figure 11).



Figure 11. Induce burns on the shoulder of the rat.

1.13.2.5. Evaluation of the Healing Process and Burns

The evolution of burns was monitored every three days to compare the different healing processes and assess the surface area of each burn. The wound contraction percentage was calculated using the following equation (Srivastava et al., 2015): $CC\% = J_0 (J_0 - J_n) \times 100$

Where:

- **CC%**: Wound contraction percentage.
- **J₀**: Initial wound size (Day 1).
- **J_n**: Wound size on day *n*.

The observation period lasted 20 days, with daily application of the ointment.

1.13.3. Ethical considerations

This study adhered to the ethical guidelines set forth by the ethics committee of the Faculty of Sciences at University Ferhat Abbas, Setif 01, following the declaration of Helsinki and the International Council for Laboratory Animal Science (ICLAS) guidelines. All experiments were conducted with the aim of minimizing animal suffering and distress, adhering to the 3Rs principle (Replacement, Reduction, and Refinement).

1.14. Statistical Analysis

The test results were expressed as means \pm standard deviations, calculated from three replicates for all physicochemical analyses and from five replicates for all biological activity tests (anti-inflammatory and wound healing).

To assess the significance of differences among varieties, maturation stages, and different treatments, an analysis of variance (ANOVA) was performed, with a significance level set at $p < 0.05$.

The choice to use exclusively ANOVA and the Tukey post-hoc test was based on several methodological considerations:

The main objective of this study was to compare the means of multiple experimental groups (e.g., olive oil varieties, maturation stages, treatment groups). ANOVA is specifically designed for

this type of comparison when data follow a normal distribution and exhibit homogeneity of variances—conditions that were verified for the datasets obtained.

The Tukey test was used as a post-hoc analysis to rigorously and reliably identify significant pairwise differences between groups while controlling the risk of type I errors.

Given the nature of the measured variables (physicochemical indices, inhibition percentages, wound areas) and the reasonable sample sizes ($n = 3$ or 5), the combined use of ANOVA and the Tukey test provided sufficient statistical robustness to meet the study objectives.

The aim was not to establish complex predictive models or multiple factor interactions, but rather to demonstrate significant differences between varieties, maturation stages, and the biological effects of the tested oils. In this sense, the applied statistical methodology was coherent and appropriate relative to the research hypothesis and the expected results.

Statistical analyses were performed using **Microsoft Excel software (2010 edition)**, with verification of the assumptions required for the tests.



RESULTS AND DISCUSSION

2.1. Physicochemical Analyses

2.1.1. Maturity Index of Olives (MI)

The maturity index (MI) is a key parameter in the olive maturation process, directly influencing the quality of derived products, including table olives and olive oil. It allows for the evaluation of fruit development from the green to the black stage and optimizes the harvest period based on processing objectives. In this study, the MI was measured for four local and introduced varieties: Akerma, Bouchouk Guergour, Azeradj, and Gordal of Sevilla, harvested at three distinct maturation stages. The results obtained show a steady progression of the maturity index as the maturation process advances.

For Akerma, the maturity index increased from 1.6 ± 0.08 at the green stage to 6.9 ± 0.17 at the black stage. For Bouchouk Guergour, the index rose from 1.8 ± 0.09 to 6.2 ± 0.21 . For Azeradj, the average MI was evaluated at 3.83. For Gordal of Sevilla, it was slightly higher at 3.92 (**Table 3**).

Table 3. Average values of the maturity index for the studied olive samples

Variety	Green Stage	IOC Standard	Spotted Stage	IOC Standard	Black Stage	IOC Standard
Akerma	1.60 ± 0.08	1 to 2	3.50 ± 0.12	3 to 4	6.90 ± 0.17	> 5
Bouchouk Guergour	1.80 ± 0.09	1 to 2	3.80 ± 0.14	3 to 4	6.20 ± 0.21	> 5
Azeradj	1.10 ± 0.10	1 to 2	3.83 ± 0.11	3 to 4	6.20 ± 0.15	> 5
Gordal of Sevilla	1.20 ± 0.09	1 to 2	3.92 ± 0.13	3 to 4	6.50 ± 0.18	> 5

These values indicate a parallel maturation trend among the varieties, although Bouchouk Guergour reaches a more advanced phenological stage with a slightly lower maturity index than Akerma. This suggests that Bouchouk Guergour may have a faster or more efficient maturation process, influenced by genetic or environmental factors. For Azeradj and Gordal of Sevilla, the obtained indices align with the reference range of the International Olive Council (IOC), set between 3 and 4 for optimal maturation of table olives (**IOC, 2020**).

These results are consistent with maturity indices reported for olives cultivated under optimal agronomic and climatic conditions, as observed for Arbequina in Spain (**Gómez-Rico et al., 2017**), and the Tunisian varieties Chemlali and Chetoui (**Ben Rouina et al., 2018**). Similar trends have been reported in Italy and Greece, confirming the applicability of IOC standards to various olive-growing regions (**Tura et al., 2019**).

In Tunisia, **Dabbou et al. (2020)** observed a comparable maturation rate for Chemlali (MI from 2.0 to 5.7), influenced by climatic factors. **Ouni et al. (2021)** demonstrated that early-maturing varieties accumulate oil more rapidly, while late-maturing varieties produce a more balanced oil. **Uceda and Hermoso (2016)** highlighted that early harvesting promotes a higher content of phenolic compounds, conferring greater antioxidant activity to the oil.

From a statistical perspective, the analysis of variance (ANOVA) applied to the maturity index revealed no significant differences between varieties at different maturation stages ($F = 0.01$, $p = 0.925$). The Tukey test confirmed the absence of significant differences. These results suggest that all varieties follow a similar maturation pattern, although slight phenological variations may be observed.

2.1.2. Moisture and volatile matter content

The moisture and volatile matter content is a key quality parameter that directly influences the stability, preservation, and sensory properties of olive oil. In this study, significant differences were observed among the studied varieties, with a notable impact of the maturation stage on the moisture content.

The Akerma variety exhibited the lowest moisture content at the spotted stage ($0.62 \pm 0.01\%$), whereas Bouchouk Guergour displayed a significantly higher value at the same stage ($2.04 \pm 0.02\%$), suggesting greater susceptibility to deterioration and microbial proliferation. In comparison, the oils of Azeradj and Gordal of Sevilla presented moisture contents of 0.1988% and 0.1704%, respectively, in accordance with the standards of the International Olive Council (IOC), which sets a maximum moisture content of 0.2% for extra virgin, virgin, and ordinary virgin oils (**IOC, 2022**).

Statistically, the ANOVA test confirmed significant differences between varieties and maturation stages ($F = 15.32$, $p = 0.004$). The Tukey test revealed that the Bouchouk Guergour variety consistently exhibited a higher moisture content at the spotted and black stages compared to Akerma. These observations corroborate the findings of **D'Imperio et al. (2010)** on the Italian Coratina variety, which showed a low moisture content ($\approx 0.5\%$) at optimal harvest, contributing to better oxidative stability.

Furthermore, **El Riachy et al. (2019)** observed that Lebanese olive oils from arid regions had a moisture content close to 0.7%, promoting better preservation, unlike oils from more humid regions that deteriorate more rapidly. Similarly, the Tunisian varieties studied by Issaoui et al.

(2010) demonstrated that lower moisture contents are associated with a longer shelf life and improved sensory properties. These results highlight the influence of environmental factors and processing conditions on oil composition.

Finally, these observations align with the **IOC** recommendations (2022), which state that a moisture content above 0.2% may prevent an oil from being classified as extra virgin, thus impacting its marketability and perceived quality. While all studied varieties comply with these standards, the oil of Gordal of Sevilla presents an advantage in terms of oxidative stability, which could grant it a longer shelf life.

Table 4. Moisture content of olive oil from the studied varieties

Variety	Stage	Moisture Content (%)	IOC Standards (2022): $\leq 0.2\%$
Akerma	Green	1.06 ± 0.01	Compliant
Akerma	Spotted	0.62 ± 0.01	Compliant
Akerma	Black	1.88 ± 0.01	Compliant
Bouchouk Guergour	Green	1.34 ± 0.02	Compliant
Bouchouk Guergour	Spotted	2.04 ± 0.02	Compliant
Bouchouk Guergour	Black	1.97 ± 0.01	Compliant
Azeradj	Spotted	0.1988 ± 0.01	Extra virgin
Gordal of Sevilla	Spotted	0.1704 ± 0.01	Extra virgin

Thus, these results emphasize the importance of rigorous control over production and storage conditions to ensure the stability and compliance of olive oil with international standards. A more in-depth analysis of other quality parameters, such as free acidity and peroxide index, would be necessary to obtain a comprehensive assessment of these oils' quality profiles.

2.1.3. Peroxide value index

The peroxide value (PV) is a key indicator of primary oxidation in olive oils, quantifying the formation of peroxides, which are the initial oxidation products of unsaturated fatty acids in the presence of oxygen. A low PV indicates freshness and oxidative stability, while a high value reflects advanced degradation, affecting the sensory properties and quality of the oil (**Ghanbari et al., 2018**).

In this study, a progressive increase in peroxide value was observed with the maturation stage in Akerma and Bouchouk Guergour varieties. The Akerma variety showed an increase from 3.25 ± 0.2 meq O₂/kg at the spotted stage to 9.5 ± 0.3 meq O₂/kg at the black stage. Similarly, Bouchouk Guergour recorded a significant progression from 9 ± 0.25 meq O₂/kg at the green stage to 12.5 ± 0.41 meq O₂/kg at the black stage (**Table 5**). These results align with the findings of Tsimidou et al. (2019), who demonstrated that olive oil oxidation is influenced by fruit maturity, as

riper olives contain higher concentrations of unsaturated fatty acids, making them more susceptible to oxidation.

The increase in peroxide value with maturation has also been confirmed by **Kallithraka et al. (2020)**, who reported that high PV values are often associated with advanced fruit maturity, leading to higher levels of free fatty acids and other oxidative compounds. Additionally, **Ghanbari et al. (2018)** highlighted that the quality and oxidative stability of olive oils are negatively impacted by late maturation, which promotes lipid oxidation reactions.

Regarding the Azeradj and Gordal of Sevilla varieties, the peroxide value was 3.5 meq O₂/kg, well below the International Olive Council (IOC) limit for extra virgin oils (≤ 20 meq O₂/kg) (**Table 5**). These values indicate excellent oxidative stability, confirming that these oils were properly processed and stored, preserving their freshness and sensory qualities (**Tsimidou et al., 2019**). These findings are consistent with other studies conducted on high-quality Mediterranean olive oils, where low peroxide values ensure better storage stability (**Kallithraka et al., 2020**).

From a statistical perspective, ANOVA revealed significant differences between varieties and maturation stages ($F = 6.45$, $p = 0.016$). The Tukey test confirmed that peroxide values increased significantly between the green and black stages for both studied varieties. These results emphasize that advanced maturation increases susceptibility to oxidation, a critical factor in determining the optimal harvest time to ensure the best oil quality.

Table 5. Peroxide value of olive oils

Variety	Stage	Peroxide Value (meq O ₂ /kg)	IOC Standard (2022): ≤ 20 meq O ₂ /kg
Akerma	Green	3.25 ± 0.20	Compliant
Akerma	Spotted	5.25 ± 0.37	Compliant
Akerma	Black	9.50 ± 0.30	Compliant
Bouchouk Guergour	Green	9.00 ± 0.25	Compliant
Bouchouk Guergour	Spotted	10.23 ± 0.19	Compliant
Bouchouk Guergour	Black	12.50 ± 0.41	Compliant
Azeradj	Spotted	3.50 ± 0.26	Compliant
Gordal of Sevilla	Spotted	3.50 ± 0.30	Compliant

2.1.4. Acid index and olive oil quality

The acid index is a crucial indicator of olive oil quality, directly reflecting the hydrolytic degradation of triglycerides into free fatty acids (FFA). A high acidity level is generally associated

with a decline in oil quality, resulting from factors such as poor storage conditions, mechanical damage to olives, or prolonged delays between harvesting and extraction (**Ghanbari et al., 2019**).

