ISSN 2611-9013 RISG on-line 101(2) 67-140 (2024)
Finito di stampare nel mese di luglio 2024

RISG

2024

VOL. 101

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

LA RIVISTA ITALIANA DELLE SOSTANZE GRASSE

N. 2 APRILE-GIUGNO 2024

La RIVISTA ITALIANA delle SOSTANZE GRASSE

La rivista edita da Innovhub Stazioni Sperimentali per l'Industria S.r.l. ha periodicità trimestrale e la scientificità dei contenuti è garantita da un Comitato Internazionale di Referee.
Pubblica lavori originali e sperimentali di autori italiani ed esteri riguardanti la chimica, la biochimica, l'analisi e la tecnologia nei settori: sostanze grasse e loro derivati, tensioattivi, detersivi, cosmetici, oli minerali. Pubblica inoltre un Notiziario con informazioni su congressi, notizie in breve e libri.
La Rivista viene consultata in Italia dalle industrie produttrici ed esportatrici di oli e grassi alimentari ed industriali, dalle industrie chimiche, da laboratori di enti statali, da istituti di ricerca e facoltà universitarie, da dove provengono diversi lavori scientifici. È inoltre consultata all'estero in vari Paesi come Spagna, Principato di Monaco, Canada, Paesi Bassi, Svizzera, Slovenia, Regno Unito, Turchia, Lussemburgo, Malaysia, Grecia, Francia, Germania, Tunisia, Nigeria, Congo, Polonia, Romania, Bulgaria, Russia, Stati Uniti, Brasile, Cina, Giappone.

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

Via Giuseppe Colombo,79
20133 Milano

Biblioteca

La storica Biblioteca *Oli e Grassi*, attiva dai primi anni del '900, raccoglie circa 400 volumi e più di 100 titoli di riviste a partire dal 1849.
È specializzata in:

chimica generale
chimica organica
chimica analitica
scienze alimentari
sostanze grasse
aromi
prodotti petroliferi
oli minerali
lubrificanti
cosmetici e tensioattivi
vernici

Servizi Offerti

Ricerche bibliografiche
La Biblioteca offre la possibilità di commissionare ricerche bibliografiche che vengono prese in carico da esperti di settore

Fornitura di articoli
La Biblioteca offre il servizio di fornitura di materiale da riviste nazionali ed internazionali sia dalla propria dotazione che attraverso i circuiti nazionali interbibliotecari (a titolo gratuito) in osservanza della vigente normativa sul Diritto d'Autore
(Legge n. 633 del 22/4/1941 e successive modifiche e integrazioni)

4duemila**ventiquattro**
APRILE/GIUGNO 2024 - ANNO CI

RISG
LA RIVISTA ITALIANA DELLE
SOSTANZE GRASSE

DI INNOVHUB
STAZIONI SPERIMENTALI PER L'INDUSTRIA S.r.l.

Indice

GRAFICA, IMPAGINAZIONE E STAMPA

Ancora srl
Via Benigno Crespi, 30
20159 Milano

DIRETTORE RESPONSABILE: P. ROVELLINI
REDAZIONE: C. ZIGLIANI

REDAZIONE

Chiara Zigliani
risg@mi.camcom.it

Autorizzazione del Tribunale di Milano 28.9.1948, N. 591

DIREZIONE E REDAZIONE
Via Giuseppe Colombo 79 – 20133 Milano

Sito web: www.innovhub-ssi.it

La **RIVISTA ITALIANA** delle **SOSTANZE GRASSE**

Impact Factor: 0.4
Subject Area: Organic Chemistry (Q4)

Indexed and Abstracted in:
- **Clarivate Analytics Company** (Philadelphia, USA)
- **Chemical Abstracts**
- **Elsevier Bibliographic Databases:** SCOPUS
- **FSTA – Food Science and Technology Abstract** (IFIS Publishing – UK)

Olive oil triglycerides separation by HPLC and on-line DAD and RID detection: a contribution to identify extra virgin oil blends with soft-deodorised olive oils

Mauro Amelio ✉

Fratelli Carli SpA Società Benefit
Imperia (Italy)

Extra virgin olive oil is one of the healthiest vegetable oils and it is the best source of fats in the Mediterranean Diet. Olive tree cultivation and olive oil consumption spread all over the world and since extra virgin olive oil is also one of the most expensive oils, often undergoes fraudulent practices by mixing it with lower grade oils. Improved knowledge of olive oils and technology may give rise to a frequent extra virgin counterfeiting by mixing authentic extra virgin olive oils with the so-called soft- or mild-deodorised oils: these are virgin oils, deodorised in a soft way to distillate unpleasant compounds so that oils can be blended with real extra virgin oils and be illegally sold as if they were fully authentic. The aim of this paper is to describe an approach that takes into consideration the ultraviolet absorbency of each triglyceride in soft-deodorised oils or micro- or ultra-filtered oils and their blends with authentic extra virgin oils. Further data elaboration by principal component analysis allowed us to clearly distinguish false extra virgin oils from authentic. Furthermore, chromatographic separation enables us to calculate the ECN42 without performing a new HPLC separation according to the Official method, as required by the law in force.

Keywords: Soft/mild-deodorised olive oil, crossflow micro/ultra- filtered oil, HPLC, DAD, RID, PCA

List of abbreviations used:

IOC: International Olive Council
EU: European Union
GC-IMS: gas-chromatography ion mobility spectrometry
FGC-Enose: flash gas-chromatography electronic nose
NIR: Near infrared
MIR: Medium infrared
MF: Crossflow microfiltration
UF: Crossflow ultrafiltration
TDR: Time Domain Reflectometry
FAEE: Fatty acid ethyl ester
TAG: Triacylglycerol
DAD: Diode Array Detector
RID: Refractive Index Detector
ECN: Equivalent Carbon Number
ΔECN: Difference between calculated ECN and experimental ECN
SPE: Solid Phase Extraction
UV: Ultraviolet
PCA: Principal Component Analysis
EV: Extra virgin olive oil
Δ: it refers to a difference
NARP-HPLC-APCI-MS: Non aqueous reverse phase-high performance chromatography-atmospheric pressure chemical ionization-mass spectrometry

✉ CORRESPONDING AUTHOR:
Mauro AMELIO
via Diano Calderina, 182
18100 Imperia (Italy)
Mob: +39 333 6727254
Email: mlaa2004@libero.it

Received: December 19, 2023
Accepted: April 3, 2023

1. INTRODUCTION

Olive oil is among the foods with a history that dates back thousands of years and is typical of the Mediterranean [1, 2], even if the olive tree seems to come from Asia Minor and, before, from the southern Caucasus, from the Iranian plateau, from the Mediterranean coasts of Syria and Palestine (IOC) [3] and, originally, from the "Fertile Crescent". It is recognised by numerous studies that olive oil is the preferable fat in human nutrition and has become perhaps the most characteristic ingredient of the Mediterranean diet [4]. Olive cultivation has spread from the Mediterranean to very distant areas such as South America (e.g. in Argentina), the United States (e.g. in California), South Africa (e.g. in the Cape Province), up to the Far East such as China and Japan, and even Australia and New Zealand. Appreciation for olive oil and its knowledge has naturally developed in these geographical areas. Where deemed necessary, the olive oil market has been regulated by laws aimed at guaranteeing its nature and authenticity. At an international level, the reference body is the International Olive Council (IOC) [5] which is based in Madrid (Spain). It currently includes 19 countries including the European Union as a single member. Although not all world markets interested in the production or trade of olive oil are part of it, this is the most important regulatory reference for international trade. In the case of the European Union, the matter is dealt with by ad hoc Regulations which comply with the requirements of the IOC. Among those in force, Reg. (EU) 1308/2013 [6] establishes the various olive oil Categories, Reg. (EU) 2104/2022 [7] their chemical-physical and sensory characteristics and the related analytical limits, and the Reg.(EU) 2105/2022 [8] the analysis methods to ascertain them (it refers to IOC Methods). Among the eight categories envisaged, the first is extra virgin olive oil which is the best for its chemical-physical and sensory properties. Among vegetable oils, olive oil has always been considered the most valuable and, therefore, also the subject of fraudulent attention aimed at marketing oils declared as olive, but containing foreign fats or, in the case of oils with chemical-physical characteristics of extra virgin, but with sensory defects, treated with processes aimed at removing those defects (e.g.: soft/mild deodorisation) and mixing them with authentic extra virgin olive oils and sold as such. The evolution of knowledge of olive oil and the progress of chemical-physical analysis techniques [9] have made it possible to increasingly refine the possibility of discovering frauds, but also applying advanced technological procedures, aimed at adulterating oils then sold as belonging to more valuable categories, such as virgin oils "transformed" into extra virgin. This transformation can be implemented, for example, through the so-called "soft deodorisation", also called "mild-deodorisation", performed under high vacuum, at much lower temperatures than for normal deodorisation of oils under refining. The aim is to remove by distillation those volatile components that give it sensory defects, without excessively altering the other chemical-physical parameters, so as to allow the oil to be mixed with authentic extra virgin olive oils and fraudulently placed on the market as entirely extra virgin olive oils, respecting the limits established for this Category. During the last twenty years or so, the problem of recognising deodorised oils mixed with extra virgin olive oils has become the subject of multiple research projects, sometimes supported by analytical checks conducted through appropriate interlaboratory proficiency testing. Below we will refer to only some of them, among the most significant. We remember the studies that considered the transformations of chlorophyll pigments combined with those of diglycerides [10, 11]. Various other research followed, among which the one that indicated a method for their determination intended to be included in the German Standard Methods [12]. Investigation on the content of fatty acid ethyl esters (FAEE), pyropheophytins and volatile compounds in oils subjected to soft-deodorisation conducted on a laboratory scale were also performed [13]. In [14] interesting results that require further investigation to be useful for the purpose are described. As already mentioned, diglycerides have been the subject of studies and research. In addition to those that studied the kinetics of transformation of 1,2- into 1,3-diglycerides [15, 16], we recall a recent work carried out within the European Oleum Project in the years 2016-2020 [17, 18], also based on diglycerides isomerisation kinetics and their relationship with the free acidity of the oil.

Among the methods aimed at finding markers of deodorisation, we recall the one that identified methyl 9(E),11(E)-octadecadienoate at trace level [19]. However, the markers which have been limited by an EU Regulation are the alkyl esters of fatty acids (methyl and ethyl). In fact, their presence is due to the formation of methyl and ethyl alcohols due to anaerobic fermentations that can occur in the olives during their storage before transformation, with consequent production of sensory defects in the oils from them, such as, for example, winey and, after oxidation in aerobic conditions, vinegary. Soft deodorisation allows the distillation of these alcohols and other compounds responsible for the defects but is less efficient in removing those alkyl esters. In this regard, among the numerous works, we remember those that use gas chromatography [20, 21, 22], while, with other analytical techniques, we recall the results obtained using TDR (Time Domain Reflectometry) [23] and others by means of gas chromatography-mass spectrometry with processing of the results via PCA [24]. The adoption by the European Union of Reg. (EU) 61/2011 [25] has introduced a limit to the content of methyl and ethyl esters of fatty acids in extra virgin olive oils. Their evolution over time has been the subject of various studies among which we mention just one [26]. Later, with Reg. (EU) 1348/2013 [27] that

limit was lowered and provided only for ethyl esters (FAEE). In fact, especially in unfiltered extra virgin oils, there may be the formation of methyl alcohol due to the degradation of the pectin present. Again, with the aim of preparing reliable methods for the recognition of mixtures of extra virgin oils with soft-deodorised oils, other studies have been conducted with different techniques. We refer to non-targeted methods, where high resolution mass detectors are used [28], and where the fingerprints of the volatile fractions are obtained with gas-chromatography ion mobility spectrometry (GC-IMS) and flash gas-chromatography electronic nose (FGC-Enose) techniques [29]. We also mention the use of NIR and MIR and chemometric analysis to process the data [30] and the use of near infrared spectroscopy (NIR) together with other traditional analytical parameters processed with a specific statistical approach [31]. Finally, we refer to the studies aimed at verifying the use of crossflow microfiltration (MF) and crossflow ultrafiltration (UF) for the purpose of removing the compounds responsible for off-flavours in oils. Among those, we mention one that dealt with the purification of lampante oils [32] and the study of the effect of membrane filtration on virgin olive oils to remove the compounds responsible for sensory defects [33].

The work presented in this publication illustrates the results obtained in the investigation on the possible variations of the specific extinctions at 270 nm (K270), increased by the conjugation of the trienes in single triglycerides (TAG), some of them perhaps more sensitive to soft-deodorisation treatments. Although these variations may be significant, the possible low concentration of the relevant TAGs may cause the effect on the specific extinction of the oil to be negligible. TAG separation was conducted by isocratic HPLC with on-line DAD and RID detectors. Furthermore, we wanted to compare the ECN42 values determined using this method under study with those obtained with the official method.

2. MATERIALS AND METHODS

2.1 CHEMICAL REAGENTS AND SOLVENTS

SPE-Si, 1g / 6mL (Strata®SI-1 Silica (55 µm, 70Å);
n-Hexane, ≥ 97.0%, ChromasolvTM for HPLC (Honeywell);
Diethyl ether, ≥ 99.8%, ACS Reagent, Reag. ISO, Reag. Ph. Eur., (Honeywell);
Acetone, ≥ 99.8%, HiPerSolv CHROMANORM® for HPLC, (VWR);
Propionitrile, ≥ 99.9%, for UV, HPLC, (PanReac AppliChem);
Nitrogen, Alphagaz 1 (Air Liquide).

2.2 SAMPLES

Oils (Table I): 56 oils were used of which: 6 extra virgin from the 2021-2022 campaign (n°10 to 15) and 9 from the 2022-2023 campaign (n°1 to 9) from Italy, Greece and Spain; 10 lampante olive oils from the 2022-2023 campaign (n°36 to 45) from Italy, Greece and Spain; 5 blends of extra virgin oils with the addition of refined olive oils at 1% (n°26 to 30) and 5 blends at 0.5% (n°31 to 35); 10 refined oils of which 5 from the 2021-2022 olive oil campaign (n°21 to 25) and 5 from the 2022-2023 campaign (n°16 to 20); 1 deodorised oil (100%, Spanish origin) (n°52) and 3 of its blends with 30%, 15% and 5% extra virgin olive oil (n°53 to 55); 1 oil, blend of extra virgin and 30% soft-deodorised (n°47); 1 blend oil (in unknown proportions) (n°46); 4 blend oils between extra virgin oils containing 30%, 20%, 10%, 4.6% of the latter in the list (n°48 to 51); 1 oil obtained from EV ultra filtered on membranes (n°56). All samples were stored in glass containers, in the dark at 18°C or some frozen at –20°C.