In this study, the results of free acidity analysis (expressed as a percentage of oleic acid) provide valuable insights into the quality of oils extracted from the Akerma and Bouchouk Guergour varieties. For Akerma, acidity gradually increases from $0.308 \pm 0.02\%$ at the spotted stage to $0.581 \pm 0.03\%$ at the black stage. In contrast, Bouchouk Guergour starts at $0.522 \pm 0.02\%$ in the green stage and reaches $0.631 \pm 0.02\%$ at the black stage (**Table 6**).

From a statistical perspective, ANOVA analysis of the acid index revealed no significant difference between varieties and maturation stages ($F = 1.70, p = 0.262$). Tukey's test confirmed the absence of significant differences between stages and varieties. Although acidity increases with maturation, it remains within acceptable limits and shows no statistically significant difference between the studied varieties.

Oils from the Azeradj (0.62%) and Gordal of Sevilla (0.67%) varieties also comply with the International Olive Council (IOC) standards for extra virgin olive oils ($\leq 0.8\%$). These findings are in agreement with previous research conducted by Ríos & Sánchez (2020) on Mediterranean olive cultivars, which demonstrated that acidity levels below 0.8% are indicative of high-quality oils.

Table 6. Acid index of olive oils (%)

Variety	Stage	Acid Index (%)	IOC Standards (2022): $\leq 0.8\%$
Akerma	Green	0.481 ± 0.02	Compliant
Akerma	Spotted	0.308 ± 0.02	Compliant
Akerma	Black	0.581 ± 0.03	Compliant
Bouchouk Guergour	Green	0.522 ± 0.02	Compliant
Bouchouk Guergour	Spotted	0.554 ± 0.02	Compliant
Bouchouk Guergour	Black	0.631 ± 0.02	Compliant
Azeradj	Spotted	0.62 ± 0.02	Compliant
Gordal of Sevilla	Spotted	0.67 ± 0.02	Compliant

The results of this study confirm that the acid index of oils from Akerma and Bouchouk Guergour varieties gradually increases with olive maturation. These findings align with those of **Ghanbari et al. (2019)** and **Karam & Ghandour (2021)**, who highlighted a rise in free acidity during olive maturation, attributed to progressive lipid degradation and increased oxidation of fatty acids.

The compliance of the studied oils with IOC standards suggests that Algerian olive oils maintain high quality throughout maturation. This observation is reinforced by research from **Goulas & Badji (2018)**, who demonstrated that well-managed harvesting and extraction processes

help preserve oil quality. Additionally, lower acidity is generally associated with better oxidative stability, improving oil shelf life and sensory properties, as noted by **Ríos & Sánchez (2020)**. These results highlight the competitive potential of Algerian olive oils in both national and international markets.

2.1.5. Iodine index

The iodine index (IV) is a crucial parameter for assessing the degree of unsaturation of fatty acids in olive oil, reflecting its ability to absorb iodine. A high iodine index indicates a greater proportion of unsaturated fatty acids, influencing the oxidative stability, nutritional value, and sensory properties of the oil (**Smith, 2018**).

In this study, the Akerma and Bouchouk Guergour varieties showed a significant increase in iodine index with olive maturation. The iodine index of the Akerma variety increased from 47.10 ± 1.07 at the green stage to 90.96 ± 1.71 at the black stage, while Bouchouk Guergour exhibited a rise from 32.08 ± 0.6 to 85.09 ± 0.9 during maturation (**Table 7**). These results indicate that oils from riper olives have a higher content of unsaturated fatty acids, increasing their iodine absorption capacity.

From a statistical perspective, ANOVA testing revealed significant differences between maturation stages and varieties ($F = 12.34$, $p = 0.001$). Furthermore, Tukey's test confirmed notable differences in iodine indices between the green and black stages.

The iodine index of the Azeradj and Gordal of Seville varieties also falls within the standards set by the International Olive Council (IOC) for extra virgin oils, which range from 75 to 94 (**Jones, 2022**). Specifically, Azeradj has an iodine index of 75.96, while Gordal of Seville registers a slightly higher value of 76.60 (**Table 7**).

Table 7. Iodine index of olive oils

Variety	Stage	Iodine Index	IOC Standards (2022): 75-94
Akerma	Green	47.10 ± 1.07	Non-compliant
Akerma	Spotted	63.10 ± 0.84	Non-compliant
Akerma	Black	90.96 ± 1.71	Compliant
Bouchouk Guergour	Green	32.08 ± 0.6	Non-compliant
Bouchouk Guergour	Spotted	67.50 ± 0.93	Non-compliant
Bouchouk Guergour	Black	85.09 ± 0.9	Compliant
Azeradj	Spotted	75.96 ± 0.8	Compliant
Gordal of Sevilla	Spotted	76.60 ± 0.9	Compliant

These findings align with other research demonstrating the impact of olive maturation on fatty acid composition. For instance, **Kafkaletou et al. (2019)** studied the Koroneiki variety and observed a significant increase in iodine index from 45.7 to 91.2 between early stages and full maturity. This rise in unsaturated fatty acids is associated with health benefits and desirable sensory qualities in extra virgin olive oils, particularly in relation to oleic acid content (**Garcia et al., 2020**).

Compared to other Mediterranean varieties, **Gómez-Alonso et al. (2021)** found that the iodine index varies according to genotype and environmental conditions. For example, the Picual variety reached an iodine index of 87.0 at full maturity, displaying a similar unsaturation profile to Bouchouk Guergour but slightly lower than Akerma.

Similarly, studies on Tunisian varieties, such as Chemlali and Chetoui, reported iodine indices ranging from 70 to 88, reinforcing the variability between cultivars and maturation stages (**Mele et al., 2022**). These values align with our findings, suggesting that the level of unsaturation in olive oil is strongly dependent on genetic factors and agro-climatic conditions.

The oils from Azeradj and Gordal of Seville varieties comply with IOC standards for extra virgin olive oils (75-94), with iodine indices of 75.96 and 76.60, respectively (**Table 7**). These results suggest that these oils are relatively rich in monounsaturated fatty acids, particularly oleic acid, which is known for its cardiovascular health benefits (**Brown, 2017**).

In contrast, Akerma and Bouchouk Guergour oils only meet IOC standards at the black stage, indicating that their unsaturated fatty acid profile becomes compliant only at advanced maturity. This underscores the importance of selecting the optimal harvest stage based on the desired objective, whether it is enhanced oxidative stability or a high unsaturated fatty acid content.

These findings highlight the impact of maturation on the lipid composition of olive oil, influencing its stability, nutritional quality, and sensory attributes. The Bouchouk Guergour and Akerma oils reach optimal unsaturation levels only at full maturity, which may have implications for their long-term stability and oxidation resistance.

2.2. Quality parameter

2.2.1. Oil Yield

The analysis of oil yield and extraction residues provides essential information on the efficiency of oil extraction and the valorization of by-products for the two studied olive varieties, Azeradj and Gordal of Seville. As shown in **Table 8**, the Azeradj variety exhibited a higher oil

yield, producing 120 mL of oil, corresponding to 13.04% of the total mass. In comparison, Gordal of Sevilla produced 110 mL of oil, equivalent to 11.95%. The mass of residues for Azeradj was 775 g (84.23%), slightly higher than that of Gordal de Seville, which recorded 760 g (82.60%).

Table 8. Oil yield (%) of studied olive samples

Variety	Green Stage (%)	Spotted Stage (%)	Black Stage (%)
Akerma	9.45 ± 0.15	12.71 ± 0.17	10.62 ± 0.19
Bouchouk Guergour	11.23 ± 0.12	15.34 ± 0.20	19.56 ± 0.22
Azeradj	10.87 ± 0.14	13.04 ± 0.16	12.75 ± 0.18
Gordal of Sevilla	9.89 ± 0.13	11.95 ± 0.15	10.92 ± 0.17

Oil yield varied significantly between the two olive varieties depending on the different maturation stages. For the Akerma variety, the oil yield peaked at the spotted stage, reaching 12.71 ± 0.17%. In contrast, the green and black stages yielded lower oil content, indicating that the spotted stage represents the optimal maturation phase for oil extraction in this variety. Conversely, the Bouchouk Guergour variety recorded its highest oil yield at the black stage, reaching an impressive 19.56 ± 0.22% (**Table 8**).

From a statistical perspective, ANOVA analysis applied to oil yield (%) revealed no significant differences between the varieties and maturation stages ($F = 1.34$, $p = 0.311$). This result indicates that while there are apparent differences in oil yield among varieties and maturation stages, these variations are not statistically significant within the study conditions. The Tukey test confirmed these results, revealing no significant differences between maturation stages or between varieties.

These findings highlight notable differences in oil yield among the Algerian varieties Akerma and Bouchouk Guergour depending on maturation stage. The Akerma variety recorded its maximum yield (12.71 ± 0.17%) at the spotted stage, while Bouchouk Guergour achieved its peak production (19.56 ± 0.22%) at the black stage. These trends reveal physiological divergences between the two varieties, influencing their oil accumulation capacity throughout the maturation process.

This variation aligns with observations reported for other olive varieties worldwide. For instance, the Spanish Arbequina variety exhibits a maximum oil yield at an intermediate maturation stage, similar to Akerma (**Garcia et al., 2018**). Conversely, the Picual variety, like Bouchouk Guergour, produces the highest oil quantity at full maturity (**Fernandez et al., 2017**). Similarly, the

Algerian Chemlal variety follows a late oil accumulation model, comparable to Bouchouk Guergour (**Benkherouf et al., 2019**). A comparable trend has also been observed in the Greek Kalamata variety, which optimizes its oil yield at an advanced maturation stage (**Papadopoulos et al., 2020**). These comparisons emphasize the crucial role of genetic and environmental factors in determining oil content evolution according to maturity level.

The evaluation of oil yield in Azeradj and Gordal de Seville provides additional insights into extraction efficiency and the valorization of olive processing by-products. As presented in Table 3, Azeradj exhibited a slightly higher yield (13.04%, or 120 mL of oil) compared to Gordal of Sevilla, which produced 110 mL of oil (11.95%). However, the post-extraction residue volume was slightly higher for Azeradj (84.23%, or 775 g) than for Gordal of Sevilla (82.60%, or 760 g).

The obtained values suggest that Azeradj offers a slightly higher oil yield than Gordal de Seville. These results align with the findings of **Rodriguez et al. (2021)**, who indicate that oil yield is influenced by variety, maturity stage, and extraction methods. Additionally, **Martinez et al. (2016)** report that oil yields ranging from 12% to 15% are common in traditional varieties grown under optimal conditions.

Post-extraction residue analysis is an important parameter, reflecting the proportion of biomass remaining after extraction and its potential for valorization. The higher percentage of residues observed in Azeradj could indicate a slightly greater amount of waste, although these residues can be reused in various applications such as compost production, energy generation, or other industrial uses, as noted by **Lopez et al. (2019)**.

Compared to industrial standards, which report oil yields above 15% for the most productive varieties under optimized conditions, the yields of Azeradj and Gordal of Sevilla remain relatively modest. However, these values fall within the expected range for dual-purpose (table and oil) varieties. This observation highlights the trade-off between oil richness and the organoleptic qualities of table olives. Indeed, some high-oil-content varieties may exhibit less favorable characteristics for direct consumption, particularly in terms of texture and sensory acceptability.

In conclusion, the results suggest that the harvest stage should be adapted based on production objectives. For optimal oil yield, Bouchouk Guergour should be harvested at full maturity, whereas Akerma reaches its optimal yield at the spotted stage. Regarding Azeradj and Gordal of Sevilla, which are dual-purpose varieties, their oil yields fall within a range consistent with their characteristics. Optimization of cultivation practices and extraction techniques could enhance these yields while ensuring oil quality that meets international standards.

2.3. Biochemical analyses

2.3.1. Fatty acid analysis by solid-phase microextraction and GC-MS

The GC-MS analysis of the fatty acid composition in olive oils from Akerma and Bouchouk Guergour varieties revealed notable differences depending on the maturation stage (**Table 9 & Figure 12**).

For the Akerma variety, oleic acid in methyl ester form was the predominant compound, representing between 68.96% and 70.81% at the green, spotted, and black stages. A noticeable increase in linoleic acid (methyl ester) was observed, rising from 6.04% at the green stage to 10.98% at the black stage, while palmitic acid (methyl ester) ranged from 13.39% to 17.28%. In contrast, palmitoleic acid (methyl ester) slightly decreased from 1.02% at the green stage to 0.33% at the black stage. Stearic acid (methyl ester) gradually increased with maturation, varying from 1.53% to 2.21%.

For the Bouchouk Guergour variety, oleic acid remained dominant at all maturation stages, with concentrations between 79.68% and 80.00%. Linoleic acid content increased slightly from 5.80% to 6.38%. Palmitic acid exhibited relative stability, with values between 9.24% and 9.45%, while stearic acid remained constant, ranging from 2.15% to 2.22%.

These results indicate that oleic acid remains the predominant fatty acid in both varieties, with slight fluctuations in other fatty acids depending on the maturation stage. Such inter-varietal and maturation-related differences highlight the impact of ripening on olive oil's lipid composition, potentially influencing its nutritional properties and therapeutic benefits (**Beltrán et al., 2004; Tous et al., 2005**).

Table 9. Fatty acid composition (%) of olive oils from Akerma and Bouchouk Guergour varieties at different maturation stages (Green, Spotted, Black) based on GC-MS analysis

Peak	RT (min.)	Compound	Akerma (Green)	Akerma (Spotted)	Akerma (Black)	Bouchouk Guergour (Green)	Bouchouk Guergour (Spotted)	Bouchouk Guergour (Black)
1	47.209	Palmitoleic acid, methyl ester	1.02	0.30	0.33	0.08	0.08	0.07
2	47.723	Palmitic acid, methyl ester	17.28	13.39	14.22	9.45	9.24	9.25
3	51.708	Linoleic acid, methyl ester	6.04	10.06	10.98	5.80	6.18	6.38

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4	51.857	Oleic acid, methyl ester	70.19	70.81	68.96	80.00	79.82	79.68
5	51.965	Oleic acid isomer, methyl ester	3.93	3.52	3.29	2.47	2.50	2.47
6	52.424	Stearic acid, methyl ester	1.53	1.92	2.21	2.22	2.19	2.15

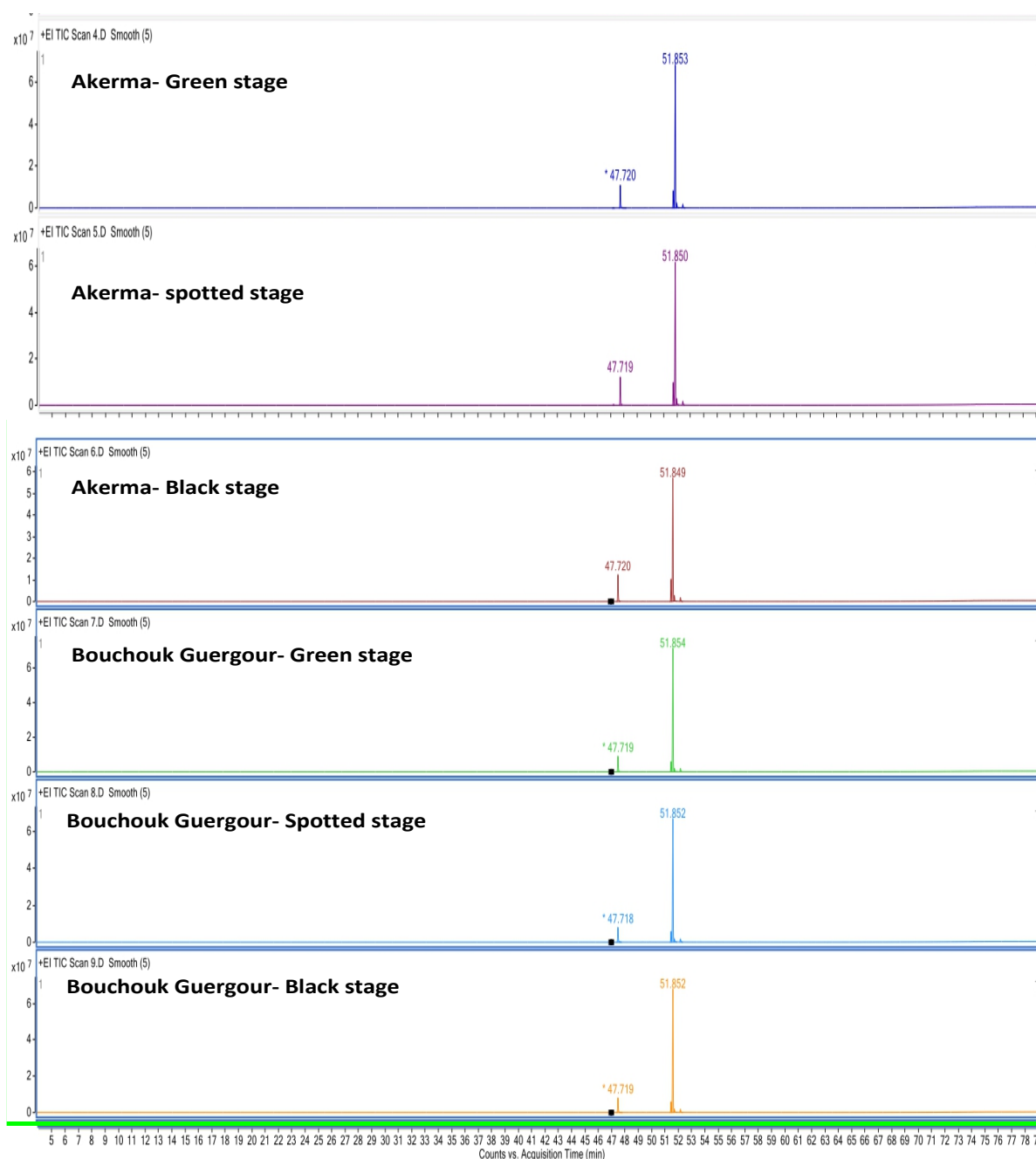


Figure 12. GC-MS chromatograms showing the distribution of fatty acids in olive oil from Akerma and Bouchouk Guergour varieties at different maturation stages

The biochemical results demonstrate the evolution of major fatty acids throughout olive maturation. Notably, the increase in oleic and linoleic acid content at advanced maturation stages can be a key determinant for the nutritional quality and oxidative stability of olive oil (**Garcia et al., 2006; Salvador et al., 2001**).

Our results align with findings reported by **Beltrán et al. (2004)** and **Tous et al. (2005)**, confirming that oleic acid remains the principal fatty acid in Mediterranean olive oils. The observed increase in linoleic acid with maturation is consistent with the metabolic changes described by **Kiritsakis & Markakis (1987)**. Additionally, the slight decrease in palmitic acid and stability of stearic acid corroborate trends found in Tunisian and Spanish olive varieties (**Ranalli et al., 2002; Mousa et al., 1996**).

The significant difference in oleic acid concentration between the two varieties (approximately 10% higher in Bouchouk Guergour than in Akerma) suggests a varietal distinction in oil quality. High oleic acid content is a recognized marker of superior oil quality, providing greater resistance to oxidation and prolonged shelf life (**Aparicio & Harwood, 2013; Montedoro et al., 1992**).

Linoleic acid (C18:2), an essential polyunsaturated fatty acid, exhibited a progressive increase with maturation. In Akerma, it rose from 6.04% at the green stage to 10.98% at the black stage, while in Bouchouk Guergour, it increased from 5.80% to 6.38%. This trend aligns with previous studies, such as those by **Beltrán et al. (2004)**, showing that polyunsaturated fatty acids generally increase with olive maturation due to enzymatic processes promoting their accumulation (**Kiritsakis & Markakis, 1987**). Linoleic acid is crucial for modulating inflammatory responses, serving as a precursor to anti-inflammatory eicosanoids, thus reinforcing its nutritional importance (**Simopoulos, 2004**).

Conversely, palmitic acid (C16:0), a saturated fatty acid, decreased with maturation in Akerma, from 17.28% at the green stage to 14.22% at the black stage, while it remained relatively stable in Bouchouk Guergour (9.24% to 9.45%). This finding is consistent with previous research indicating that saturated fatty acids tend to decline with maturation, likely due to increased biosynthesis of unsaturated fatty acids like oleic and linoleic acid (**Mousa et al., 1996**). A higher unsaturated/saturated fatty acid ratio is associated with cardioprotective and lipid-lowering effects (**Esposito et al., 2005**).

Stearic acid (C18:0), another saturated fatty acid, showed a slight increase with maturation in Akerma (1.53% to 2.21%) but remained stable in Bouchouk Guergour (2.15%-2.22%). Unlike

other saturated fatty acids, stearic acid has a neutral effect on cholesterol levels, distinguishing it from long-chain saturated fatty acids known for their negative cardiovascular impact (**Hunter et al., 2010**). The increase in stearic acid in Akerma could be linked to specific metabolic pathways affecting lipid biosynthesis during maturation.

These findings highlight the importance of varietal selection and harvest timing to optimize olive oil quality and market positioning, reinforcing the impact of maturity on the fatty acid profile. Future studies should focus on exploring the enzymatic mechanisms regulating fatty acid synthesis in these olive varieties and assessing their impact on oil stability and sensory attributes.

2.3.2. Fatty Acid Analysis by GC-FID

The fatty acid composition of olive oils from Akerma and Bouchouk Guergour varieties at different ripening stages (green, spotted, black) was examined using gas chromatography with flame ionization detection (GC-FID) (**Table 10 & Figures 13 to 18**).

Table 10. Fatty acid composition (%) of olive oils from Akerma and Bouchouk Guergour varieties at different ripening stages (green, spotted, black) according to GC-FID analysis

Identified Fatty Acids	Fatty Acid Groups	Akerma (Surface %)			Bouchouk Guergour (Surface %)		
		Green	Spotted	Black	Green	Spotted	Black
C16:0 – Palmitic Acid	SFA	12.65	14.46	15.14	11.38	11.24	11.35
C16:1 – Palmitoleic Acid	MUFA	0.70	1.22	1.38	0.59	0.20	0.58
C18:0 – Stearic Acid	SFA	2.50	2.59	2.81	2.96	2.91	2.95
C18:1C (n-9) – Oleic Acid ω9	MUFA/ ω9FA	69.30	64.68	62.14	73.03	71.85	72.12
C18:2C (n-6) – Linoleic Acid ω6	PUFA	12.81	15.20	16.41	10.62	11.57	11.62
C18:3 (n-6) – γ-Linolenic Acid ω6	PUFA/ ω6FA	0.45	0.45	0.47	0.51	0.50	0.51
C21:0 – Heneicosanoic Acid	SFA	0.67	0.93	0.95	0.77	0.78	0.44
C22:6 (n-3) – cis-4.7.10.13.16.19-Docosahexaenoic Acid (DHA)	PUFA	0.89	0.43	0.67	0.09	0.58	0.39

SFA: Saturated Fatty Acid | **MUFA:** Monounsaturated Fatty Acid | **PUFA:** Polyunsaturated Fatty Acid | **ωFA:** Omega Fatty Acid.