2.3 INSTRUMENTS AND SOFTWARE

Aspec XL Solid Phase Extraction Autosampler (Gilson, USA) with SW: 735 Sampler Software v.6.10 installed on PC with Microsoft Windows XP operating system.
HPLC 1260 Infinity with Degasser (1260 Degasser), Quaternary Pump (1260 Quat Pump VL), Autosampler (1260 ALS), Thermostated Column Chamber (1260 TCC) at 23°C, Diode Array Detector (1260 DAD VL) set at 270 nm, Refractive Index Detector (1260 RID) thermostated at 35°C (Agilent Technologies, USA);
HPLC columns: double column, InfinityLab Poroshell 120 EC-C18 (4.6 × 250 mm, 4 µm) (Agilent Technologies) thermostated at 23°C;
Vibrating shaker: Vortex mixer ZX3 (Velp Scientifica, Italy)
Micropipette: Eppendorf Research 10 - 100 µL (Eppendorf, Germany);
Vials: Chromacol 03-FIV with 300 µL fixed insert (Thermo Scientific, USA);
Vial closures: Ø 11 mm, with Silicone/PTFE septum (Microcolumn, Italy);
Common laboratory glassware;
Chromatogram acquisition and processing software: ChemStation for LC 3D system, Rev. B.04.03 (16) (Agilent Technologies, USA), installed on PC with Microsoft Windows 7 Professional operating system, Service Pack 1 (Microsoft);
Data collection and processing: Microsoft® Excel® 2019 MSO (Version 2307 Build 16.0.16626.20086) 64 bit (Microsoft Office 2019);
Principal Component Analysis (PCA): CAT (Chemometric Agile Tool) software, R version 3.1.2 [34] installed on PC with Microsoft Windows 10 Home operating system, Ver. 22H2;

2.4 METHODS

Oil clean-up.

Each aliquot of approximately 140 µL of oil was subjected to clean-up conducted automatically with As-

pec XL Solid Phase Extraction Autosampler in compliance with what is described in § 4.3.3 of the official method COI/T.20/Doc. No 20 /Rev. 4 2017 [35]. Once the eluate was collected, the solvent was evaporated in a stream of nitrogen. Approximately 50 µL of oil was added to the vial containing 70 µL of acetone, then closed and briefly vortexed.

Separation of TAGs.

Isocratic elution with propionitrile solvent at 1 mL/min, with the columns thermostated at 23°C. As already mentioned, we opted for the use of the double column to improve the separation of the peaks. DAD was set to signal recording at 270 nm (bandwidth = 4 nm) vs. 500 nm (bandwidth = 10 nm) as reference, while RID with Optical Unit Temperature set at 35°C. Injection volume = 5 µL. The chromatograph is first conditioned to a stable baseline, then the injection is performed. Run duration = 80 min. The RID signal is delayed by 0.2 min compared to that of the DAD, that is the first of the two detectors, due to the tube line connecting them. The same oils were also analysed according to the official method [35] for the determination of ECN42 to be compared with those determined with the method described here.

Integration of chromatograms.

The RID chromatogram shows, as expected, the separation of the TAGs according to the various ECNs and the use of the double column allows for better resolution (Figure 1).

In particular, ECN42 and ECN44 are extremely interesting for the purposes of this research, since they contain TAGs with triene fatty acids, namely linolenic acid (C18:3). As is known, because of the treatments to which the oil may have been subjected, part of those trienes conjugates, increasing the specific extinction at 270nm. In fact, it is in their correspondence that the greatest variations in the signals recorded by the DAD are detected, while for the higher ECN there is practically no response. The integration of the RID chromatograms was done by tracing the respective baselines underlying the ECN42 and ECN44. From ECN46 to ECN50 a single baseline was drawn and any peaks belonging to higher ECNs were integrated individually. Additionally, for ECN42 and ECN44, perpendiculars to the baseline were drawn at the valleys between incompletely resolved peaks. As already mentioned, the DAD chromatogram corresponds almost entirely to the first two ECN and was integrated by tracing the baselines under each of them as done for the RID signal, while the correspondence with the RID peaks was given by tracing the perpendiculars at the same times as the valleys of the relevant RID signal realigned for the 0.2 min time gap (Figure 2). The areas of the corresponding peaks were deduced from the integration reports and the A_{DAD}/A_{RID} ratios on which this work is based were calculated.

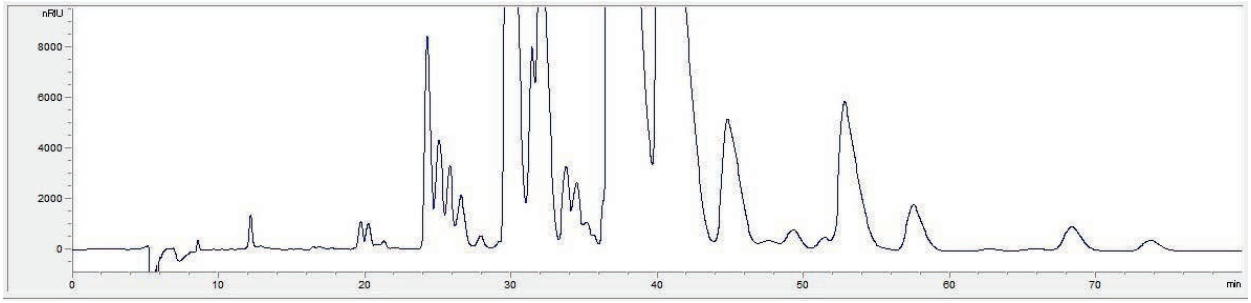


Figure 1 - olive oil triglyceride HPLC separation according to the method described in this research

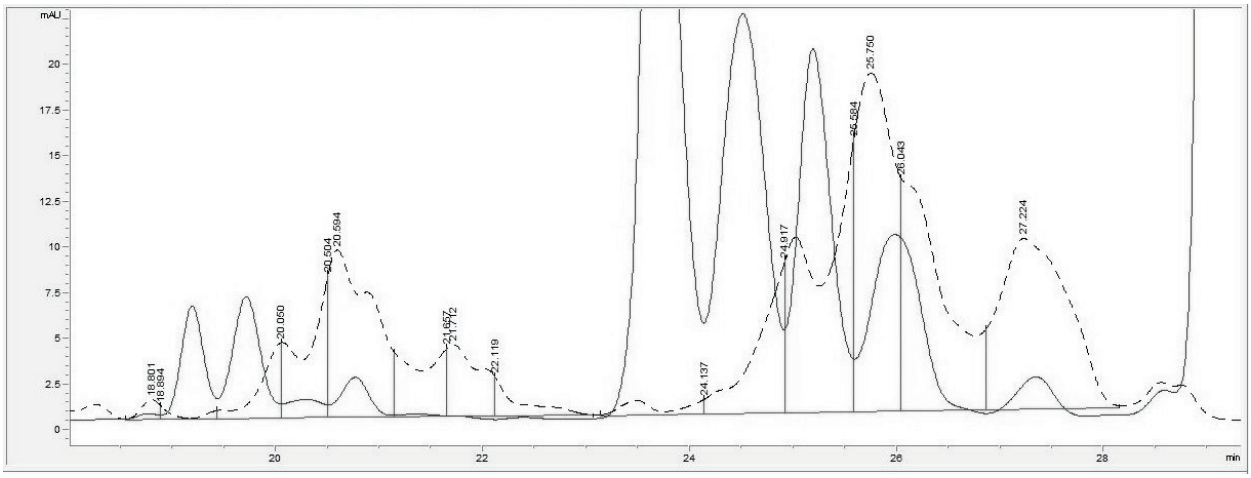


Figure 2 - DAD (dotted line) and RID (continuous line) signals integration of TGs belonging to ECN42 and ECN44

3. DISCUSSION

As already anticipated in the Introduction, the treatments to which an oil is subjected can increase the value of the specific extinction K, which is determined at 268 nm or 270 nm, according to the method requirements [36]. The reference spectrophotometric law is the Lambert-Beer Law which, as readers may remember, was first formulated in the 18th century, thanks to the studies of Bouguer in 1729 [37], of Lambert in 1760 [38] and of Beer in 1852 [39].
One of the most common formulations of this Law is taken from the official method:

$$E_\lambda = K_\lambda \times c \times s$$

where:
E_λ = extinction (or absorbance) measured at wavelength λ in nm; K_λ = specific extinction (or extinction coefficient) at wavelength λ; c = solution concentration, in g/100 mL; s = optical path of the measurement cell, in cm.

During the recording of the DAD signal due to the i^{th} compound eluted and completely resolved by the others, the absorbance Ei expressed by the Lambert-Beer law at wavelength λ, can be written in differential form, as follows:

$$dE_i = K_i \times N_i(t) \times dt$$

where $N_i(t)$ is the function that describes the elution trend of the moles of the i^{th} compound over the time t of passage through the detector. The integration between the start and end of the peak, ti_0 - ti_1, can be expressed:

$$E_i = \int_{ti0}^{ti1} Ki\, Ni(t)dt$$

Since the specific extinction K_i is a constant, it results:

$$E_i = Ki \int_{ti0}^{ti1} Ni(t)dt$$

The integral gives the number of total eluted moles of i:

$$E_i = K_i N_i \qquad (1)$$

Similarly, the integration of the RID signal of the same i^{th} compound (Ai) gives a value proportional to the number of moles eluted, Ni:

$$A_i = f_i \times N_i \qquad (2)$$

where f_i is a constant of proportionality. The ratio between the two relations (1)/(2), gives:

$$E_i / A_i = K_i \times f_i^{-1} \qquad (3)$$

In other words, the ratio between the DAD and RID signals gives a value proportional to the specific extinction coefficient of the compound considered, K_i. The constant f_i could have negligible variation with triene conjugation respect to the isolated triene compared to K_i changes. Thus, for our purposes we can consider it constant.

All data (E_i / A_i) were PCA processed by R-CAT software [34]. It is an R-based chemometric software that makes use of NIPALS algorithm. It opens a "RGui (32-bit)" window to load the file containing the data to be processed. In case of an Excel file: "Data Handling -> Load -> XLS/XLSX". Calculation is activated from the pull-down menu "PCA", then "Model Computation -> PCA". An "Input Choice" window opens to specify the "Matrix Name", the "Rows to be selected", the "Columns to be selected", the "Number of Components" and then "OK" to start the calculation. The file that collects and processes the relationships between the signals used in this work is Appendix I [40]: it is an Excel file made of 4 sheets: "DAD area pks" that collects the area of each peak of interest from the DAD signal integrated as described above; "RID area pks" that collects those corresponding from the RID signal; "DAD RID ratios" that calculates the E_i / A_i ratios that are multiplied by 10^5 to have all numbers not less than 0.1 and keeping unchanged each other proportion; "Sample C1 score calculation" that calculates the C1 score of a single new sample with two choices: including or not Refined olive oils in the original data base used for PCA. It must be underlined that this calculation is just a rough approximation of the new sample proper score, because its data are not included and processed with the full database to obtain a proper PCA calculation: this is just to have an idea about the sample position in the proper PCA score plot.

3.1 RESULTS

The parameters used in the processing of the experimental data presented in this work are the E_i / A_i ratio of each peak belonging to ECN42 or ECN44.
The relationships considered are 14 in total, of which 8 belong to ECN42 and 6 to ECN44. None of them, considered individually, allows us to unambiguously discriminate soft-deodorised oils or filtered with membranes and their blends with genuine extra virgin olive oils from the authentic ones. In contrast, their elaboration by PCA, which, as is known, also considers any existing relationships between the processed parameters belonging to the same sample, was much more efficient. In fact, the application of PCA to the entire set of results shows a clear discrimination of refined oils from others (Figure 3) with 96.7% of the total variance explained by the first two principal components (C1, C2) and as much as 94.4% explained by the component C1 along which samples are mainly separated.
If refined oils are excluded from the analysis, the score plot becomes the one shown in Figure 4, with 79.5% of the total variance explained by the first two component C1 and C2, with 70.8% by the C1 along which samples are mainly separated: those containing soft-deodorised oils (no. 46, 47, 53, 54, 55) or are entirely made up of it (n° 52), as well as the oil which has undergone membrane filtration (n° 56) are

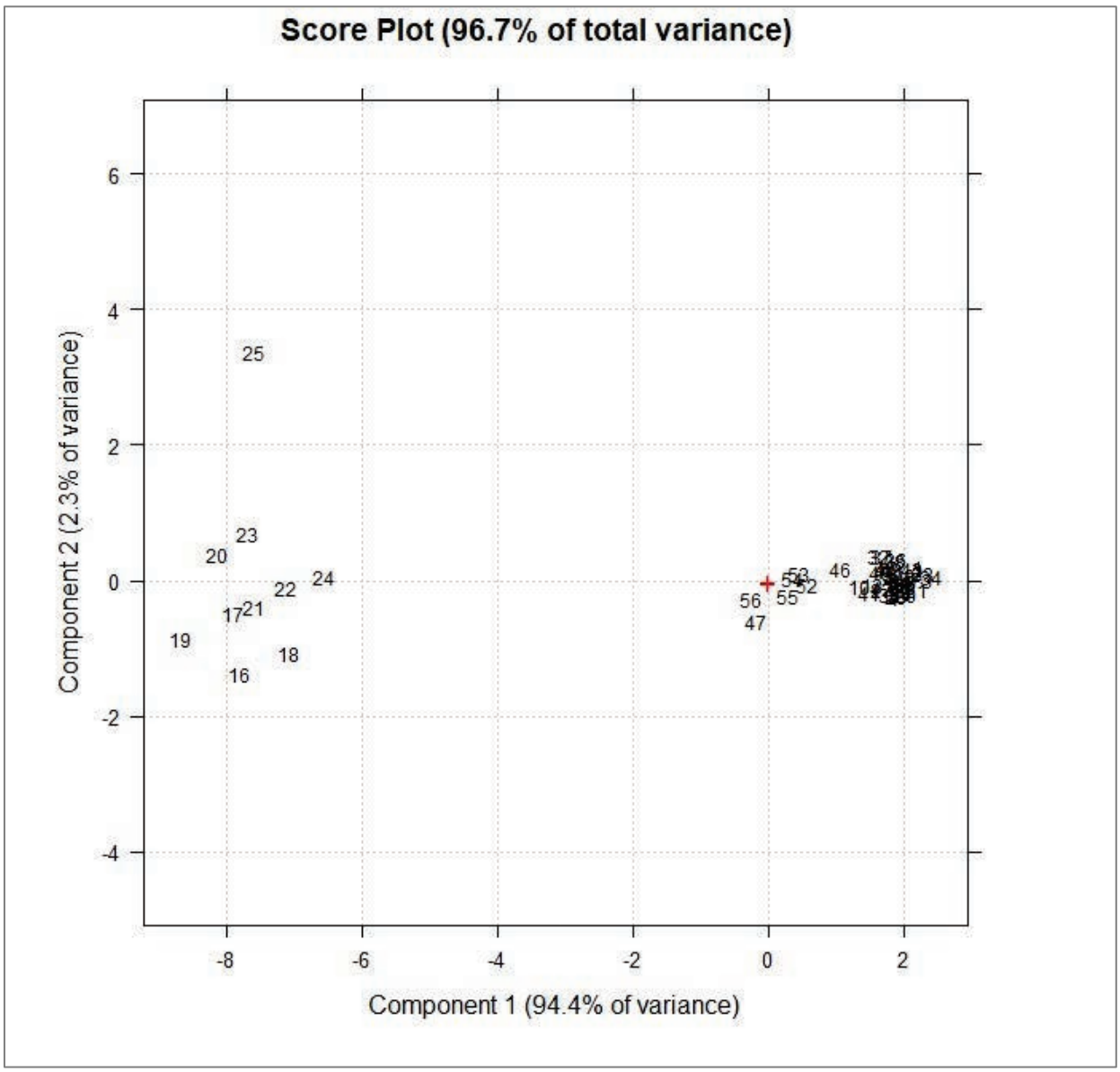


Figure 3 - PCA score plot of all samples. Samples from 16 to 25 are refined olive oils

well separate from the others. In particular, we remind the reader that samples 53, 54 and 55 are respectively dilutions of deodorised oil (n°52) in EV at 30%, 15% and 5%. N° 47 contains 30% of deodorised oil. However, neither EV, nor lampante oils, nor EV mixtures containing 1% or 0.5% of refined oil are distinct from each other, as well as n° 48, 49, 50 and 51 that are dilutions of n° 46, which is made of an unknown dilution of soft-deodorised oil in genuine extra virgin olive oil.