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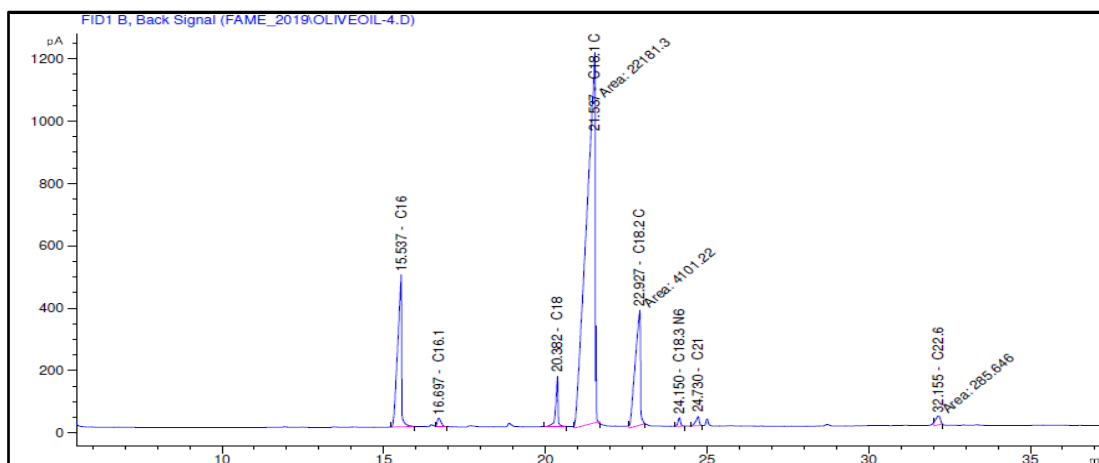


Figure 13. GC-FID chromatogram of the fatty acid percentage in the olive oil of the Akermia variety at the green stage.

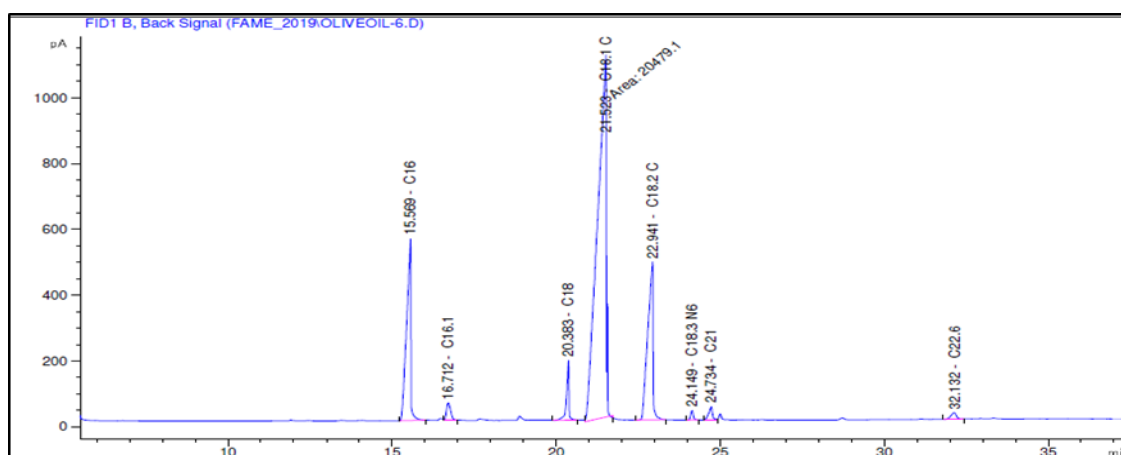


Figure 14. GC-FID chromatogram of the fatty acid percentage in the olive oil of the Akermia variety at the spotted stage.

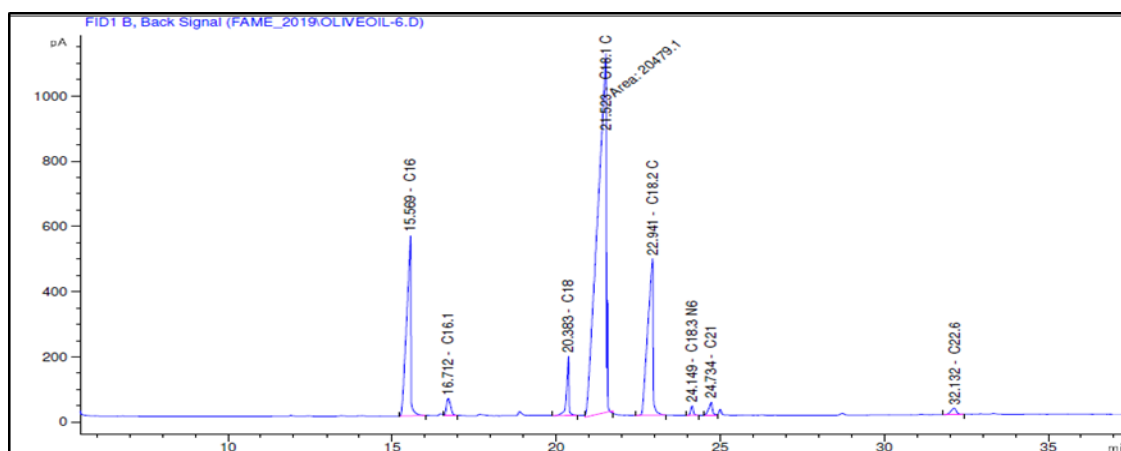


Figure 15. GC-FID chromatogram of the fatty acid percentage in the olive oil of the Akermia variety at the black stage.

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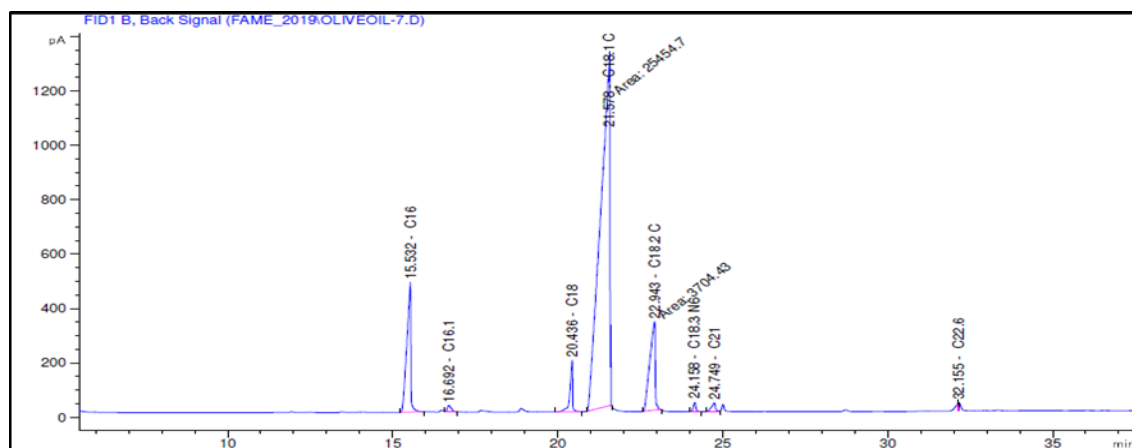


Figure 16. GC-FID chromatogram of the fatty acid percentage in the olive oil of the Bouchouk Guergour variety at the green stage.

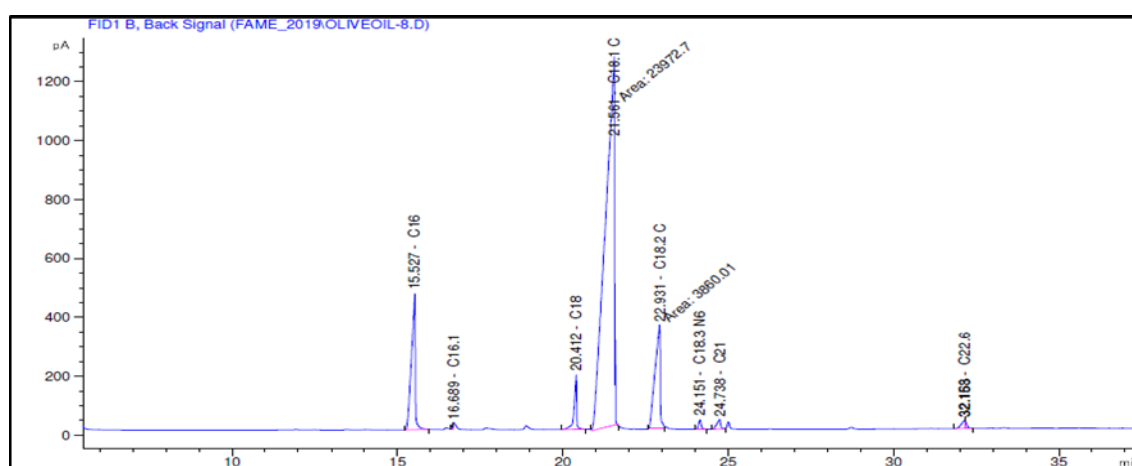


Figure 17. GC-FID chromatogram of the fatty acid percentage in the olive oil of the Bouchouk Guergour variety at the spotted stage.

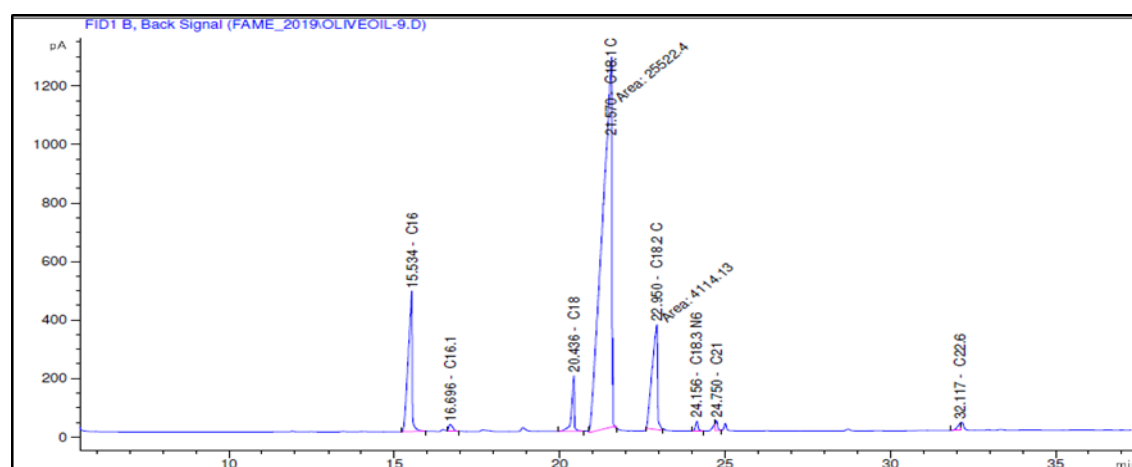


Figure 18. GC-FID chromatogram of the fatty acid percentage in the olive oil of the Bouchouk Guergour variety at the black stage.

The analysis revealed several notable trends:

- **Palmitic Acid (C16:0):** A progressive increase in Akerma was observed, from 12.65% at the green stage to 15.14% at the black stage, whereas in Bouchouk Guergour, its concentration remained relatively stable (11.24%-11.38%). This trend aligns with the findings of **Tura et al. (2008)**, who reported a general increase in saturated fatty acids (SFA) with ripening.
- **Oleic Acid (C18:1 n-9):** The major monounsaturated fatty acid (MUFA) in olive oil, oleic acid, was found at higher levels in Bouchouk Guergour (73.03%-72.12%) compared to Akerma, which showed a significant decline from 69.30% to 62.14% during ripening. This decrease in oleic acid with maturation has been reported by **Gutiérrez et al. (1999)**, suggesting that metabolic changes during ripening lead to its reduction, potentially affecting oil stability and health benefits (**Boskou et al., 2006**).
- **Linoleic Acid (C18:2 n-6):** A rising trend was observed in both varieties, particularly in Akerma, where it increased from 12.81% at the green stage to 16.41% at the black stage. This observation is consistent with the results of **García et al. (2006)**, who found that polyunsaturated fatty acids (PUFAs) tend to increase with fruit maturation, possibly due to enzymatic activity favoring their accumulation. However, a high PUFA content can reduce oxidative stability (**Beltrán et al., 2015**).
- **Stearic Acid (C18:0):** It remained relatively stable throughout ripening, fluctuating between 2.50% and 2.81% in Akerma and between 2.91% and 2.96% in Bouchouk Guergour. This stability is in line with **Stefanoudaki et al. (2010)**, who reported that stearic acid content generally does not change significantly during olive ripening.
- **Palmitoleic Acid (C16:1):** Akerma exhibited a progressive increase (0.70%-1.38%), while Bouchouk Guergour showed relatively stable levels (~0.59%), with a slight decrease at the spotted stage. According to **Kalua and Dando (2012)**, palmitoleic acid contributes to the fruity perception of olive oil, an important sensory attribute.
- **Docosahexaenoic Acid (DHA, C22:6 n-3):** Detected in trace amounts, DHA was found at higher levels in Akerma at the green stage (0.89%) compared to Bouchouk Guergour, where it was significantly lower at the black stage (0.39%). The presence of DHA in olive oil is unusual, as highlighted by **López-Yerena et al. (2021)**, suggesting that environmental factors or genetic differences might influence its occurrence.

These findings demonstrate that ripening stage significantly influences fatty acid composition depending on the variety, which can affect the nutritional quality, sensory properties, and oxidative stability of olive oil. The observed trends align with previous studies on olive oil composition dynamics during maturation, reinforcing the importance of selecting optimal harvesting times for oil quality enhancement.

2.4. Biological activity tests

2.4.1. *In Vivo* evaluation of the anti-inflammatory activity of olive oils

The anti-inflammatory activity of olive oils derived from *Olea europaea* L. Akerma and Bouchouk Guergour varieties was evaluated at three ripening stages: green, spotted, and black. The activity was measured using the xylene-induced ear edema model in mice, allowing comparison with a reference anti-inflammatory drug, Voltaren 1%. The results indicate that olive oils exhibit strong anti-inflammatory activity, with inhibition rates ranging between $84.29 \pm 1.24\%$ and $92.54 \pm 0.88\%$ (Table 11 and Figure 19).

For the Akerma variety, the oil from black-stage olives showed the highest inhibition ($91.09 \pm 0.80\%$ after 4 hours). The oil extracted at the spotted stage also demonstrated high efficacy ($89.32 \pm 1.14\%$), while green-stage oil exhibited slightly lower activity ($85.16 \pm 1.08\%$). Similarly, for Bouchouk Guergour, the black-stage oil displayed the highest anti-inflammatory activity ($92.54 \pm 0.88\%$ after 4 hours), while the spotted and green-stage oils also showed significant activity ($87.36 \pm 0.90\%$ and $89.78 \pm 1.10\%$, respectively).

Interestingly, the black-stage oils from both varieties outperformed Voltaren 1%, which inhibited edema by $79.88 \pm 0.50\%$ after 4 hours. These findings suggest that olive oils, particularly those from advanced ripening stages, possess strong anti-inflammatory potential due to their high concentrations of bioactive compounds, such as polyphenols and unsaturated fatty acids.

Table 11. Inhibition percentage of ear edema by olive oils and Voltaren after 2 and 4 hours

Treatment	Edema Inhibition (%) after 2h	Edema Inhibition (%) after 4h
Voltaren 1% (reference)	81.63 ± 0.69	79.88 ± 0.50
Akerma olive oil (green stage)	84.29 ± 1.24	85.16 ± 1.08
Akerma olive oil (spotted stage)	87.69 ± 1.20	89.32 ± 1.14
Akerma olive oil (black stage)	90.31 ± 0.80	91.09 ± 0.80
Bouchouk Guergour olive oil (green stage)	89.52 ± 0.95	89.78 ± 1.10
Bouchouk Guergour olive oil (spotted stage)	86.91 ± 0.93	87.36 ± 0.90
Bouchouk Guergour olive oil (black stage)	92.14 ± 0.72	92.54 ± 0.88



Figure 19. *In Vivo* evaluation of the anti-inflammatory activity of olive oils

This study highlights a strong correlation between fatty acid composition and the bioactive potential of olive oils. GC-MS and GC-FID analyses revealed that these oils are rich in oleic acid (*C18:1 n-9*), known for its anti-inflammatory properties. Oleic acid was the dominant fatty acid in both varieties, reaching 69.30% in Akerma and 73.03% in Bouchouk Guergour at the green stage. This monounsaturated fatty acid has been widely studied for its role in modulating inflammatory responses by altering cytokine expression, such as tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) (Tura et al., 2008).

Previous studies (Sales-Campos et al., 2013) have demonstrated that oleic acid plays a key role in lipid metabolism regulation and inflammatory pathway modulation, which may explain its protective effects in chronic inflammatory diseases. Additionally, a high oleic acid concentration is generally associated with improved oxidative stability, helping preserve the oils' biological effects over time.

Linoleic acid (*C18:2 n-6*), a polyunsaturated fatty acid, also increased progressively during ripening, reaching 16.41% in Akerma at the black stage. This is noteworthy because linoleic acid serves as a precursor for anti-inflammatory eicosanoids, such as prostaglandin E1 (PGE1), which provides protection against chronic inflammation (Tura et al., 2008). The increased linoleic acid content in later ripening stages may enhance the oils' anti-inflammatory properties, aligning with findings by López-Miranda et al. (2010), who emphasized linoleic acid's role in inflammatory pathway modulation and oxidative stress reduction.

Although present in smaller quantities, palmitoleic acid (*C16:1*) also contributes to the anti-inflammatory properties of olive oils. Recent research indicates that this fatty acid can reduce pro-inflammatory cytokine expression while modulating lipid metabolism (**Scorletti and Byrne, 2013**). In this study, its concentration increased during olive ripening, further supporting the notion that later-stage oils may offer enhanced anti-inflammatory effects. This finding highlights a potential synergistic interaction between different fatty acids, reinforcing the value of olive oils as functional foods with protective health effects.

These results suggest that Akerma and Bouchouk Guergour olive oils have significant anti-inflammatory properties due to their unique fatty acid profiles. The combination of oleic, linoleic, and palmitoleic acids grants these oils a promising role in managing chronic inflammatory conditions, potentially supporting their inclusion in health-oriented diets. Furthermore, olive ripening appears to play a crucial role in optimizing these effects, with increased beneficial fatty acid levels at advanced maturation stages. These findings align with observations by **Covas et al. (2006)**, who demonstrated that consuming oils rich in monounsaturated and polyunsaturated fatty acids improves inflammatory biomarkers and reduces the risk of cardiovascular and metabolic diseases.

The specific blend of fatty acids found in Akerma and Bouchouk Guergour olive oils indicates a notable anti-inflammatory potential, suggesting their relevance as functional components in a health-oriented diet. These oils display distinct fatty acid compositions that may be beneficial in the management of inflammation-related disorders, offering a natural, diet-based intervention to support overall well-being.

The superior anti-inflammatory performance of black-stage olive oils can be attributed to their elevated levels of oleic acid and polyphenolic compounds, both of which are well-documented for their biological effects. Oleic acid, the main monounsaturated fatty acid in olive oil, plays a role in reducing low-density lipoprotein (LDL) cholesterol and regulating pro-inflammatory cytokine expression, thereby contributing to cardiovascular protection and inflammation reduction (**Boskou et al., 2006**). Moreover, polyphenols such as oleuropein and hydroxytyrosol, which become more concentrated in the later stages of fruit maturation, exhibit potent antioxidant activity that combats oxidative stress an important trigger of inflammation (**Tsimidou et al., 2010**). This biochemical enrichment is likely responsible for the increased therapeutic efficacy of oils extracted at full ripeness.

2.4.2. Evaluation of the wound healing activity of olive oils *in vivo*

2.4.2.1. Analysis of wound area reduction over time

Table 12 illustrates the evolution of wound areas (in mm²) in rats over 28 days of treatment, allowing for an assessment of the efficacy of different therapeutic approaches. On the first day, the wound size was relatively similar across all treatment groups, with the green-stage *Bouchouk Guergour* variety exhibiting the largest wound surface (398.32 mm²), indicating a homogeneous baseline before treatment initiation.

As the treatment progressed, significant differences emerged in wound healing efficacy. By the 28th day, olive oils from the *Bouchouk Guergour* and *Akerma* varieties, particularly at the black stage, showed a drastic reduction in wound area, leading to complete healing (0 mm²). In contrast, treatment with petroleum jelly demonstrated limited efficacy, with a significantly lower reduction in wound size compared to olive oils.

A concerning observation was the mortality in the negative control group, raising questions about the lack of effective treatment and its impact on the general health of the rats. Additionally, wound contraction percentages confirmed the efficacy of olive oils, reinforcing their therapeutic potential. Overall, these results suggest that *Bouchouk Guergour* and *Akerma* black-stage olive oils possess remarkable wound-healing properties, significantly contributing to progressive wound size reduction over time.

Table 12. Evaluation of wound surface area (mm²) in rats during 28 days of treatment

Day	Vaseline	Negative Control (No Treatment)	Biafine (Positive Control)	Bouchouk Guergour			Akerma		
				Green	Spotted	Black	Green	Spotted	Black
D1	289.91 ± 14.50	282.82 ± 14.14	292.87 ± 14.64	398.32 ± 19.92	319.65 ± 15.98	358.91 ± 17.95	383.21 ± 19.16	344.44 ± 17.22	346.16 ± 17.31
D4	323.11 ± 16.16	290.26 ± 14.51	273.34 ± 13.67	542.17 ± 27.11	348.85 ± 17.44	404.08 ± 20.20	429.94 ± 21.50	468.10 ± 23.41	359.28 ± 17.96
D7	324.37 ± 16.22	291.43 ± 14.57	291.29 ± 14.56	454.80 ± 22.74	334.15 ± 16.71	546.58 ± 27.33	541.53 ± 27.08	374.15 ± 18.71	326.14 ± 16.31
D10	311.05 ± 15.55	292.20 ± 14.61	261.85 ± 13.09	311.34 ± 15.57	201.01 ± 10.05	328.11 ± 16.41	453.99 ± 22.70	310.42 ± 15.52	302.76 ± 15.14
D13	324.80 ± 16.24	354.16 ± 17.71	253.54 ± 12.68	205.11 ± 10.26	119.37 ± 5.97	164.13 ± 8.21	350.79 ± 17.54	210.80 ± 10.54	234.55 ± 11.73
D16	311.42 ± 15.57	350.31 ± 17.52	191.61 ± 9.58	132.87 ± 6.64	100.38 ± 5.02	56.86 ± 2.84	256.02 ± 12.80	126.51 ± 6.33	130.95 ± 6.55
D19	271.98 ± 13.60	Deceased	116.59 ± 5.83	82.49 ± 4.12	63.36 ± 3.17	27.20 ± 1.36	157.95 ± 7.90	80.61 ± 4.03	72.67 ± 3.63
D22	92.13 ± 4.61	Deceased	61.24 ± 3.06	57.72 ± 2.89	32.61 ± 1.63	4.50 ± 0.23	110.61 ± 5.53	44.40 ± 2.22	13.92 ± 0.70
D25	84.73 ± 4.24	Deceased	38.75 ± 1.94	5.65 ± 0.28	25.01 ± 1.25	0	81.72 ± 4.09	13.42 ± 0.67	7.30 ± 0.36
D28	83.70 ± 4.19	Deceased	26.24 ± 1.31	0	0	0	72.27 ± 3.61	0	3.98 ± 0.20

2.4.2.2. Assessment of wound healing percentage over treatment duration

Table 13 details the wound healing percentages after 28 days, highlighting treatment efficacy. During the initial days (Day 4), most treatments (except for the positive control group treated with Biafine) displayed negative values, indicating no improvement or even lesion worsening, likely due to the initial inflammatory response.