Because more than 70% of the total variance is explained by the component C1 in both cases (Refined olive oils included or not), it could be thought to find a sample data linear combination to detect blends with deodorised oils: Figure 3 shows all refined olive oil scores less than -6, while the other oils show scores greater than -1. Figure 4 shows oils containing deodorised or ultrafiltered oils with scores less than -3.60, while the others have scores greater than -1.50. This is why we included the fourth sheet "Sample C1 score calculation" into Appendix I. It makes use of C1 loadings from PCA calculation and, in order to z-standardise (mean=0, std.dev.=1) the new data, for each variable their mean value and standard deviation of data listed in "DAD RID ratios" sheet. The new sample "score" is calculated multiplying the new z-standardised data by the corresponding loading values. Again, we want to repeat that the "score" calculated in this way is a rough approximation of the real one, and just avoids the very basic and simple use of PCA that, on the contrary, we strongly advice to.

3.2 DETERMINATION OF ECN42

As anticipated in the Introduction, we also wanted to ensure that the quantitative results necessary to determine ΔECN42 could be obtained from the RID

chromatogram, as required by the current standard [7], without having to repeat the separation of the TAGs according to the official method [35]. The analyses conducted with the official method and by the one proposed in this work gave the results shown in Table I. This table also shows the differences between the results of the two methods (Δ (prop.-Offic.) for each sample. Among these, those differences of samples 1 and 3 are to be considered outliers according to the Grubbs test at both 95% and 99% level of confidence. The average value of these differences, excluding outliers, is equal to 0.02%. If we compare this result with the reproducibility value (R) reported in the official method for extra virgin oils, equal to 0.12% [35], it can be said that the two compared methods are consistent.

4. CONCLUSIONS

The method presented in this work is a preliminary one and gave encouraging results in detecting mixtures of extra virgin oils with soft-deodorised oils even to concentrations as low as only 5% of the latter. Membrane-filtered EV oil was also clearly discriminated. However, these results were achieved thanks to data processing via PCA, thus demonstrating that the effect of those treatments on the extinction coefficients of individual TAG is not nonspecific, but structured. In fact, no single E_i / A_i ratio allows us to clearly discriminate those samples. It was also observed that the inclusion of data related to refined olive oils in the PCA analysis produces a clear distinction between them from the others, with over 94% of the variance explained, described by the main component C1, along

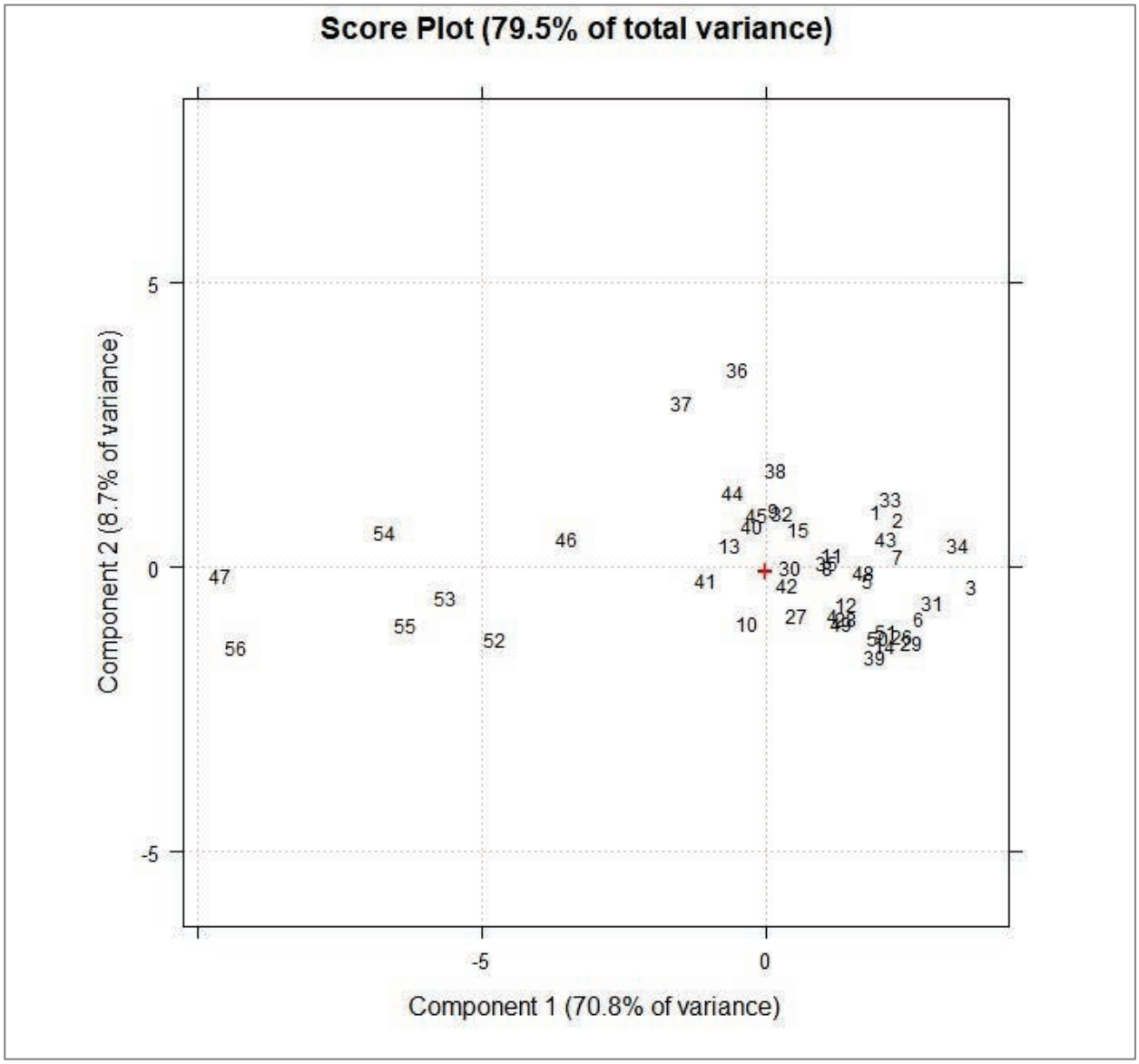


Figure 4 - PCA score plot after excluding refined olive oils from all sample set. Samples 46 and 47 are blends of EV with soft-deodorized oils; sample 52 is a soft-deodorized oil; samples from 53 to 55 are blends of EV with sample 52 at 30%, 15% and 5%; sample 56 is MF/UF oil

Table I - Comparison of ECN42 determined by the proposed method and by the Official Method

sample n°			Proposed method	Official Method	Δ (prop.-Offic.)	*sample n°*			Proposed method	Official Method	Δ (prop.-Offic.)
			%	%	%				%	%	%
1	Italy	EV 2022-2023	0,47	0,61	-0,14	29	EV n° 4 + 1% Ref n° 24		0,53	0,48	0,05
2	Italy	EV 2022-2023	0,37	0,31	0,06	30	EV n° 5 + 1% Ref n° 25		0,62	0,61	0,01
3	Italy	EV 2022-2023	0,38	0,64	-0,26	31	EV n° 1 + 0.5% Ref n° 21		0,36	0,37	-0,01
4	Greece	EV 2022-2023	0,34	0,34	0,00	32	EV n° 2 + 0.5% Ref n° 22		0,38	0,34	0,04
5	Greece	EV 2022-2023	0,29	0,29	0,00	33	EV n° 3 + 0.5% Ref n° 23		0,38	0,36	0,02
6	Italy	EV 2022-2023	0,37	0,36	0,01	34	EV n° 4 + 0.5% Ref n° 24		0,38	0,38	0,00
7	Spain	EV 2022-2023	0,38	0,37	0,01	35	EV n° 5 + 0.5% Ref n° 25		0,28	0,31	-0,03
8	Italy	EV 2022-2023	0,43	0,38	0,05	36	Greece	Lampante olive oil 2022-2023	0,38	0,30	0,08
9	Greece	EV 2022-2023	0,40	0,37	0,03	37	Italy	Lampante olive oil 2022-2023	0,37	0,37	0,00
10	Italy	EV 2021-2022	0,38	0,33	0,05	38	Italy	Lampante olive oil 2022-2023	0,39	0,36	0,03
11	Greece	EV 2021-2022	0,27	0,26	0,01	39	Italy	Lampante olive oil 2022-2023	0,37	0,36	0,01
12	Spain	EV 2021-2022	0,36	0,36	0,00	40	Greece	Lampante olive oil 2022-2023	0,30	0,29	0,01
13	Italy	EV 2021-2022	0,36	0,33	0,03	41	Italy	Lampante olive oil 2022-2023	0,70	0,70	0,00
14	Spain	EV 2021-2022	0,30	0,30	0,00	42	Italy	Lampante olive oil 2022-2023	0,51	0,44	0,07
15	Italy	EV 2021-2022	0,36	0,36	0,00	43	Spain	Lampante olive oil 2022-2023	0,46	0,51	-0,05
16	Refined olive oil	2022-2023	0,43	0,34	0,09	44	Spain	Lampante olive oil 2022-2023	0,83	0,79	0,04
17	Refined olive oil	2022-2023	0,31	0,26	0,05	45	Spain	Lampante olive oil 2022-2023	0,62	0,56	0,06
18	Refined olive oil	2022-2023	0,44	0,41	0,03	46	EV+soft deod.		0,52	0,52	0,00
19	Refined olive oil	2022-2023	0,47	0,44	0,03	47	EV+30% soft deod.		0,42	0,43	-0,01
20	Refined olive oil	2022-2023	0,37	0,36	0,01	48	EV n°3 + 30% n°46		0,48	0,48	0,00
21	Refined olive oil	2021-2022	0,45	0,42	0,03	49	EV n°3 + 20% n°46		0,70	0,67	0,03
22	Refined olive oil	2021-2022	0,57	0,50	0,07	50	EV n°3 + 10.5% n°46		0,64	0,60	0,04
23	Refined olive oil	2021-2022	0,52	0,43	0,09	51	EV n°3 + 4.6% n°46		0,21	0,24	-0,03
24	Refined olive oil	2021-2022	0,51	0,49	0,02	52	Soft deod.		0,25	0,21	0,04
25	Refined olive oil	2021-2022	0,54	0,49	0,05	53	EV + 30% n°52		0,34	0,32	0,02
26	EV n° 1 + 1% Ref n° 21		0,55	0,52	0,03	54	EV + 15% n°52		0,36	0,35	0,01
27	EV n° 2 + 1% Ref n° 22		0,58	0,52	0,06	55	EV + 5% n°52		0,35	0,35	0,00
28	EV n° 3 + 1% Ref n° 23		0,57	0,51	0,06	56	Ultra Filtered EV		0,37	0,35	0,02

Δ (prop.-Offic.) mean value, % = 0,02

which they are separated from other oils. Appendix I can be used as a database to which other data can be added to be processed as a single new set (up to row n° 1000). As regards the analytical part, in particular the chromatographic one, an UHPLC application of this method using appropriate DAD and RID detectors is considered desirable: a greater resolution of the TAG peaks would allow a more accurate determination of the relationship between the DAD and RID signals to the benefit of the analytical results and a possible verification of the proposed method. It is important to note that there is no full correspondence between the peaks of the DAD signal with those of the RID signal, despite the realignment of the two chromatograms for the 0.2 min gap already mentioned. The DAD plot maxima often do not match those of the RID plot. This is evidence of the incomplete resolution of the TAGs observed in the RID plot, whose peaks are however attributed to TAG as indicated by the official method [35]. In this regard, it is useful to refer to the research where the incomplete resolution of those peaks and their more correct identification is demonstrated through NARP-HPLC-APCI-MS [41]. As regards the integration of chromatograms, the choice of the method used to delimit the peaks of the DAD signal based on the integration of the RID chromatogram was explained in the "Method" part. It could be interesting to try the opposite, taking the integration of the DAD chromatogram as a reference, which constitutes an in-depth topic to be developed in the future. The method presented also proved to be accurate in the determination of ECN42, as proven by the comparison with the values obtained from the separation according to the official method. The possible control of the authenticity of an oil using the method presented would also allow us to have the data necessary to ascertain the value of ΔECN42 without any further HPLC separation according to the official method.

Acknowledgements
I would like to thank my colleague Andrea Bellone for performing the TAGs analysis according to the official method. I am also grateful to Dr Emanuele Farinini (Chemistry and Industrial Chemistry Dept., Genoa University) for his advice on the use of some R-CAT software feature.