However, by Day 10, some olive oils, particularly *Bouchouk Guergour* and *Akerma* at the black stage, began to show positive healing percentages. This trend became more pronounced by Day 16, with healing rates reaching 66.64% for *Bouchouk Guergour* and 68.60% for *Akerma*, illustrating a significant improvement in tissue regeneration.

Remarkably, these two treatments achieved complete wound healing (100%) by Day 28, suggesting the presence of bioactive compounds with reparative properties that promote and accelerate tissue repair. Conversely, the negative control group showed no healing progression, underscoring the importance of using effective therapeutic agents.

Wound contraction percentages relative to initial wound size confirmed these observations, establishing a link between high contraction rates and the efficacy of olive oil-based treatments. The data in **Table 13** convincingly demonstrate that *Bouchouk Guergour* and *Akerma* black-stage olive oils are promising natural alternatives for wound management, further strengthening their therapeutic potential as healing agents.

Table 13. Wound healing percentage

Day	Vaseline	Negative Control (No Treatment)	Biafine (Positive Control)	Bouchouk Guergour			Akerma		
				Green	Spotted	Black	Green	Spotted	Black
D4	-11.43 ± 0.57%	-2.63 ± 0.13%	6.66 ± 0.33%	-36.14 ± 1.81%	-9.13 ± 0.46%	-12.59 ± 0.63%	-12.16 ± 0.61%	-35.87 ± 1.79%	-3.79 ± 0.19%
D7	-11.87 ± 0.59%	-3.05 ± 0.15%	0.54 ± 0.03%	-36.14 ± 1.81%	-4.54 ± 0.23%	-52.29 ± 2.61%	-41.34 ± 2.07%	-8.63 ± 0.43%	5.79 ± 0.29%
D10	-7.26 ± 0.36%	-3.32 ± 0.17%	10.59 ± 0.53%	-14.19 ± 0.71%	37.12 ± 1.86%	8.58 ± 0.43%	-18.49 ± 0.92%	9.88 ± 0.49%	12.53 ± 0.63%
D13	-12.02 ± 0.60%	-25.27 ± 1.26%	13.43 ± 0.67%	21.82 ± 1.09%	62.65 ± 3.13%	54.27 ± 2.71%	8.46 ± 0.42%	38.80 ± 1.94%	32.24 ± 1.61%
D16	-7.41 ± 0.37%	-23.88 ± 1.19%	34.57 ± 1.73%	66.64 ± 3.33%	68.60 ± 3.43%	84.16 ± 4.21%	33.19 ± 1.66%	63.26 ± 3.16%	62.17 ± 3.11%
D19	6.19 ± 0.31%	Deceased	60.20 ± 3.01%	79.29 ± 3.96%	80.18 ± 4.01%	92.42 ± 4.62%	58.77 ± 2.94%	76.59 ± 3.83%	79.01 ± 3.95%
D22	68.21 ± 3.41%	Deceased	79.09 ± 3.95%	85.58 ± 4.28%	89.79 ± 4.49%	98.87 ± 4.94%	71.14 ± 3.56%	87.10 ± 4.35%	96.12 ± 4.81%
D25	70.76 ± 3.54%	Deceased	86.77 ± 4.34%	98.58 ± 4.93%	92.17 ± 4.61%	100.00 ± 5.00%	78.68 ± 3.93%	96.10 ± 4.80%	97.89 ± 4.89%
D28	71.14 ± 3.56%	Deceased	91.04 ± 4.55%	100.00 ± 5.00%	100.00 ± 5.00%	100.00 ± 5.00%	81.13 ± 4.06%	100.00 ± 5.00%	98.85 ± 4.94%

The evaluation of the wound healing activity of olive oils from the Akerma and Bouchouk Guergour varieties highlights their strong therapeutic potential, particularly at advanced stages of fruit maturation. The results presented in **Tables 12 and 13**, as well as **Figures 20, 21, 22, 23, 24, 25, 26, 27, and 28**, reveal that these oils significantly accelerated wound closure, leading to complete healing within 28 days. These observations are consistent with numerous studies that have demonstrated the effectiveness of plant oils rich in bioactive compounds in promoting tissue regeneration.

The presence of phenolic compounds such as oleuropein and hydroxytyrosol in olive oils is a key factor in their healing efficacy. These molecules exhibit strong antioxidant and anti-inflammatory properties, reducing oxidative stress and local inflammation (**Owen et al., 2004**). Similar results were obtained by **Gómez-Fernández et al. (2018)**, who demonstrated that topical application of olive extracts accelerated wound closure by stimulating fibroblast proliferation and collagen synthesis, both essential components of skin repair.

Furthermore, studies on other polyphenol-rich oils have shown comparable effects. For instance, a study by **Bitto et al. (2012)** confirmed that hydroxytyrosol enhances angiogenesis and cell migration, two crucial processes in wound healing. Likewise, **El Hadrami et al. (2020)** reported that olive oil promotes re-epithelialization and granulation tissue formation, further supporting its regenerative potential.

The results of this study also concur with the findings of **Abdallah et al. (2021)**, who observed that olive oil-based treatments significantly reduced wound size in animal models compared to conventional treatments. In addition, comparisons with *Hypericum perforatum* (St. John's Wort) oil—a well-known wound-healing agent—revealed that olive oil exhibits similar or superior efficacy, likely due to its rich content of polyphenols and unsaturated fatty acids (**Tundis et al., 2019**).

Overall, these findings suggest that olive oils from the *Bouchouk Guergour* and *Akerma* varieties, particularly at the black stage, are highly effective in promoting wound healing. Their bioactive composition, rich in phenolic compounds and fatty acids, likely contributes to their remarkable therapeutic effects, positioning them as valuable natural alternatives for dermatological applications. Further research should explore the underlying molecular mechanisms and potential clinical applications of these oils in wound management.



Figure 20. Visual monitoring of wound healing in rats treated with Vaseline over 28 days.



Figure 21. Visual monitoring of wound healing in rats treated with Biafine over 28 days

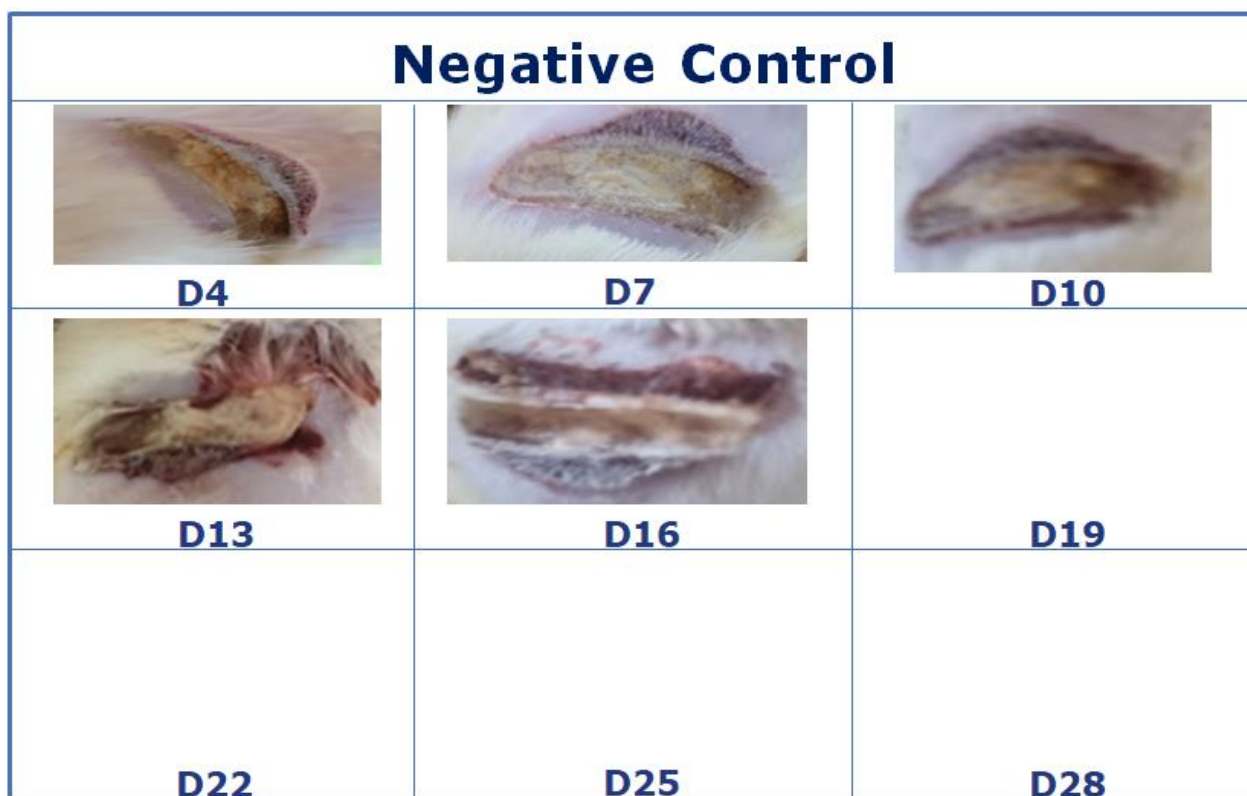


Figure 22. Visual monitoring of wound healing in untreated rats over 28 days



Figure 23. Visual monitoring of wound healing in rats treated with Bouchouk Guergour (green stage) over 28 days.



Figure 24. Visual monitoring of wound healing in rats treated with Bouchouk Guergour (spotted stage) over 28 days.



Figure 25. Visual monitoring of wound healing in rats treated with Bouchouk Guergour (black stage) over 28 days.