Conflict of interest
The author declares that there is no conflict of interest

Funding sources
No fundings were granted

REFERENCES

[1] S. Vannucci, *Storia dell'olio*, in *L'ulivo e l'olio*, (Ed. Script), Bologna, Italy (2009), Ch. storia e arte
[2] D. Giugliano, *La dieta mediterranea alle origini del mito*, Idelson-Gnocchi, Casoria, Italy (2000)
[3] International Olive Council (IOC), https://www.internationaloliveoil.org/olive-world/olive-tree/, last access 12/2023
[4] A. Keys, *Coronary heart disease in seven countries*, Circulation, 41(Suppl.1) (1970)
[5] International Olive Council (IOC), https://www.internationaloliveoil.org/, last access 12/2023
[6] Reg.(UE)1308/2013, https://eur-lex.europa.eu/legal-content/IT/TXT/?uri=CELEX%3A32013R1308&qid=1691428259817, last access 12/2023
[7] Reg.(UE)2104/2022 https://eur-lex.europa.eu/legal-content/IT/TXT/?qid=1691503785219&uri=CELEX-%3A32022R2104, last access 12/2023
[8] Reg.(UE)2105/2022 https://eur-lex.europa.eu/legal-content/IT/TXT/?uri=CELEX%3A32022R2105&qid=1691428858856, last access 12/2023
[9] L. Conte, OCL - Oilseeds and fats, Crops and Lipids, *27*, 1, (2020)
[10] A. Serani, D. Piacenti, J of the American Oil Chemists' Society, *69*,469,(1992)
[11] A. Serani, D. Piacenti, La Rivista Italiana delle Sostanze Grasse, *78*, 459, (2001)
[12] C. Gertz, H.J. Fiebig, Eur. J. Lipid Sci. Technol., *108*, 1062, (2006)
[13] R. Aparicio-Ruiz, I. Romero, D.L. García-González, C. Oliver-Pozo, R. Aparicio, Food Chemistry, *220*, 42 (2017)
[14] R.B. Gómez-Coca, M. Alassi, W. Moreda, M.C. Pérez-Camino, Foods *9*, 978 (2020)
[15] A. Serani, D. Piacenti, G. Staiano, La Rivista Italiana delle Sostanze Grasse, *78*, 525 (2001)
[16] A. Serani, D. Piacenti, La Rivista Italiana delle Sostanze Grasse, *78*, 567 (2001)
[17] R.B. Gómez-Coca, M.C. Pérez-Camino, A. Bendini, T. Gallina Toschi, W. Moreda, Food Chemistry, *330*, 127226 (2020)
[18] Oleum Project: http://www.oleumproject.eu/about-oleum/aims-and-objectives, last access 12/2023
[19] A. Saba, F. Mazzini, A. Raffaelli, A. Mattei, P. Salvadori, Journal of Agricultural and Food Chemistry, *53*, 4867 (2005)
[20] M.C. Pérez-Camino, W. Moreda, R Mateos, A. Cert, Journal of Agricultural and Food Chemistry, *50*, 4721 (2002)
[21] M.C. Pérez-Camino, A. Cert, A. Romero-Segura, R. Cert-Trujillo, W. Moreda, Journal of Agricultural and Food Chemistry, *56*, 6740 (2008)
[22] A. Bendini, L. Cerretani, E. Valli, G. Lercker, C. Mazzini, Ind. Alim., *48*, 46 (2009)
[23] A. Berardinelli, L. Ragni, A. Bendini, E. Valli, L. Conte, A. Guarnieri, T. Gallina Toschi, Journal of Agricultural and Food Chemistry, *61*, 10919 (2013)

[24] R. Boggia, C. Borgogni, V. Hysenaj, R. Leardi, P. Zunin, Talanta, *119*, 660 (2014)
[25] Reg.(EU) 61/2011 https://eur-lex.europa.eu/legal-content/IT/TXT/?qid=1691508089436&uri=CELEX-%3A32011R0061, last access 12/2023
[26] L. Conte, C. Mariani, T. Gallina Toschi, S. Tagliabue, La Rivista Italiana delle Sostanze Grasse, *91*, 21 (2014)
[27] Reg. (EU)1348/2013 https://eur-lex.europa.eu/legal-content/IT/TXT/?uri=CELEX%3A32013R1348&qid=1691508089436, last access 12/2023
[28] D. Cavanna, K. Hurkova, Z. Džuman, A. Serani, M. Serani, C. Dall'Asta, M. Tomaniova, J. Hajslova, M. Suman, ACS Omega, *5*, 24169 (2020)
[29] T. Damiani, D. Cavanna, A. Serani, C. Dall'Asta, M. Suman, Microchemical Journal, *159*, 105374 (2020)
[30] K. Wójcicki, I. Khmelinskii, M. Sikorski, F. Caponio, V.M. Paradiso, C. Summo, A. Pasqualone, E. Sikorska, Eur. J. Lipid Sci. Technol., *117*, 92 (2015)
[31] C. Gertz, B. Matthäus, I. Willenberg, European Journal of Lipid Science and Technology, *122*, 1900355 (2020)
[32] A. Hafidi, D. Pioch, H. Ajana, Food Chemistry, *92*, 17 (2005)
[33] A. Bottino, G. Capannelli, A. Mattei, P. Rovellini, P. Zunin, Eur. J. Lipid Sci. Technol., *110*, 1109 (2008)
[34] R. Leardi, C. Melzi, G. Polotti, *CAT (Chemometric Agile Tool*), R version 3.1.2, freely downloadable from http://gruppochemiometria.it/index.php/software, last access 12/2023
[35] COI/T.20/Doc. No 20 /Rev. 4 2017– *Determination of the difference between actual and theoretical content of triacyglycerols with ECN42*, https://www.internationaloliveoil.org/wp-content/uploads/2019/11/Method-COI-T.20-Doc.-No-20-Rev.-4-2017-.pdf, last access 12/2023
[36] COI/T.20/Doc. No 19/Rev. 5 2019 - *Spectrophotometric investigation in the ultraviolet,* https://www.internationaloliveoil.org/wp-content/uploads/2019/11/Method-COI-T.20-Doc.-No-19-Rev.-5-2019-2.pdf, last access 12/2023
[37] P. Bouguer, *Essai d'optique sur la gradation de la lumiere*, Claude Jombert, Paris, (1729)
[38] J.H. Lambert, *Photometria sive de mensura et gradibus luminis, colorum et umbrae*, Eberhard Klett, Augustae Vindelicorum, Germany, (1760)
[39] A. Beer, Annalen der Physik und Chemie, *62*, 78 (1852)
[40] link to Appendix I: https://share.mail.libero.it/ajax/share/0bbf41f301c82675b3cd05f-1c8264f1b9f396c0a1deb1052/1/8/MjQ/MjQvMTM
[41] M. Beccaria, E. Moret, G. Purcaro, L. Pizzale, A. Cotroneo, P. Dugo, L. Mondello, L.S. Conte, Food Chemistry, *190*, 216 (2016)

Effect of geographical and edaphic factors on the quality of some olive oils from different varieties of olives grown in western Algeria

Mounsif Charaf-eddine Bendi Djelloul[1 2] ✉
Pierangela Rovellini[3]
Roza Chenoune[4]
Mohammed Rida Bouzebboudja[2]

[1] Laboratory of Functional Agrosystems & Technologies of Agronomic Sectors, Faculty of Natural and Life Sciences, Earth and Universe Sciences, Tlemcen University Tlemcen, Algeria.

[2] Laboratory of Ecology and Natural Ecosystems Management, Faculty of Natural and Life Sciences, Earth and Universe Sciences, Tlemcen University Tlemcen, Algeria.

[3] INNOVHUB SSI Via Giuseppe Colombo, 79 Milano, Italy

[4] Mediterranean Agronomic Institute of Montpellier, France

✉ CORRESPONDING AUTHOR:
charafagro13@gmail.com
Tel +213 542 708 241

Received: May 13, 2023
Accepted: October 5, 2023

In this study, our objective was to investigate the effect of soil and altitude on the quality of some olive oils from different varieties of olive trees. The quality parameters, the chemical composition of the oils in fatty acids, as well as the profile in phenolic compounds by HPLC are determined and soil analyses are carried out. Statistical analysis of the data was performed using multivariate analysis (PCA). The results obtained showed that the highest concentrations of oleuropein derivatives and ligstroside derivatives were observed in chemlal oil (SBA) and the recorded values were 105.97 mg/Kg and 83.49 mg/Kg. Oleocanthal was found in all the tested samples, and it was higher in Chemlal oil (102.43 mg/kg). Phenolic compounds have affinities with soil clay content and altitude, palmitic and palmitoleic acids are influenced by organic matter content and soil pH.
Key words: Algerian varieties, olive oil, quality, altitude, soil.

1. INTRODUCTION

More than 75% of the worldwide olive oil production is concentrated in the Mediterranean area [15]. Among the sectors having benefited from financial and technical support, in Algeria there is an olive growth that currently represents 4% of the useful agricultural area and 40% of the total arboreal area [16]. Olive oil production was the highest during the last fifteen years, reaching over 900,000 hl across the country, with more than 25% of the previous season production [17]. Algeria, one of the main producers of olive oil in the world (9th in the world ranking), has a wide range of varieties with two dominant ones: *Chemlal* and *Sigoise* [15]. Olive oil is a natural product known for its virtues and health benefits; it is composed of 99% fatty acids and 1% of the minor fraction (antioxidants). Olive oil healthy properties are well known in the Mediterranean diet [21]. The quality of olive oil begins at the time of planting of a particular variety, continues through the cultivation of the olive tree, the harvesting methods and duration, preliminary work, and the duration of storage at the olive grove, the transport conditions of the fruit to the unit, the storage duration before transformation and the technological management of extraction, as well as the conditions of storage and distribution of the oil. The fine composition of an olive oil, besides being strongly dependent on the cultivar used for its production, is influenced by several other factors like climate, soil conditions and agricultural practices [11]. Moreover, it is widely known that the quality of virgin olive oil is influenced by various agronomic factors, such as olive cultivar, climatic conditions, production process, and the degree of maturation and agronomic practices related to irrigation [18]. The production of olive oil is slowly moving beyond Mediterranean countries, and olive trees (*Olea europaea L.*) are being planted in countries such as Chile and New Zealand. This expansion, which is primarily due to new agricultural practices devised by

farmers to increase the olive oil yield without a loss of sensory and nutritional properties, is based on the adaptation of cultivars to climates associated with latitudes and altitudes different from their autochthonous regions [14].
Many research works are dedicated to the study of the influence of these different factors on the phenolic fraction of olive oil. Other studies have focused on determining the phenolic compound profile of virgin olive oil by various analytical techniques, in particular by HPLC. This research work was carried out to investigate the effect of soil composition and altitude on the quality of seven olive oils from different varieties of olives namely Chemlal, Sigoise and Oleaster in western Algeria.

2. MATERIALS AND METHODS

2.1 MATERIALS

Two dominant varieties in the west of Algeria, namely *Sigoise* and *Chemlal* and a wild variety *Oleaster*, are the subject of this study. Seven olive samples were collected by hand during the month of December 2016 in the regions of Zenata, Bordj Arima, Bensekrane, Sidi Belabbes (SBA), Sebra and Sig. The quantities of olive harvested are approximately 20 Kg for each sample. After harvesting fruits were quickly transported in plastic crates for oil extraction (Figure 1).
The olive oils *Chemlal* Zenata, *Chemlal* Bordj Arima and *Chemlal* Sidi Belabbes were extracted by the

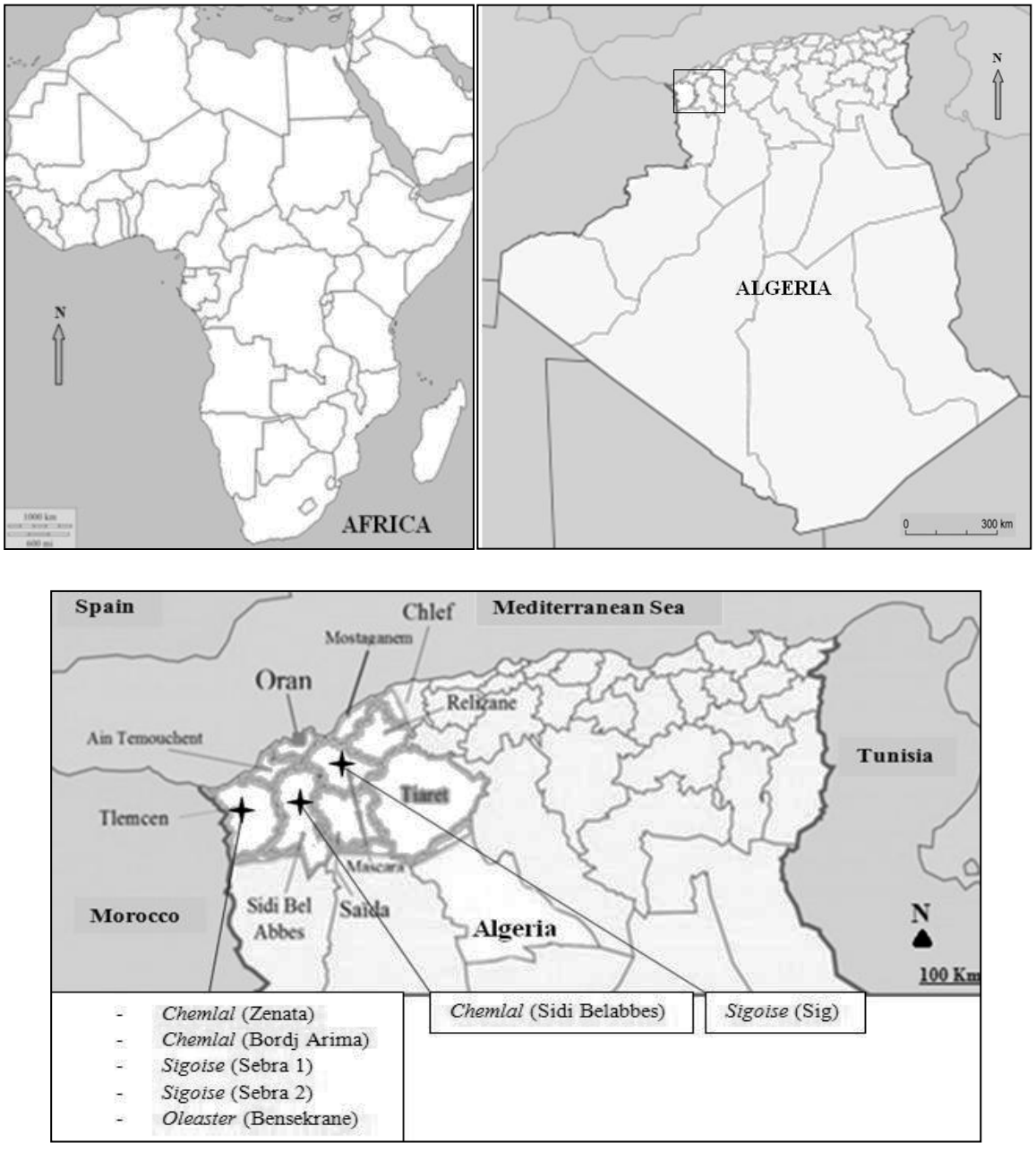


Figure 1 - Geographical location of the regions of study in Algeria

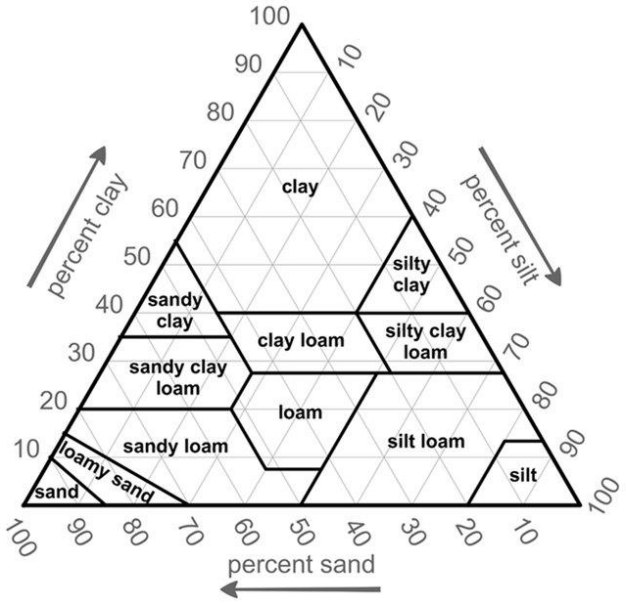


Figure 2 - USDA Soil Texture Triangle

continuous three-phase system while the olive oils *Oleaster* Bensekrane, *Sigoise* Sebra 1, *Sigoise* Sebra 2 and *Sigoise* Sig are extracted by the batch system by super press. The oils are collected in smoked glass bottles, filled, labelled and stored at a temperature of 4°C while waiting to be analysed.

2.2. QUALITY INDICES DETERMINATION

The determination of the free fatty acids, peroxide value (PV) and the UV absorption characteristics at 232 nm and 270 nm (K_{232} and K_{270}, respectively) of virgin olive oil were carried out following the analytical methods described in the European Union Commission (EEC/2568/91) [28].

2.3 TOCOPHEROL ANALYSIS

Tocopherols were analysed using a HPLC according to the method developed by Rovellini *et al* [27]. 500 mg of oil was dissolved in 10 ml of acetone. 20 µl of this solution was injected. A Column Allsphere ODS2 (Alltech) (250 mm × 4,6 mm, i.d. 4 mm) with a particle size of 5 µm and UV detectors at 292 nm were used. The mobile phase was acetonitrile/methanol (50/50) with a flow rate of 1.3 ml/min.

2.4 FATTY ACID ANALYSIS

The fatty acid composition was determined as methyl ester derivatives by gas chromatography according to methods described in EC methods [10]. Fatty acid methyl esters were prepared by vigorous shaking of a solution of each olive oil sample in n-hexane (0.5 g in 5 mL) with 0.5 mL of 2 N methanolic potassium hydroxide solution. Chromatographic analysis was performed on a CHROMPACK C 9002 gas chromatograph equipped with a FID detector, using a capillary column DB 23 (30m × 0.32mm i.d. × 0.25 µm film thicknesses). The injector and detector temperatures were maintained at 250°C; the oven temperature was set at 200°C. Nitrogen was employed as a carrier gas with a flow rate of 1 mL/min.

2.5 ANALYSIS OF THE PHENOLIC COMPOUND

The extraction of the minor polar compounds of phenolic compounds was made from 2 g of olive oil by methanol/water (80/20) solution. Identification and quantification were performed using an HPLC equipped with UV detector (λ 280 nm). A volume of 20 µl of sample was injected into a Spherisorb ODS-2 C18 column (4.6 mm × 250 mm, particle size: 5 µm). The mobile phase is composed of water/orthophosphoric acid (99.8/0.2, v/v), methanol and acetonitrile. The contents of the total and individual polyphenols are expressed in mg/kg. The internal standard is syringic acid [26].

2.6 SOIL ANALYSIS

This part begins the physico-chemical analyses of the soils of the seven olive groves where we have to collect our samples of olives, the parameters taken into consideration are: Texture, pH, total limestone and organic matter.

To carry out soil analyses, we took samples from each olive grove taking into account: sampling equipment, timing of sampling, location, depth and Conditioning of samples. After drying the samples of soils, we sieved manually using a 2 mm opening sieve, we recovered the elements passing through the sieve and that are said to be fine earth, useful for carrying out the analyses.

The purpose of the granulometric analysis is to determine the texture of the soil, the sand, and the clay, and silt content was evaluated. For this we used the Casagrande method which is based on the phenomenon of variation over time of the density of the

"soil-water" mixture measured using a hydrometer; then we used the triangle of textures (Figure 2) which allows us to determine the textural class of the soil.
The measurement of the reaction of the soil (acidity; basicity) was done using a pH meter. Among the chemicals that go into the composition of the soil, limestone plays an essential role not only in plant nutrition but also in pedogenesis. In this analysis, we used Bernard calcimeter, which allows us to measure the volume of (CO_2) released by the action of hydrochloric acid (Hcl) on the calcium carbonate ($CaCO_3$) of a sample and to measure the scale total.
$\%CaCO_3$ = (p*V)/(P*v)*100. Let v be the volume of CO_2 released by the $CaCO_3$ outlet p and V the volume of CO_2 released by the earth outlet P
For the determination of organic carbon, we used the method of Tjurin [25], which consists in knowing the quantity of potassium dichromate that will oxidise the carbon of the organic matter in the presence of sulfuric acid. The percentage of (Co) is calculated by the formula:
%Cox = [(40-d*f)*0,3/g]*100
- % Cox: percentage of carbon oxidised;
- 0,3: convert to mg;
- 40 ml: potassium dichromate 0,1M;
- d: volume of Mohr salt solution;
- f = 40% a;
- a: titration of the control solution containing only $K_2Cr_2O_7$.

To pass from the carbon rate to the total organic matter rate, the Welte coefficient is used:
% humus = %Cox*1,72

2.7 DATA ANALYSIS BY PCA
The PCA provides a graphical representation of the similarities and differences between the data in the space defined by the main components. A statistical study was carried out on all the parameters studied, to understand the results and highlight the relationships between soil parameters, altitude, and the quality of olive oil. (PCA) was applied to the dataset using TANAGRA 2.0 software.

3. RESULTS AND DISCUSSION

3.1 QUALITY INDICES
In the absence of the results of sensory analysis of the oils and according to IOC standards, the values of the quality indices (see appendix), correspond to those of the "extra virgin" category for olive oils. Chemlal Zenata, Chemlal Bordj Arima and Chemlal SBA and the "virgin" category for Sigoise Sebra 1, Sigoise Sig and Oleaster Bensekrane olive oils. As well as the "ordinary" category for Sigoise Sebra 2 oil.
The peroxide value and acidity of studied olive oil were in the ranges of 6,60-14,60 meq of O_2/Kg and 0,6-2,8 (% oleic acid), respectively. K_{270} was between 0,20 and 0,13 and K_{232} was in the range of 1,873 to 2,429.

3.2. TOCOPHEROLS
Indeed, the seven oils studied have a fairly high percentage of alpha tocopherol, the highest rate is in *Chemlal* oil SBA with 228,12 mg/Kg, followed by *Sigoise* oil Sebra 2 and *Oleaster* Bensekrane 202,9 and 201,71 mg/Kg respectively. While the oils *Chemlal* Bordj Arima, *Chemlal* Zenata and *Sigoise* Sig display rates of 193,55, 179,72 and 156,36 mg/Kg respectively. *Sigoise* oil Sebra 1 registers the low value which is 108,77 mg/Kg (Table I).
These results are in agreement with several studies which indicate that the tocopherol content is highly dependent on the variety [12-7]. According to Alasalvar *et al* [1], the proportion of tocopherols is a function of several factors such as the nature of the oil, geographical origin, culture, and climate.

3.3. COMPOSITION OF FATTY ACIDS
The analysis of the composition of fatty acids (Table II) is qualitatively similar between the samples. Quantitatively, all the oils studied have different fatty acid contents that meet the standards established by the IOC [4]. Oleic acid (C18: 1) is the dominant fatty acid, all the oils studied have proportions greater than 60%. The highest values are recorded respectively for *Oleaster* oil Bensekrane, *Sigoise* Sebra 1, *Chemlal* Bordj Arima and *Chemlal* Zenata 72,80%, 72,26%, 70,41% and 70,31% followed by other oils, the lowest value being noted for *Chemlal* oil SBA 67,78%.
The percentages of linoleic acid (C18: 2) vary between 10,24% for *Oleaster* oil Bensekrane and 12,23% for *Sigoise* Sig. While the palmitic (C16: 0) and stearic (C18: 0) acid levels vary between 11,30% for *Sigoise* oil Sebra 1 and 15,86% for *Chemlal* SBA and 2,29% for *Chemlal* oil SBA and 3.96% for *Sigoise* Sig respectively. *Oleaster* oil Bensekrane has the highest

Table I - Tocopherol content (mg/kg) of different olive oils

Olive oils	*Chemlal* Zenata	*Sigoise* Sig	*Sigoise* Sebra 2	*Chemlal* SBA	*Sigoise* Sebra 1	*Oleaster* Bensekrane	*Chemlal* Bordj Arima
Delta Tocopherol	0,56±0,13	0,51±0,02	0,69±0,09	0,58±0,13	0,35±0,06	0,49±0,04	0,57±0,09
Gamma Tocopherol	7,11±0,06	5,8±0,49	8,86±0,2	9,29±0,33	5,86±0,14	11,33±0,14	6,58±0,49
Beta Tocopherol	1,18±0,05	1,69±0,09	3,16±0,27	2,11±0,22	1,37±0,17	1,98±0,26	1,65±0,11
Alfa Tocopherol	179,72±1,67	156,36±1,27	202,9±2,74	228,12±1,58	108,77±1,77	201,71±4,95	193,55±1,97
Total Tocopherols	188,55±1,8	164,35±1,66	215,6±2,89	240,1±1,60	116,35±2,14	215,49±7,37	202,35±1,47

Means ± standard deviation (n = 3)

Table II - Fatty acid compositions (%) of the different oils

Fatty acids (%)	*Chemlal* Zenata	*Sigoise* Sig	*Sigoise* Sebra 2	*Chemlal* SBA	*Sigoise* Sebra 1	*Oleaster* Bensekrane	*Chemlal* Bordj Arima
C14 : 0	0.02 ± 0,0	0.02 ± 0,0	0.03 ± 0,0	0.02 ± 0,0	0.03 ± 0,0	0.02 ± 0,0	0.02 ± 0,0
C16 : 0	12.81 ± 0,30	12.09 ± 0,01	14.54 ± 0,04	15.86 ± 0,05	11.30 ± 0,03	11.55 ± 0,08	12.09 ± 0,07
C17 : 0	0.05 ± 0,0	0.04 ± 0,01	0.05 ± 0,0	0.04 ± 0,0	0.04 ± 0,0	0.04 ± 0,0	0.04 ± 0,0
C18 : 0	2.58 ± 0,02	2.80 ± 0,27	2.95 ± 0,06	2.29 ± 0,02	2.83 ± 0,16	2.51 ± 0,02	2.80 ± 0,02
C20 : 0	0.34 ± 0,01	0.36 ± 0,02	0.35 ± 0,02	0.34 ± 0,01	0.32 ± 0,01	0.32 ± 0,01	0.36 ± 0,01
C22 : 0	0.09 ± 0,01	0.09 ± 0,01	0.11 ± 0,01	0.06 ± 0,05	0.08 ± 0,01	0.08 ± 0,0	0.09 ± 0,0
C24 : 0	0.04 ± 0,0	0.04 ± 0,01	0.05 ± 0,01	0.04 ± 0,01	0.04 ± 0,0	0.04 ± 0,01	0.04 ± 0,01
SFA	15.93 ± 0,05	15.44 ± 0,05	18.08 ± 0,02	18.65 ± 0,02	14.64 ±0,03	14.56 ± 0,02	15.44 ± 0,02
C16 : 1	1.41 ± 0,07	1.21 ± 0,06	1.76 ± 0,01	2.11 ± 0,02	1.01 ± 0,0	1.16 ± 0,0	1.21 ± 0,02
C17 : 1	0.08 ± 0,0	0.06 ± 0,02	0.08 ± 0,01	0.09 ± 0,01	0.06 ± 0,0	0.07 ± 0,01	0.06 ± 0,01
C18 : 1	70.31 ± 0,37	70.41 ± 0,07	68.04 ± 0,17	67.78 ± 0,06	72.26 ± 0,29	72.80 ± 0,05	70.41 ± 0,07
C20 : 1	0.28 ± 0,01	0.30 ± 0,01	0.28 ± 0,01	0.25 ± 0,01	0.31 ± 0,01	0.28 ± 0,01	0.30 ± 0,0
MUFA	72.08 ± 0,11	71.98 ± 0,04	70.16 ± 0,05	70.23 ±0,03	73.64 ± 0,08	74.31 ± 0,02	71.98 ± 0,03
C18 : 2	11.21 ± 0,01	11.66 ± 0,08	10.96 ± 0,06	10.34 ± 0,02	10.86 ± 0,06	10.24 ± 0,0	11.66 ± 0,02
C18 : 3	0.81 ± 0,01	0.87 ± 0,02	0.82 ± 0,01	0.75 ± 0,0	0.88 ± 0,01	0.93 ± 0,01	0.87 ± 0,0
PUFA	12.02 ± 0,01	12.53 ± 0,05	11.78 ± 0,04	11.09 ± 0,01	11.74 ± 0,06	11.17 ± 0,01	12.53 ± 0,01
C18 : 1/C18 : 2	6.27 ± 0,36	6.03 ± 0,01	6.2 ± 0,11	6.55 ± 0,04	6.65 ± 0,23	7.1 ± 0,05	6.03 ± 0,02

Means ± standard deviation (n = 3)

oleic acid / linoleic acid ratio 7,1 and the lowest remains for *Sigoise* oil Sig 5,67.

The levels of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) vary depending on the oils and variety, the oils *Chemlal* SBA and *Sigoise* Sebra 2 have a percentage of acids saturated fat of 18,65% and 18.08% respectively, of monounsaturated fatty acids of 70,23% and 70,16% and of polyunsaturated fatty acids of 11,09% and 11,78%. While the oils *Sigoise* Sig, *Chemlal* Zenata, *Chemlal* Bordj Arima, *Sigoise* Sebra 1 and *Oleaster* Bensekrane record respectively a total of saturated fatty acids of 15,96, 15,93, 15,44, 14,64 and 14,56%, monounsaturated fatty acids of 70,79, 72,08, 71,98, 73,64 and 74,31% and polyunsaturated fatty acids of 13,3, 12,02, 12,53, 11,74 and 11,17%.