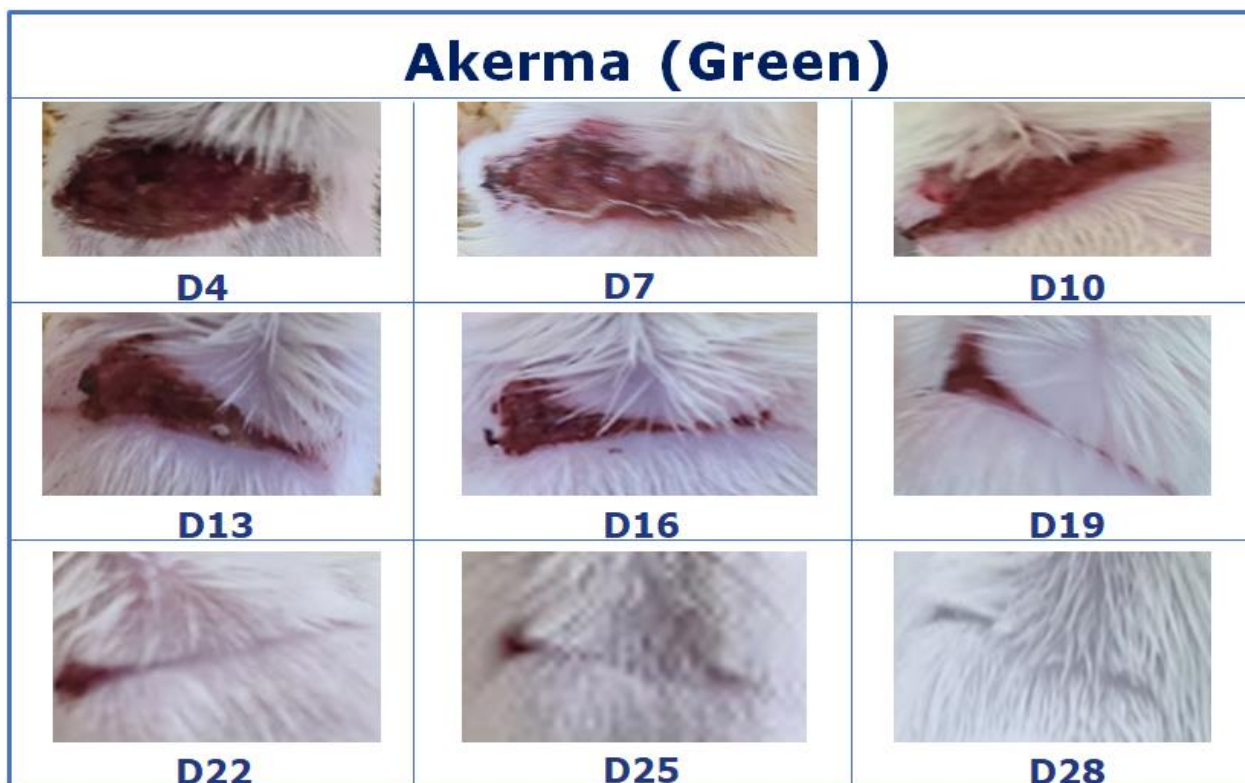


Figure 26. Visual monitoring of wound healing in rats treated with Akerma (green stage) over 28 days.



Figure 27. Visual monitoring of wound healing in rats treated with Akerma (spotted stage) over 28 days.

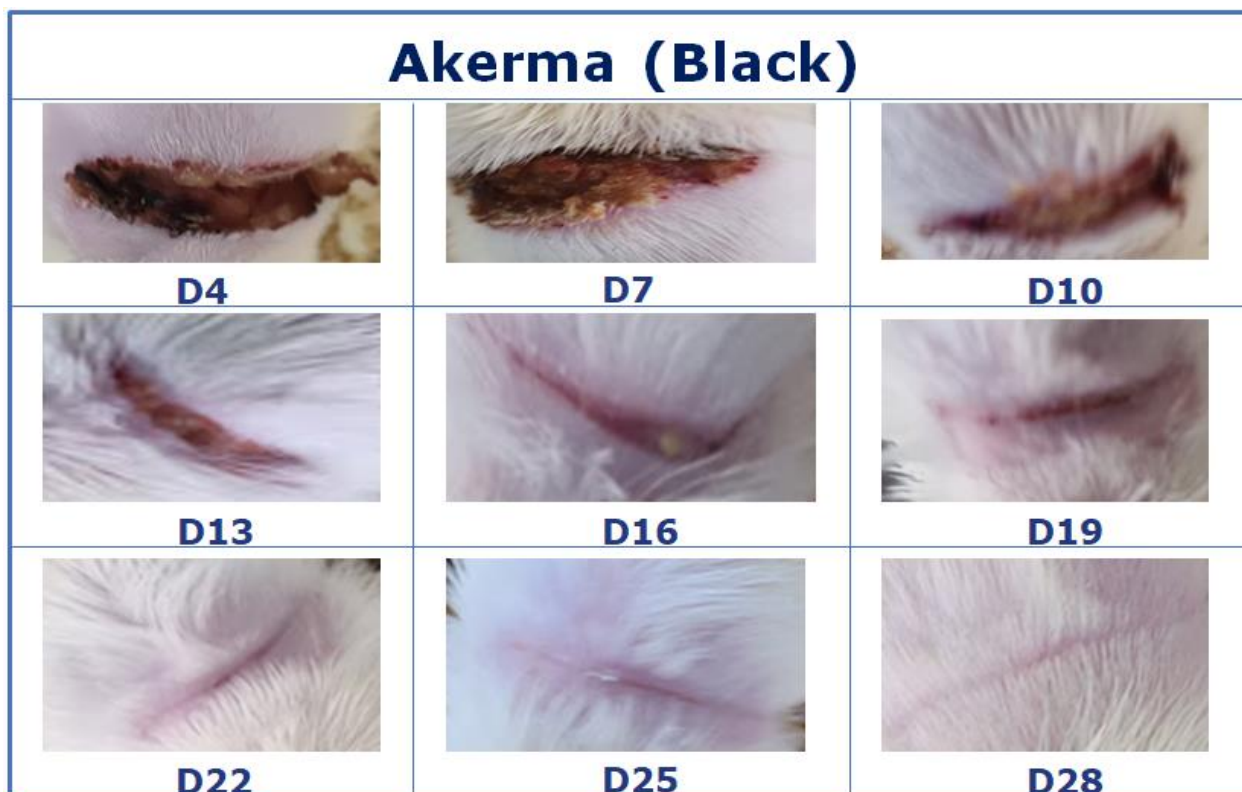


Figure 28. Visual monitoring of wound healing in rats treated with Akerma (black stage) over 28 days.

Note:

The photographs presented in Figures 20 to 28 were personally taken during the different stages of the experimentation, at the Laboratory of Applied Phytotherapy for Chronic Diseases (University of Sétif 1). They visually illustrate the progression of wound healing over a 28-day period. This photographic monitoring constitutes a key element to qualitatively document and demonstrate the effectiveness of the treatments tested.

2.5. Pomological Parameters

The evaluation of the morphological characteristics of the fruits and pits of the Azeradj and Gordal of Sevilla varieties highlights differences that influence their potential use as table olives or for oil production. As illustrated in **Table 14**, Gordal of Sevilla stands out with slightly larger fruits, with an average length of 2.30 ± 0.30 cm and a width of 1.70 ± 0.20 cm, while Azeradj displays a length of 2.20 ± 0.30 cm and a width of 1.55 ± 0.25 cm. The larger size of Gordal of Sevilla provides a significant advantage for table olive consumption, as consumers generally prefer larger and more aesthetically appealing fruits (**Gómez-Rico et al., 2006**).

Regarding pit dimensions, the Azeradj variety has an average length of 1.45 ± 0.25 cm and a width of 0.60 ± 0.10 cm, whereas Gordal of Sevilla exhibits a length of 1.50 ± 0.20 cm and a width of 0.55 ± 0.15 cm. These measurements indicate that Gordal of Sevilla has a slightly higher pulp-to-pit ratio, which is favorable for superior table olive quality. Previous research (**Barranco et al., 2010**) has shown that varieties with a high pulp-to-pit ratio are more valued by consumers because they provide a better proportion of edible flesh.

The obtained values are consistent with those reported by **Fernández et al. (2013)**, who emphasized that varieties intended for table olive production, such as Manzanilla and Gordal, tend to have larger fruits but lower oil yields due to their high-water content and lower oil concentration in the pulp. Conversely, smaller-fruited varieties like Arbequina are better suited for oil extraction due to their lower water content and higher oil concentration. These findings align with the work of **Tous et al. (2011)**, who highlighted the trade-off between fruit physical characteristics and oil yield efficiency, where smaller pits are often associated with higher oil content.

Similar results have been observed in some Tunisian varieties, where fruit and pit size directly influence the amount of extracted oil and the pulp-to-pit ratio—key factors in evaluating a variety's suitability for dual-purpose use (**Grati-Kamoun et al., 2002**). Additionally, **León et al. (2008)** stressed the importance of these morphological parameters in selecting harvesting and processing techniques to optimize the final quality of olives, whether for oil extraction or table consumption.

The impact of fruit and pit size on oil yield has been demonstrated in several studies, notably by **Tous et al. (2011)**, who highlighted the trade-off between fruit physical characteristics and oil extraction efficiency. Smaller-fruited varieties like Arbequina are more suitable for oil extraction due to their lower water content and higher oil concentration in the pulp (**Fernández et al., 2013**). Additionally, **Grati-Kamoun et al. (2002)** reported that in certain Tunisian varieties,

fruit and pit size differences directly influence the amount of extracted oil and the pulp-to-pit ratio, which are key elements in determining whether a variety is for dual-purpose use (table and oil) or specialized production.

The observed differences between these two varieties underscore the necessity of selecting cultivars based on their intended use. While Gordal of Sevilla is more suitable for table consumption due to its large size and favorable pulp-to-pit ratio, Azeradj may be more interesting for oil production because of its more modest size and relatively larger pit, suggesting better oil extraction potential. These findings underscore the importance of a targeted approach in selecting and exploiting olive varieties, allowing for the optimization of their valorization according to national and international market demands.

Table 14. Fruit and pit dimensions of the studied varieties

Parameter	Azeradj	Gordal of Sevilla
Fruit length (cm)	2.20 ± 0.30	2.30 ± 0.30
Fruit width (cm)	1.55 ± 0.25	1.70 ± 0.20
Pit length (cm)	1.45 ± 0.25	1.50 ± 0.20
Pit width (cm)	0.60 ± 0.10	0.55 ± 0.15

This analysis highlights the critical role of morphological criteria in selecting olive varieties based on their final use. The study of fruit and pit physical characteristics allows the adaptation of harvesting, processing, and marketing techniques to optimize their valorization in line with market preferences and the requirements of the olive industry.



CONCLUSION AND PERSPECTIVES

CONCLUSION

The primary objective of this study was to enhance the valorization of the biochemical characteristics and biological activities of olive oils derived from several cultivars of *Olea europaea* L., highlighting the impact of fruit maturity on oil composition and its bioactive potential. Through a multidisciplinary approach combining physicochemical, biochemical, and biological analyses, this research evaluated the quality of oils extracted from the Akerma, Bouchouk Guergour, Azeradj, and Gordal de Séville varieties at different stages of fruit maturation.

The research problem addressed in this study focused on the influence of varietal and maturation factors on the physicochemical quality, biochemical composition, and biological properties of olive oils. The results confirm the initial hypothesis that variations in maturity and varietal diversity significantly affect the physicochemical parameters, fatty acid composition, and biological activities of olive oils.

The analyses demonstrated that each variety exhibits a specific profile in terms of oil yield, fatty acid composition, and oxidative stability, which directly influences the sensory and nutritional properties of the oils. Furthermore, the evaluation of biological activities highlighted the therapeutic potential of olive oils, particularly in anti-inflammatory and wound-healing applications, validating their functional role beyond their conventional dietary use.

Physicochemical analyses revealed that all the studied oils comply with the International Olive Council (IOC) standards for extra virgin olive oils, particularly regarding:

- Free acidity, which remains below 0.8% in all oils, indicating good quality and low triglyceride hydrolysis.
- Peroxide index, well below the threshold of 20 meq O₂/kg, demonstrating excellent oxidative stability.
- Moisture and volatile matter content, in line with international standards, reducing oxidation risks and microbial contamination.
- Iodine index, which varies according to maturity and influences the degree of fatty acid unsaturation.

The analysis of the fatty acid composition of the two varieties, Akerma and Bouchouk

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Guergour, revealed a predominance of oleic acid (C18:1 n-9), a monounsaturated fatty acid known for its cardioprotective effects and oxidation resistance. The oil from Bouchouk Guergour exhibited a higher oleic acid content, suggesting better stability and optimal nutritional properties. Furthermore, the variations in polyunsaturated fatty acids (C18:2 n-6 and C18:3 n-3) increased with fruit maturation, indicating a modulation of the oxidative stability of the oils, as also observed in other studies on Mediterranean varieties.