3.4. DETERMINATION OF PHENOLIC COMPOUND BY (HPLC)

The quantitative data concerning the phenolic content of the seven samples are given in (Table III). Five main phenolic groups have been detected: phenolic alcohols (hydroxytyrosol and tyrosol), secoiridoids (Mainly derived from oleuropein and ligstroside and elenolic acid), Lignans, flavonoids (luteolin and apigenin) and phenolic acids. The results relating to the content of total phenolic compounds in olive oils are shown in (Table III). The contents are between 93,32 mg/Kg for *Sigoise* oil Sig and 328,99 mg/Kg for *Chemlal* oil SBA, while the oils *Chemlal* Zenata, *Sigoise* Sebra 1 and *Chemlal* Bordj Arima display the values of 216,64, 191,3 and 188,49 mg/Kg respectively. The oils *Sigoise* Sebra 2 and *Oleaster* Bensekrane record rates of 169,56 and 141,68 mg/Kg. The significant difference noted between the polyphenol contents of the oils *Chemlal* SBA, *Chemlal* Zenata and *Chemlal* Bordj Arima, is explained by the difference in the region of olive production. The work of Essalami *et al* [20] has shown that the virgin olive oil obtained from the (*Roghiani*) cultivar from different regions of northern Libya shows a variation in its phytochemical contents and antioxidant properties. Other research has shown that the profile of phenolic compounds is also affected by the geographical origin of the variety of olive oil [2]. It is influenced by genotyping and other agro-climatic parameters [19].

For phenolic alcohols their quantity varies between 38,31 mg/Kg for *Sigoise* oil Sebra 1 and 7,01 mg/Kg for *Chemlal* Bensekrane. Hydroxytyrosol (3,4-DHPEA) and tyrosol (p-HPEA) are the main phenolic alcohols present in the oils studied. The Secoiridoide derivatives, mainly represented by oleuropeine and ligstroside derivatives, were the most abundant group of phenolic compounds in all the samples analysed, whatever their geographical origin and variety. In our case, the highest concentrations of oleuropein derivatives were observed in *Chemlal* oil SBA with 105,97 mg/Kg followed by *Chemlal* Zenata, *Sigoise* Sebra 1 and *Chemlal* Bordj Arima with 89,6, 87,48 and 60,08 mg/Kg respectively. While the lowest concentration is recorded by *Sigoise* oil Sig with 32,85 mg/Kg. As for ligstroside derivatives, the oils *Sigoise* Sig, *Sigoise* Sebra 2 and *Oleaster* Bensekrane record the lowest values 41,43, 44,78 and 54,97 mg/Kg respectively. While the highest value remains for *Chemlal* oil SBA in suite come the oils *Chemlal* Bordj Arima, *Chemlal* Zenata and *Sigoise* Sebra 1 with 83,49, 79,13 and 64,66 mg/Kg respectively.

The content of elenolic acid varies between 5 mg/Kg for *Sigoise* oil Sig and 42 mg/Kg for *Chemlal* Bordj Arima. In addition, another secoiridooid acid, name-

ly decarboxymethylelenolic acid, ranging from 1,56 mg/Kg for *Sigoise* Sig to 10,39 mg/Kg for *Chemlal* Zenata. The seven samples studied contain oleocanthal, found a considerable amount in *Chemlal* oil SBA 102,43 mg/Kg the lowest values were recorded by the oils *Sigoise* Sebra 2 and *Sigoise* Sig 13,26 mg/kg and 14,03 mg/Kg respectively. The amount of lignans varies between 6,48 mg/Kg for *Sigoise* oil Sig and 35,93 mg/Kg for *Chemlal* oil SBA followed by *Sigoise* oils Sebra 1, *Chemlal* Bordj Arima and *Chemlal* Zenata with contents of 25,91, 25,39 and 24,97 mg/Kg respectively, remainder *Sigoise* oil Sebra 2 with 20,06 mg/Kg and *Oleaster* Bensekrane with 17,65 mg/Kg.
Besides, flavonoids were in the range of 3,91 to 15,6 mg/Kg detected in the oils *Sigoise* Sig and *Chemlal* SBA respectively. Luteolin and apigenin were the most relevant compounds in this group. Luteolin, the most abundant flavonoid in the samples analysed, varies from 2,45 mg/Kg for *Sigoise* Sig to 10,16 mg/Kg for *Chemlal* SBA, while the concentrations of apigenin vary between 1,46 mg/Kg for *Sigoise* Sig and 5,44 mg/Kg for *Chemlal* SBA. Regarding phenolic acids, the oils studied have shown quantities of phenolic acids which vary between 2,12 mg/Kg for *Sigoise* Sig and 4,35 mg/kg *Oleaster* Bensekrane, except *Sigoise* oil Sebra 2 which records in quantity acceptable from 16,97 mg/Kg.

3.5 SOIL ANALYSIS RESULTS

Concerning the texture of the soils of the different olive groves (Table IV) and based on the triangle of textures (Figure 2), it appears that the soils of the olive groves Sebra 1, Sebra 2 and Sig are of silty texture, while the soils of olive groves Zenata and SBA have a silty-clay texture. However, the olive grove Bensekrane has a silty-clay-sandy texture and the latest olive grove Bordj Arima has a sandy-silty texture.
Regarding the pH, all soils have an alkaline pH, except the pH of the olive grove Bensekrane which is neutral. The load in ($CaCO_3$%) was estimated to be an average for most soils except for the soils of olive groves Sebra 1, Sebra2 and SBA which have a high load. Finally, organic matter was estimated to be high for the soils of olive groves Bensekrane and SBA and medium for the olive groves Sebra1 and Sebra2. While it is estimated to be very low for the olive groves Zenata and Bordj Arima and low for the olive grove Sig.
The olive tree grows poorly on clay soils because of the suffocation suffered by the roots during the rainy seasons. The harmful consequences of such a soil can be summed up in a significant drop in fruit and a reduced size of the olives, which affects the quality and yield of the oil extracted. Unlike clay soils, deep soils adapt much better to the olive tree by their ac-

Table III - Content of phenolic compound (mg/kg) of olive oils by HPLC

Phenolic compounds	***Chemlal* Zenata**	***Sigoise* Sig**	***Sigoise* Sebra 2**	***Chemlal* SBA**	***Sigoise* Sebra 1**	***Oleaster* Bensekrane**	***Chemlal* Bordj Arima**
Total Flavonoids	8,44 ± 0,18	3,91 ± 0,64	11,58 ± 0, 22	15,6 ± 0,01	4,86 ± 0,04	13,33 ± 0,01	6,97 ± 0,01
Apigenin	2,75 ± 0,16	1,46 ± 0,38	4,53 ± 0,09	5,44 ± 0,01	1,72 ± 0,0	3,8 ± 0,04	2,03 ± 0,01
Luteolin	5,69 ± 0,02	2,45 ± 0,26	7,05 ± 0,15	10,16 ± 0,01	3,14 ± 0,04	9,53 ± 0,04	4,94 ± 0,01
Oleuropein derivatives	89,6 ± 2,87	32,85 ± 0,6	48,6 ± 0,72	105,97 ± 0,03	87,48 ± 0,2	34,37 ± 0,33	60,08 ± 0,08
Hydroxytyrosol	12,32 ± 0,19	2,47 ± 0,09	5,28 ± 0,09	2,51 ± 0,03	22,42 ± 0,11	1,07 ± 0,03	3,75 ± 0,03
Tyrosol	9,17 ± 0,06	4,99 ± 0,04	9,68 ± 0,11	6,44 ± 0,03	15,89 ± 0,06	5,94 ± 0,07	9,48 ± 0,01
Oleuropein	0,16 ± 0,01	1,11 ± 0,07	1,66 ± 0,14	0,06 ± 0,0	0,77 ± 0,07	0,24 ± 0,08	0,11 ± 0,02
Ligstroside derivatives	79,13 ± 1,95	41,43 ± 0,17	44,78 ± 0,05	147,56 ± 0,35	64,66 ± 0,32	54,97 ± 0,03	3,49 ± 0,1
Oleocanthal	42,76 ± 0,9	14,03 ± 0,02	13,26 ± 0,06	102,43 ± 0,3	23,69 ± 0,13	24,43 ± 0,09	40,93 ± 0,01
Total secoiridoid Acids	39,18 ± 0,26	6,8 ± 0,01	22,68 ± 0,23	24,93 ± 0,1	30,19 ± 0,23	18,29 ± 0,1	49,72 ± 0,11
Decarboxymethylel enolic acid	10,39 ± 0,12	1,56 ± 0,01	5,82 ± 0,08	2,63 ± 0,02	6,48 ± 0,1	2,74 ± 0,02	7,72 ± 0,01
Elenolic acid	28,79 ± 0,14	5,24 ± 0,02	1,86 ± 0,15	22,3 ± 0,08	23,71 ± 0,13	15,55 ± 0,08	42 ± 0,08
Total Lignans	24,97 ± 0,36	6,48 ± 0,01	25,91 ± 0,29	35,93 ± 0,18	20,06 ± 0,13	17,65 ± 0,01	25,39 ± 0,08
Total Phenolic Acids	2,2 ± 0,03	2,12 ± 0,04	16,97 ± 0,14	3,35 ± 0,06	3,45 ± 0,08	4,35 ± 0,06	3,53 ± 0,01
Total Biophenols	216,64 ± 4,57	93,32 ± 0,13	169,56 ± 0,5	328,99 ± 0,64	191,3 ± 0,63	141,68 ± 0,33	188,49 ± 0,38
Total Aromatic Alcohols	21,49 ± 0,25	7,46 ± 0,05	14,95 ± 0,19	8,95 ± 0,01	38,31 ± 0,17	7,01 ± 0,04	13,23 ± 0,04
Total Natural Biophenoles	204,33 ± 4,97	86,77 ± 0,11	147,85 ± 2,06	308,4 ± 0,62	180,49 ± 0,5	124,67 ± 0,24	179,45 ± 0,11
Biophenols oxidized	12,31 ± 0,40	6,55 ± 0,02	21,71 ± 0,44	20,59 ± 0,02	10,83 ± 0,13	17,01 ± 0,09	9,04 ± 0,27

Means ± standard deviation (n = 3)

Table IV - Results of soil analyzes of the olive groves used in the study in Algeria

Granulometry %	Olive grove Sabra 1	Olive grove Sabra 2	Olive grove Zenata	Olive grove Bordj Arima	Olive grove Bensekrane	Olive grove SBA	Olive grove Sig
Sand	32	37	23	37	49	28	31,5
Silt	42	40	40	25	27	40	37,5
Clay	26	23	37	23	29	32	14,5
Texture	silty	silty	silty clay	sand silty	silty-clay-sand	silty clay	silty
pH	7,60	7,51	7,70	7,39	7,40	8,89	8
Appreciation	alkaline	alkaline	alkaline	neutral	neutral	very alkaline	alkaline
$CaCO_3$ %	28	37	11,73	3	13	24	20,27
$CaCO_3$ Charge	strong	strong	average	average	average	strong	average
Organic matter %	2,56	2,96	0,4	1	3,48	5,16	1,94
Estimate	average	average	very low	Very low	strong	strong	low
Altitude (m)	593	533	207	730	295	483	50

tion of retention of rainwater which will be exhausted by the tree during the spring to feed its vegetation, which improves the oil yield and quality [13]. As far as texture is concerned, the most suitable soils for the olive tree are those characterised by a balance between sand, silt and clay (Table V).

The predominantly sandy soils have a low capacity for retaining water and minerals but allow good aeration of the soil and constitute an advantage for the olive tree when water is available, provided that relevant fertilisation is ensured to satisfy nutritional requirements of mineral elements. The amounts of clay should not be excessive as they could constitute an obstacle to the circulation of air and the conduct of the soil.

The particles must form glomerulus structures to give a certain porosity to the soil, which is possible if the soil contains enough organic matter and if a rational soil management is carried out to avoid the phenomena of compaction or erosion.

Regarding the chemical properties, it should be noted that the olive tree tolerates a good pH margin. However, attention should be paid to acidic soils with pH levels below 6.5. Based on (Table V), it is judged that the soil of the olive grove Bensekrane is the most suitable for olive growing.

Table V - Characteristics of a soil deemed suitable for olive growing [5]

Texture	Sand 20-75%
	Silt 5-35%
	Clay 5-35%
Structure	Friable
Water retention capacity	30 - 60 %
Permeability	10 - 100 mm/h
pH	7-8
Organic matter	> 1%
Nitrogen	> 0,10 %
Phosphorus available (P_2O_5)	5 - 35 ppm
Exchangeable potassium (K_2O)	50 - 150 ppm
Exchangeable Calcium ($CaCO_3$) CO_3	1 650 - 5 000 ppm
Exchangeable Magnesium	10 - 200 ppm

3.6 INFLUENCE OF SOIL AND ALTITUDE ON THE QUALITY OF OLIVE OIL

The analysis in Principal Components (PCA) (Figure 3), is established from thirty-eight measured variables and seven individual, axis 1 (PC1, 35,86%) and axis 2 (PC2, 22,89%) carries 58,75% information from the datasets, we will therefore focus on these two dimensions for interpretation.

The circle of correlation of the variables in the plane of the main components PC1 and PC2 revealed that the first group of phenolic compounds (total biophenols, natural biophenols, ligstrosides, lignans, oleuropein derivatives, secoiridoides and oleocanthal) was in affinities with a parameter soil which is clay and the geographical parameter which is the altitude of olive groves. The second group composed of flavonoids, tocopherols, palmitic acid, palmitoleic acid, heptadecenoic acid, was in affinities with the level of organic matter in the soil and their pH, next to this group distinguish phenolic acids and lignoceric acids which have an affinity with the level of limestone in the soil.

While aromatic alcohols hydroxytyrosol and tyrosol, oleic acid (C18: 1), linoleic acid (C18: 2), gadoleic acid (C20: 1) and peroxide value (PV) were not related to soil and altitude factors. However fatty acids (C18: 0 (stearic acid), C18: 2 (linoleic acid), C14: 0 (myristic acid), C17: 0 (heptadecanoic acid), C20: 0 (arachidic acid), C22: 0 (acid behenic) with acidity, UV absorbance (K_{270}) and oleuropein level, were negatively correlated with soil parameters and altitude. According to Demnati [8], the soil nature and composition and the pH influence the quality of the oil. So, fatty soils produce fewer aromatic oils than lean soils, and oils from calcareous soils have a lower acidity than those from clay soils. Olive oil was affected by altitude, especially its fatty acid composition and the polyphenol content.