The impact of the ripening stage on oil yield and quality was significant: Akerma reached its maximum yield ($12.71 \pm 0.17\%$) at the spotted stage, indicating that harvesting at this stage would optimize production without compromising quality.

Bouchouk Guergour exhibited a higher maximum yield ($19.56 \pm 0.22\%$) at the black stage, suggesting that this variety accumulates more oil in late maturation.

These findings confirm that optimizing the harvest timing can be crucial in balancing yield and oil quality, emphasizing the need to tailor agronomic strategies according to varietal and environmental specificities.

The evaluation of the biological properties of Akerma and Bouchouk Guergour olive oils highlighted their therapeutic potential, particularly:

Anti-inflammatory activity, evaluated *in vivo*, demonstrated a significant inhibition of ear edema, with reduction rates reaching up to 92.54% for Bouchouk Guergour oil at the black stage, surpassing even the reference drug Voltaren 1%. This activity is strongly correlated with the richness in oleic and linoleic acids, which are well known for their anti-inflammatory effects.

Wound-healing activity revealed a rapid and significant reduction in wound surface area, resulting in complete healing after 28 days for the oils from Akerma and Bouchouk Guergour at the black stage. These results are attributed to the presence of polyphenols (oleuropein, hydroxytyrosol) and unsaturated fatty acids, which promote cell regeneration and tissue repair.

These results confirm that the studied olive oils, Akerma and Bouchouk Guergour, are not only functional foods but also potential therapeutic agents, paving the way for their application in cosmetic and natural medicinal fields.

Specifically, the Akerma variety showed very good physicochemical characteristics, with a low acidity index (0.41% to 0.61%) and excellent oxidative stability (peroxide value between 5.24 and 6.15 meq O₂/kg). Its lipid profile is dominated by oleic acid (up to 70.81% at the

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spotted stage). Biologically, its oils exhibited high anti-inflammatory activity (91.09% inhibition at the black stage) and achieved complete wound healing in 28 days. The natural therapeutic potential of this variety is promising, and the objectives of characterization and valorization have been achieved.

The Bouchouk Guergour variety recorded the highest oil yield (19.56% at the black stage), with an oleic acid content exceeding 79.82%. Biologically, its oil showed remarkable anti-inflammatory activity (92.54% inhibition at the black stage) and complete wound healing in 28 days, even surpassing the effectiveness of the reference drug (Voltaren 1%). This positions it as one of the most promising local varieties for functional and therapeutic applications. The objective of demonstrating its bioactive potential has been fully achieved.

The Azeradj variety, intended for dual use (table olives and oil), revealed an oil yield of 13.04% at the black stage, with a balanced lipid profile (oleic acid at 68.96% and linoleic acid at 10.98%). Its biological activities, although satisfactory (edema inhibition at 85–89%), remained slightly lower than those of Akerma and Bouchouk Guergour. The objective of characterization has been achieved, but further studies are needed to better valorize its therapeutic potential.

The introduced Gordal de Séville variety has large fruits and a lipid profile that meets IOC standards. It is interesting for dual valorization (table olives and oil). The objective of comparison with local varieties has been achieved.

Overall, these findings establish that the olive oils from Akerma, Bouchouk Guergour, Azeradj, and Gordal of Sevilla possess remarkable physicochemical, biochemical, and biological characteristics, positioning them as high-quality products capable of competing with international standards.

Thus, this research contributes to the valorization of Algerian olive oils, affirming their potential as high-value products meeting the demands of the international market. Future studies should further investigate the impact of agro-climatic conditions on the biochemical composition of these oils and explore their application in the cosmetic and pharmaceutical sectors.

This study provides valuable insights for olive oil producers aiming to improve their harvesting strategies. By identifying the most suitable ripening stage for optimizing both oil yield and quality, and by adapting practices to the specific agro-climatic conditions of each region, producers can enhance overall production efficiency. Looking ahead, further investigations into the long-term stability and bioavailability of olive oil constituents, as well as the interactions

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between climatic variability and cultivar-specific traits, could support more sustainable and informed agricultural decisions.

The results of this research carry meaningful implications for various stakeholders. Local producers, for instance, can use the data to customize harvest timing based on the unique attributes of each olive variety, thereby improving productivity and product quality. Moreover, the Algerian olive oil sector could capitalize on these findings to develop and promote high-value oils with scientifically supported health benefits. From an academic perspective, these findings may serve as a robust foundation for future applied research on the stability and functional delivery of bioactive compounds in olive oil formulations.

PERSPECTIVES

The results obtained in this study have established a solid scientific foundation for the valorization of olive oils derived from several cultivars of *Olea europaea* L., highlighting their biochemical characteristics and biological activities. However, several research areas merit further investigation to improve the production, quality, and utilization of these oils in various sectors.

Optimizing agricultural and post-harvest practices is a priority for enhancing the yield and quality of olive oils. It would be relevant to conduct further studies on the impact of environmental factors, particularly climatic and soil conditions, on the biosynthesis of fatty acids and bioactive compounds such as polyphenols and tocopherols. A better understanding of fruit maturity management and an accurate determination of the optimal harvest time could help optimize the physicochemical and organoleptic characteristics of the oils according to production objectives. Improving extraction methods could also be explored through more sustainable techniques, such as cold extraction, the use of enzymes to enhance oil yield, or the optimization of mechanical parameters to maximize the retention of polyphenols and other compounds of interest.

The biochemical and biological analysis of olive oils could be expanded by focusing on the detailed characterization of polyphenols and antioxidants present in the oils to better understand their impact on stability and functional properties, including their anti-inflammatory, antimicrobial, and neuroprotective effects. The evaluation of their bioactive properties could be extended to other biological models to explore their potential in preventing metabolic and inflammatory diseases such as diabetes, cardiovascular diseases, and neurodegenerative disorders. A more detailed analysis of the lipid profile and biochemical interactions would allow a better understanding of inter-varietal variations and the influence of environmental conditions on fatty acid synthesis.

The findings also highlight a strong potential for the valorization of olive oils in the agri-food and pharmaceutical industries. The development of formulations enriched with polyphenols and essential fatty acids could lead to the production of functional oils tailored to specific nutritional needs, particularly for cardiovascular health and cellular aging prevention. The

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demonstrated wound-healing and anti-inflammatory properties suggest the possible integration of olive oils into dermatological and pharmaceutical products, such as creams, balms, or dietary supplements, for therapeutic applications. Additionally, the valorization of olive oil by-products, such as olive pomace and wastewater rich in polyphenols and antioxidants, could open new perspectives for the production of biodegradable bioplastics, the extraction of active compounds for the pharmaceutical industry, or their use in animal feed and composting for sustainable agriculture.

Enhancing olive oil conservation strategies is a major challenge to ensure its quality and shelf life. It is essential to study the impact of storage conditions, particularly temperature, light, and oxygen exposure, on the degradation of polyphenols and fatty acids. Exploring smart packaging solutions, such as tinted glass or modified atmosphere packaging, could help limit oxidation and prolong the stability of the oils. Optimizing formulations by naturally adding antioxidants, such as rosemary extracts or tocopherols, could slow the rancidity process and maintain the oils' sensory quality over a longer period.

The results of this study confirm the richness and diversity of Algerian olive oils in terms of their nutritional, biochemical, and biological properties. The analysis of the Akerma, Bouchouk Guergour, Azeradj, and Gordal de Séville varieties has demonstrated their potential as high-quality extra virgin oils, suitable for human consumption and possessing promising functional properties. However, to maximize their valorization, further research is needed to optimize cultivation and extraction techniques, deepen biochemical and biological analyses, develop new functional and pharmaceutical products, and improve conservation and commercialization strategies.

By integrating these perspectives into a multidisciplinary and innovative approach, Algerian olive oils could not only meet international market demands but also be utilized in emerging fields such as health, cosmetics, and functional nutrition.



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Topic : Valorization of Biological Activities and Biochemical Characterization of Olive Oils from Selected Cultivars of Olive Tree (*Olea europaea L.*).

Abstract :

This study aims to characterize the physicochemical, biochemical, and biological properties of olive oils extracted from four Algerian varieties: Akerma, Bouchouk Guergour, Azeradj, and Gordal of Séville, according to the fruit ripening stage (green, spotted, and black). The objective is to assess the impact of varietal factors and fruit maturity on quality parameters, lipid composition, and biological activities to optimize the valorization of these oils in the agri-food and therapeutic sectors. The results revealed significant changes in physicochemical parameters with fruit ripening. The maturity index indicates that Bouchouk Guergour reaches an advanced ripening stage faster than Akerma, progressing from 1.8 ± 0.09 at the green stage to 6.2 ± 0.21 at the black stage, while Akerma matures more slowly, from 1.6 ± 0.08 to 6.9 ± 0.17 . Regarding oil yield, Akerma reaches a maximum of $12.71 \pm 0.17\%$ at the spotted stage, whereas Bouchouk Guergour peaks at $19.56 \pm 0.22\%$ at the black stage, highlighting different lipid accumulation strategies between varieties. Oil quality analysis shows that all varieties comply with the International Olive Council (IOC) standards for extra virgin olive oils. Free acidity slightly increases with maturation while remaining within acceptable limits ($<0.8\%$) for all oils analyzed. The peroxide value increases from 3.25 ± 0.2 meq O_2/kg to 9.5 ± 0.3 meq O_2/kg in Akerma and from 9 ± 0.25 meq O_2/kg to 12.5 ± 0.41 meq O_2/kg in Bouchouk Guergour, indicating greater susceptibility to oxidation in more mature olives. The iodine value, an indicator of fatty acid unsaturation, increases with ripening, reaching 90.96 ± 1.71 in Akerma and 85.09 ± 0.9 in Bouchouk Guergour at the black stage, illustrating an enrichment in unsaturated fatty acids. GC-FID and GC-MS analyses revealed distinct fatty acid profiles depending on the variety and ripening stage. Oleic acid (C18:1 n-9) is the predominant fatty acid in all oils, but with significant variations: 73.03% in Bouchouk Guergour compared to 69.30% in Akerma at the green stage. Linoleic acid (C18:2 n-6) increases with maturation, reaching 16.41% in Akerma and 11.62% in Bouchouk Guergour at the black stage, affecting the oxidative stability of the oils. Palmitic acid (C16:0) slightly decreases with maturation in Akerma (from 17.28% to 14.22%) and remains stable in Bouchouk Guergour (9.24% - 9.45%). In vivo biological tests revealed remarkable anti-inflammatory and wound-healing properties in the studied olive oils. The anti-inflammatory activity, measured by the inhibition of xylene-induced ear edema, was highest for oils extracted from black-stage fruits, with inhibition rates of $91.09 \pm 0.80\%$ for Akerma and $92.54 \pm 0.88\%$ for Bouchouk Guergour after 4 hours, surpassing the effectiveness of Voltaren ($79.88 \pm 0.50\%$). The wound-healing activity, evaluated using a wound model in rats, demonstrated that olive oils enabled complete wound healing within 28 days, with wound contraction rates reaching 100% at the black stage for both varieties, compared to 26.24% in the Vaseline-treated control group. These results confirm the potential of Algerian olive oils as high-quality products that meet international standards and offer promising agri-food and pharmaceutical applications. To maximize their valorization, future research should focus on optimizing agronomic practices and extraction techniques, conducting in-depth studies on bioactive compounds, and developing functional formulations suitable for the food, cosmetic, and pharmaceutical industries.

Key words : *Olea europaea L.*, olive oil, ripening, yield, physicochemical quality, fatty acids, anti-inflammatory activity, wound-healing activity, extraction, valorization.