Our results agree with those of Romero *et al* [14], who worked on the influence of soil, climate, and geographic origin on the phenolic compounds of Chil-

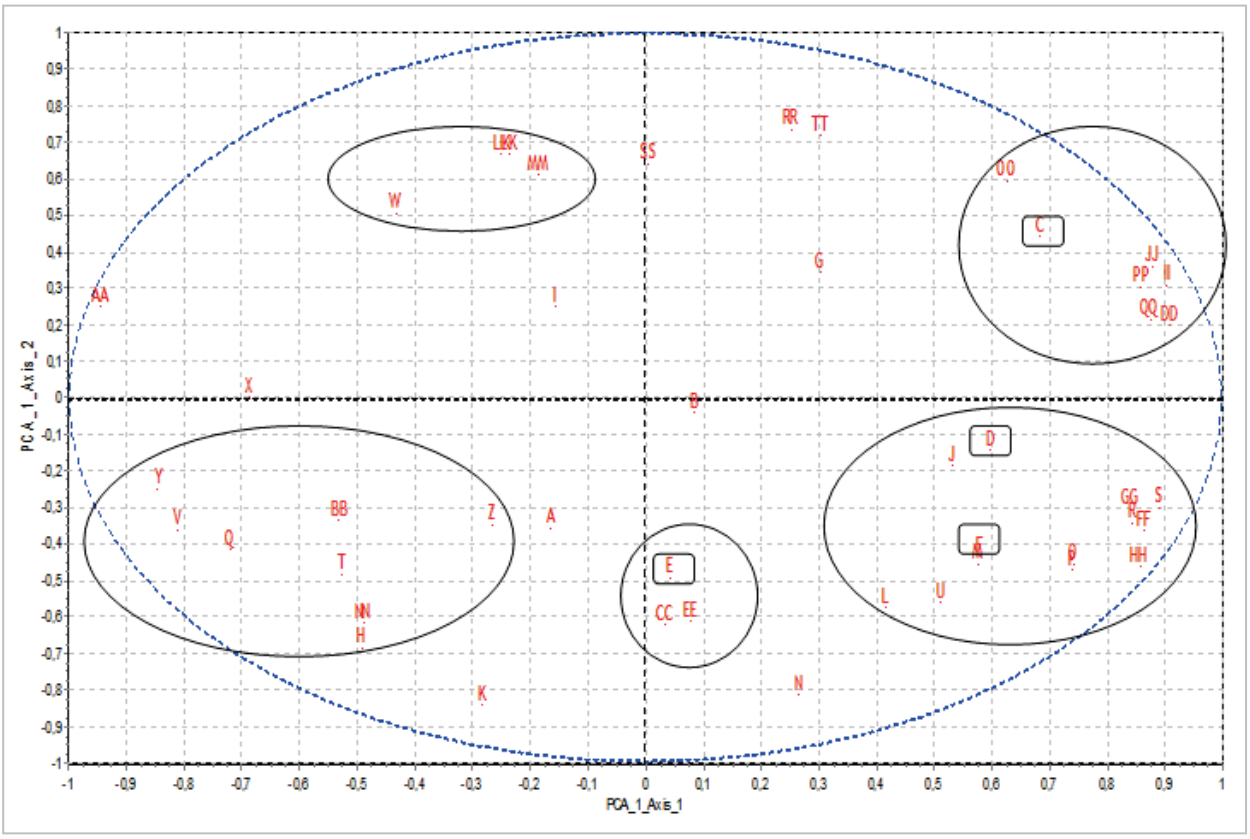


Figure 3 - Correlation circle of the variables in the main component plane PC1 and PC2

Legends :
sand: A, silt: B, clay: C, pH: D, limestone: E, organic matter: F, Altitude: G, acidity: H, PV: I, K_{232}: J, K_{270}: K, delta tocopherol: L, gamma tocopherol: M, beta tocopherol: N, alpha tocopherol: O, total tocopherol: P, C14: O: Q, C16: O: R, C16: 1: S, C17: O: T, C17: 1: U, C18: O: V, C18: 1: W, C18: 2: X, C18: 3: Y, C20: O: Z, C20: 1: AA, C22: O: BB, C24: O: CC, lignans: DD, Phenolic acids: EE, flavonoids: FF, luteolin: GG, apigenin: HH, biophenols: II, natural biophenols: JJ, aromatic alcohols: KK, hydroxytyrosol: LL, tyrosol: MM, oleuropein: NN, oleuropein derivatives: OO, ligstrosides: PP, oleocanthal: QQ, cloiridoide acids: RR, decarboxymethylelenolic acid: SS, elenolic acid: TT.

ean olive oils from cultivars *Arbequina*, *Arbosana* and *Koroneiki* and found that climate and soil have a great influence on the phenolic compounds of the oils, and they did not stress the influence of variety. Cetinkaya and kulak [3] also revealed that the level of soil limestone and organic matter influence the level of some fatty acids in olive oils from Turkish cultivars.
According to Laincer *et al* [11] The fine composition of an olive oil, in addition to being highly dependent on the cultivar used for its production, is influenced by several other factors such as climate, soil conditions and farming practices.
Also, the works of Essiari *et al* [23] on the influence of the variety and the culture medium on the fatty acid and polyphenol composition of Moroccan varieties have shown the accentuated effect of the variety, and the area of culture. Douzane *et al* [9], have shown that there is a significant effect of variety on the quality of olive oil. Haddam *et al* [22] have estimated that in addition to the cultural conditions, soil-climatic conditions and extraction methods, the varietal profile contributes with 20% to the physico-chemical and organoleptic quality of an olive oil.

4. CONCLUSION

The tocopherol composition of the seven oils studied have a fairly high percentage of alpha tocopherol, the highest rate being found in *Chemlal* oil SBA with 228,12 mg/Kg, *Sigoise* oil Sebra 1 registers the low value which is from 108,77 mg/Kg. The highest gamma tocopherol content is found in *Oleaster* oil Bensekrane with 11,33 mg/Kg. Variations in the fatty acid profiles of olive oils are noted. Oleic acid is the dominant fatty acid in the composition of the oils studied, it has proportions greater than 60%, the highest value being recorded in *Oleaster* oil Bensekrane with 72.80%. Analysis of the polyphenol composition of the olive oil samples by HPLC reveals a similar qualitative composition in individual phenolic compounds, but different from a quantitative point of view, which allowed us to distinguish between the oils; *Chemlal* oil SBA is distinguished from other varieties by the highest polyphenol contents (328,99 mg/Kg), followed by *Chemlal* Zenata with (216.64 mg/Kg). Our results have shown that the cultivar is an important factor influencing the quantitative composition of total polyphenols in olive oil.

The correlation circle of the variables in the plane of the main components PC1 and PC2 reveals that the first group of phenolic compounds total biophenols, natural biophenols, ligstrosides, lignans, oleuropein derivatives, secoiridoides and oleocanthal, with affinities with a soil parameter which is clay and the geographic parameter which is the altitude of olive groves. The second group composed of flavonoids, tocopherols, palmitic acid, palmitoleic acid, heptadecenoic acid, with affinities with the level of organic matter in the soil and their pH, next to this group distinguish phenolic acids and lignoceric acids which have an affinity with the level of limestone in the soil. While aromatic alcohols (hydroxytyrosol and tyrosol), oleic acid (C18: 1), linoleic acid (C18: 2), gadoleic acid (C20: 1) and peroxide value (PV) was not related to soil and altitude factors. However, fatty acids (C18: 0 (stearic acid), C18: 2 (linoleic acid), C14: 0 (myristic acid), C17: 0 (heptadecanoic acid), C20: 0 (arachidic acid), C22: 0 (acid behenic) with acidity, UV absorbance (K_{270}) and the level of oleuropein were negatively correlated with soil parameters and altitude.

From all the results obtained, we can conclude that the seven samples studied showed an interesting quality in terms of phenolic compounds and fatty acids and provided a lot of information on the quality of olive oils from western Algeria. The results also showed that the contents of phenolic compounds and fatty acids are dependent on the variety, the change in geographical origin, the altitude, and the composition of the soil.

Appendix: Results of oil quality analyzes

Olive oils	A %	PV	K_{232}	K_{270}
Chemlal Zenata	0.60 ± 0.01	6.70 ± 0.02	1.873 ± 0.02	0.137 ± 0.00
Chemlal Bordj arima	0.80 ± 0.00	7.80 ± 0.01	1.887 ± 0.00	0.14 ± 0.00
Oleaster Bensekrane	2.00 ± 0.01	8.40 ± 0.05	1.90 ± 0.00	0.201 ± 0.00
Chemlal (SBA)	0.60 ± 0.00	11.80 ± 0.02	2.429 ± 0.00	0.17 ± 0.00
Sigoise Sebra 1	1.70 ± 0.00	14.60 ± 0.15	2.07 ± 0.01	0.168 ± 0.00
Sigoise Sebra 2	2.80 ± 0.01	6.60 ± 0.1	1.991 ± 0.00	0.201 ± 0.00
Sigoise Sig	2.00 ± 0.00	11.7 ± 0.2	2.086 ± 0.00	0.207 ± 0.00

Means ± standard deviation (n = 3)

REFERENCES

[1] C. Alasalvar, F. Shahidi, T. Ohshima, U. Wanasundara, H.C. Yurttas, C.M. Liyanapathirana, F.B. Rodrigues, Turkish Tombul Hazelnut. 2. Lipid characteristics and oxidative stability. Journal of Agricultural and Food Chemistry 51, 3797-3805, (2003)

[2] D. Arslan, Y. Karabekir, M. Schreiner, Variation of phenolic compounds, fatty acids and some qualitative characteristics of Sariulak olive oil as induced by growing area. Food Research International 54, 1897-1906, (2013)

[3] H. Cetinkaya, M. Kulak, Soil characteristics influence the fatty acid profile of olive oils. Series B, Horticuture 60*,* 53-57, (2016)

[4] IOC, Classification des huiles d'olive. Normes internationales applicables à l'huile d'olive et à l'huile de grignon d'olive. Conseil Oléicole International, (2003)

[5] IOC, Techniques de production en oléiculture. Conseil Oléicole International, Espagne, (2007)

[6] N. Cortesi, P. Rovellini, E. Fedeli, Cultivars, technologie et qualité des huiles d'olive. Olivae 81, 26-35, (2000)

[7] A. Dağdelen, G. Tümen, M.M Ozcan, E. Dündar, Determination of tocopherol contents of some olive varieties harvested at different ripening periods. Natural Product Research 26(15), 1454-1457, (2012)

[8] D. Demnati, L'huile d'olive vierge: Qualité et dégustation. Publication. IAV. Hassan II (Rabat) : Technologie Alimentaire, Analyse Sensorielle et Gestion de la Qualité. Royaume du Maroc, (2008)

[9] M. Douzane, A. Nouani, E. Dako, M. Bellal, Influence of the variety, the crop year and the growing on the fatty acid and tocopherols composition of some Algerian virgin olive oils. African Journal of Agricultural Research 7 (34), 4738-4750, (2012)

[10] E.C, Regulation n° 796 of 6 May 2006 on changes EC- Regulation. 2568/91, Official J.L.128/815/05/02. 2002. Bruxelles, Belgium, (2002)

[11] F. Laincer, N. Laccarino, J. Amato, B. Pagano, A. Pagano, T. Tenore, A. Tamendjari, P. Rovellini, S. Venturini, G. Bellan, A. Ritieni, L. Mannina, E. Novellino, A. Randazzo, Characterization of monovarietal extra virgin olive oils fromthe province of Béjaïa (Algeria). Food Research International 89, 1123-1133, (2016)

[12] H. Manai-Djebali, D. Krichene, Y. Ouni, L. Gallardo, J. Sanchez, E. Osorio, M. Zarrouk, Chemical profiles of five minor olive oil varieties grown in central Tunisia. Journal of Food Composition and Analysis 27(2), 109-119, (2012)

[13] G.F. Montedero, M. Servili, M. Baldioli, R. Salvaggini, G. Peretti, C. Maganarini, L. Cossignani, P. Damiani, The use of biotechnologie means during oil mechanical ectraction process: Relatioship with sensory and nutritional parameters of virgin olive oil quality. Riv. Ital. Sostanze Grasse 72(403), (1995)

[14] N. Romero, J. Saavedra, F. Tapia, B. Sepúlveda,

R. Aparicio, Influence of agroclimatic parameters on phenolic and volatile compounds of Chilean virgin olive oils and characterization based on geographical origin, cultivar and ripening stage. Journal of the Science of Food and Agriculture 96(2), 583-592, (2015)
[15] A. Kavallari, S. Maas, M. Schmitz, Examining the determinants of olive oil demand in nonproducing countries: Evidence from Germany and the UK. Journal of Food Products Marketing 17(2), 355-372, (2011)
[16] H. Amrouni Sais, R. Fethallah, M. Fahas, Les performances techniques et économiques de la filière de l'huile d'olive en Algérie. Le secteur oléicole contraintes, enjeux et défis 35, (2017)
[17] ONFAA, Observatoire national des filières agricole et agroalimentaire. Note de conjoncture n°4: Le suivi de compagne de l'huile d'olive. 07, (2016)
[18] F. Mansouri, A. Benmoumen, G. Richard, M.L. Fauconnier, M. Sindic, H. Serghini-Caid, A. Elamrani, Characterization of monovarietal virgin olive oils from introduced cultivars in eastern Morocco. Riv. Ital. Sostanze Grasse 93, 21-30, (2016)
[19] A. Ragusa, C. Centonze, M.E. Grasso, M.F. Latronico, P.F. Mastrangelo, F.P. Fanizzi, M. Maffia, Composition and Statistical Analysis of Biophenols in Apulian Italian EVOOs. Foods 6(10), 1-15, (2017)
[20] S.M. Esalami, E.B. Dimic, B.B. Rabrenovic, Phytochemical profile and antioxidant capacity of virgin olive oil obtained from the olive cultivar 'Roghiani' from different regions of northern Libya. Grasas y Aceites 69(2), 2-10, (2018)
[21] N. Fares, I. Karoui, S. Sifi, M. Abderrabba, N. Jouini, The effect of extra virgin olive oil enrichment by rosemary leaves and lemon peels on its sensorial characteristics, chemical composition, and oxidative stability under storage conditions. Riv. Ital. Sostanze Grasse 95, 261-273, (2018)
[22] M. Haddam, H. Chimi, A. Amine, Formulation d'une huile d'olive de bonne qualité. OLC 21(5), 1-10, (2014)
[23] M. Essiari, S. Bachir, R. Zouhair, H. Chimi, H. Misbahi, M. Boudkhili, Influence de la Variété et du Milieu de Culture Sur la Composition en Acide Gras, en Stérols et en Polyphénols Totaux Pour les Huiles Vierges de Quatre Variétés D'olives de la Région de Saïs (Maroc). European Journal of Scientific Research 125, 95-114, (2014)
[24] F. Laincer, R. Laribi, A. Tamendjari, L. Arrar, P. Rovellini, S. Venturini, Olive oils from Algeria: Phenolic compounds, antioxidant and antibacterial activities. Grasas y Aceites 65 (1), (2014)
[25] I.V. Tjurin, Soil organic matter and its role in pedogenesis and soil productivity. Study of soil humus. Moskva, Sel'skozgiz (in Russian), (1937)
[26] N. Cortesi, P. Rovellini, P. Fusari, Dosaggio dei biofenoli degli oli vergini di oliva: idrossitirosolo, tirosolo, agliconisecoriridoidi, acidi secorididoidi, lignai e flavonoidi. Riv. Ital. Sostanze Grasse 79, 145-150, (2002)
[27] P. Rovellini, N. Cortesi, E. Fedeli, Analysis of flavonoids from *Olea europaea* by HPLC-UV and HPLC-ES-MS. Riv. Ital. Sostanze Grasse 74, 273-279, (1997)
[28] EEC, European Commission Regulation 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis, and subsequent amendments. Official Journal of European Community 1991, July 11, L248, 1-102, (1991)

The Italian modern oil mill: extraction efficiency, olive oil quality, diversification, and sustainability

Luciano Di Giovacchino

retired researcher of ISE-CRA,
Pescara, Italy
Via Livenza 21
65015 Montesilvano (PE)
Tel. +39 335.6565964
e-mail: lucianodigiovacchino@gmail.com

Received: March 16, 2023
Accepted: August 4, 2023

In the last two decades, Italian oil mill modified its structures assuming a more industrial aspect, yet assuring good yields, olive oil quality and an economic and sustainable utilisation of the by-products. The modern oil mill adopts the continue centrifugation system, two- or three-phase, and carries out the crushing of olives by the metallic crushers, having different characteristics able to influence the oil yield and the organoleptic properties of virgin olive oil. The following operation of olive paste malaxation, if carried out at rational time and temperature, favours the coalescence phenomenon and "frees" most of the oil contained in the cell vacuoles, allowing to extract up to 85-86% of the oil contained in olive fruits, without a negative effect on olive oil quality. Then, olive paste is sent to the centrifugal decanter to separate virgin olive oil from the aqueous and solid phases (decanter at 3 phases) or from the solid phase only (decanter at 2 phases). Some oil mills, having a larger loading capacity, with the end to increase oil extraction yield, carry out the double extraction of olive oil, using another specific decanter. Moreover, several oil mills have also the machine able to separate the stone fragments from olive pomace with the end to utilize them as fuel. Finally, oil mill equipped with the three phases centrifugal decanter utilizes the liquid by-product (oil mill wastewater) as fertiliser of the soil, in particular, by its controlled spreading on olive grove. In the region where there aren't industrial structures to extract pomace oil, oil mill can utilise the wet olive pomace spreading it on cultivated soil, of course, after the separation of the stone fragments.

INTRODUCTION

In Italy, the restructuring of the industrial machines of olive oil mill started at the end of 1960s, with the partial replacement of the traditional press by the continuous centrifugal decanter. The same trend was followed later in Spain, but it was rather fast, as proved by the total replacement of the pressing system, with the 3-phases centrifugation system, within the end of 1980s. In Italy, instead, the change was slower and actually some oil mills are still equipped with the presses, whereas most of oil mills carry out the extraction of oil from olives by centrifugal decanter. Today, all Spanish oil mills adopt the 2-phase decanter, instead the 3-phase decanter is mostly used in Italy. This is due to the Italian law in force which allows using the oil mill liquid by-product (oil mill wastewater) as a fertliser of agricultural soil by its controlled spreading on an olive grove, or on another cultivated soil. In Italy, most oil mills have a small-medium size, and many of them are private (more than 95%) and process olives on behalf of third parties, processing separately the olive batches of each olive grower and, therefore, carrying out a service for which they are payed. Only few oil mills, instead, have a cooperative structure, contrarily to what happens in Spain, where the number of oil mills is lower than the Italian ones, and most of them have a cooperative structure and a very large size. Anyway, the Italian sector is well organised and equipped with effective machines able to assure high oil yield and also a very good quality of oil, because

olive farmers are used to picking olives when they are not ripe, when they are green-yellow or begin to turn dark. Today, a problem of Italy is the reduction of olive production (Table I), due in part to the neglect of the culture, especially in some areas in South Italy, where the rational cultivation of olive grove was not possible because of the size and structure of old and too tall olive trees. Other reasons are the size of olive farms, in general too small to assure a right income to the farmer, also due to the high cultivation costs, or the European subsidies to the oil production, regardless of the production of olive fruits. Another problem of Italian oil mills is the selling of olive pomace, their solid by-product, because the industrial sector of olive pomace manifacturing is enduring a crisis due to the reduction of the consumption of olive pomace oil in many countries. Moreover, the production costs of pomace oil increased due to the strict regulations on industrial safety and on environment protection, and to the characteristics of raw material (olive pomace from a centrifugal decanter, two- or three-phase) that is very wet and with a low oil content. For these reasons, some oil mills, with the aim to recover the lost income (non-sale of olive pomace to the industry), tried to increase the oil yield, by a second extraction of oil using another decanter, and to recover, via a stoner machine, the wooden fragments of the pomace, to use as fuel. Therefore, the current Italian way to process olives in an oil mill is as described below.

FIRST OPERATIONS AFTER OLIVE PICKING: OLIVE STORAGE, LEAF-REMOVAL AND OLIVE WASHING

Olive farmers carry olives every day, using suitable means, to the oil mill where olives are stored in the bins, the large plastic cases able to contain about 300 kg of olives. This is a right way to store olives, waiting for their process, because it helps avoiding that the olives heat due to the presence of leaves, to small layers of olives, not above 30-40 cm, or to holes in the bins which are useful for air to go through. Moreover, the use of bins allows storing and processing the olives of each farmer separately, as they want the oil of their olives, a very common usage in Italy. Finally, the method allows storing 1.5-2.0 t of olives, taking up a small surface (only 1 m^2).

The following operations, represented by the removal of leaves and the washing of the olives, are carried out via a machine that separates the leaves by means of a strong suction, due to an aspirator pump. The presence of leaves with olives during the crushing operation, carried out using the metallic crushers, affects the organoleptic properties of oil increasing the content of the trans-2-hexenal, having a pleasant aroma of fresh cut grass, as reported in some papers [1-2]. The content of phenolic compounds of oil, instead, doesn't change because their concentration in

Table I - Production (t x 1000) of virgin olive oil in the different countries of the Mediterranean Sea

COUNTRY	Year 2015-16	Year 2016-17	Year 2017-18	Year 2018-19	Year 2019-20	Average 2015-19
Spain	1403	1291	1282	1790	1125	1378
Italy	474	182	429	174	366	325
Greece	320	195	346	185	275	264
Portugal	108	69	135	100	140	110
France	5.4	3.3	6.2	5.8	3.4	4.8
Cyprus	6.0	6.0	6.0	4.7	4.3	5.4
Croatia	5.5	5.0	3.9	3.4	4.1	4.4
Slovenia	0.5	0.4	0.4	0.9	0.3	0.5
Morocco	130	110	140	200	145	145
Algeria	82	63	82	97	128	90
Tunisia	140	100	325	140	440	229
Egypt	16.5	30.0	38.5	41.0	40.0	33.2
Libya	18.0	16.0	18.0	16.0	17.0	17.0
Israel	18.0	18.0	17.0	14.0	19.0	17.2
Palestine	21.0	20.0	19.5	15.0	39.5	23.0
Lebanon	23.0	25.0	17.0	17.5	14.0	19.3
Syria	110	110	100	154	118	118
Jordan	29.5	20.0	21.0	21.0	34.5	25.2
Turkey	150	178	283	193	230	207

Source: Document IOC. Session of November 2021

the olive paste and in the leaves is almost equal, as reported in the mentioned paper [1]. After the removal of the leaves, the same machine carries out the washing of olives via a forced flow of water useful to remove all the mineral material, as sand, earth, stones, and dust. Moreover, the washing operatiion allows to remove possible residue of pesticides, or their metabolites, if any.

OLIVE PASTE PREPARATION

When the oil mill is equipped with the pressing system, which is not that common nowadays, it is better to use the granite millestones to have an olive paste with a granulometry suitable for a better yield and an organoleptic quality of oil that is more appreciated by the traditional consumers. When the oil mill is equipped with the centrifugation system, now very widespread in Italy, the preparation of olive paste is carried out by the metallic crusher, consisting of a revolving body at high speed and a fixed part, generally consisting of a grid with holes having a diameter variable between 5 and 7 mm. The metallic crushers have a high hourly working capacity and the most common are those with fixed hammers, discs, and knives, fixed or mobile. The crushing operation, carried out by the different metallic crushers, is very important because it can influence the oil yield and the organoleptic quality of virgin olive oil as well. In particular, the variable characteristics of the metallic crushers are the speed of the revolving body, that can be changed between 1000 and 2500 (or more) rpm, and the diameter of holes of the grid. In general, when the preparation of olive paste is carried out at high speed and with a grid having small holes, it is possible to obtain an increasing of oil yield and an oil with a greater content of the phenolic compounds, in particular the secoiridoides, and, therefore, more bitter, and pungent. Continuing, the results of some studies [3, 4], carried out to ascertain the influence of the use of millstones and the metallic crushers to prepare olive paste, have indicated that the use of the metallic crusher helped to obtain an olive oil with a higher content of total phenols, thus more bitter and pungent.

OLIVE PASTE MALAXATION

The olive paste obtained after the crushing operation, carried out by the metallic crushers, is generally emulsified due to the strength with which the revolving elements crush the olives at high speed through the small holes of the grid. The emulsified oil is difficult to separate from the solid olive paste causing, therefore, a reduction of oil extraction yield. To obtain satisfying yields it needs to reduce, or remove, the emulsion by a suitable operation of malaxation, carried out at right temperature, between 24 and 30°C, and for a time variable between 30 and 60 minutes. The malaxation consists in a slow movement of olive paste that helps the small drops of oil merge into larger drops [5] and form a continuous liquid phase, the free oil, easy to separate from other phases by the centrifugal decanter. The malaxation operation carried out in an industrial oil mill, adopting the suggested values of time and temperature, helps increase oil yield and doesn't influence the commercial quality of olive oil [6-8], as shown in the Tables II [6] and III [7]. In particular, the oil oxidation does not occur because the oxygen in the olive paste is consumed by the oxidation of phenols (lesser part) and by the respiration of microrganisms (greater part), as verified in a specific paper [9]. The commercial quality of virgin olive oil depends above all on the soundness of the olives, whereas its organoleptic quality depends on the cultivar and, above all, on the ripening degree of the olives and on the adopted crushing method.

Table II - Average values of some qualitative parameters of oils obtained by a 3-phases centrifugal decanter from olive pastes mixed for different times in an open mixer

Determinations	Malaxation time (minutes)		
	15	45	90
Free fatty acids (%)	0.42[a]	0.43[a]	0.41[a]
Peroxide value (meq/kg)	5.4[a]	5.3[a]	5.3[a]
K_{232}	1.50[a]	1.51[a]	1.51[a]
K_{270}	0.11[a]	0.11[a]	0.11[a]
Organoleptic assessment (score) *	7.2[a]	7.2[a]	7.2[a]
Total phenols (mg/L, as gallic acid)	269[a]	267[a]	225[b]
Induction time (Rancimat) (hours)	12.9[a]	12.5[a]	11.5[a]
Chlorophyill pigments (mg/kg)	5.9[a]	5.9[a]	5.8[a]

* Values variable between 1 and 9; extra virgin olive oil: score ≥ 6.5
Different letters along the same row indicate significant differences (P≤0.05)

Table III - Variation of some qualitative parameters of oils obtained by a 2-phases centrifugal decanter from olive pastes (cv. Cornicabra) malaxed for 60 minutes at different temperatures

Determinations	Malaxation temperature (°C)		
	20	28	40
Free fatty acids (%)	0.21[a]	0.21[a]	0.25[a]
Peroxide value (meq/kg)	5.9[a]	6.2[a]	6.8[a]
K_{232}	1.60[a]	1.67[a]	1.70[a]
K_{270}	0.14[a]	0.15[a]	0.16[a]
Total phenols (mg/kg)	450[a]	650[b]	788[c]
o-Diphenols (mg/kg)	205[a]	326[b]	390[c]
Oxidative stability Rancimat (hours)	14.7[a]	21.8[b]	24.7[c]
α-Tocopherol (mg/kg)	205[a]	219[b]	221[b]
β-Carotene (mg/kg)	0.63[a]	0.87[b]	1.56[c]
K_{225}	0.46[a]	0.52[b]	0.56[c]

Different letters along the same row indicate significant differences (P≤0.05)

SEPARATION OF OIL FROM OLIVE PASTE

In Spain, the extraction of oil from olives has been carried out only by the centrifugation system for over thirty years, first by the three-phase centrifugal decanter and, since the end of the past century, only by at the two-phase one that does not produce wastewater. As said before, the replacement of the pressing system was slow in Italy, but today most of oil mills carry out the oil separation from the malaxed olive paste by the continuous centrifugation system, as in other countries of the Mediterranean basin. However, in Italy the three-phase decanter is the method used the most, because it allows an easier control of the efficiency and the performance of the plant when it needs to process many batches of olives, belonging to different farmers and with different rheological characteristics, separately. Moreover, the use of the three-phase centrifugal decanter is also preferred because it is possible to utilise the oil mill wastewater to fertilise the agricultural soil, as permitted by the Italian law in force (Law 574/1996). Several published papers have highlighted the good quantitative, qualitative, and economic results obtained by using the three and two-phase centrifugation system and, therefore, it is possible to state that the Italian oil mill sector has reached an optimum technological standard. In fact, the centrifugation system does not cause a pollution of oil which may preserve all the original characteristics depending, above all, on the quality of the olives. Even more so occurs when the two-phase decanter is used, because it doesn't need to add water to olive paste, and, therefore, the obtained oil will have the maximum content of phenolic compounds, some of which are partially water-soluble, as shown in Table IV [10]. However, when the two-phase centrifugal decanter is used, it needs to reduce the hourly loading of olive paste into the decanter to obtain a satisfying oil yield. That is due to the viscosity of the olive paste (too high because not diluted by adding water) that causes the reduction of the sedimentation rate of the solid phase and, as consequence, the increase of the corresponding sedimentation time [11]. To assure the efficiency of the process, the time of the olive paste into the decanter needs to be extended by reducing its hourly loading to a value equal to 50-60% of the theoretical capacity suggested by the manufacturer. When the oil mill processes olives by the two-phase decanter, the non-extracted oil remains into the solid by-product and, sometimes, its percentage may be too high, especially when the amount of olive paste that is sent hourly to decanter is excessive. This often happens when the oil mill has to process large amount of olives and needs to feed the decanter with a high quantity of olive paste every hour. These circumstances that occur mainly in the countries that have a high olive production, suggested to recover, in the same oil mill, part of oil by means of a further immediate centrifugation of the partially deoiled olive paste, coming from the first extraction. The aim of the second extraction of oil from the olive paste (in the same oil mill) is just that of obtaining a higher oil yield for the benefit of the olive grower. Of course, this process is expensive and, therefore, could be convenient only for a large size oil mill, especially for a cooperative oil mill as it needs to process a large amount of olives on a daily and yearly base. This explains why it is more common in Spain and not widespread in other countries. In Italy, infact, only a few oil mills, private or a cooperative, carry out the double extraction of oil from olives by centrifugation of olive paste. Not many papers have been published on the theme of the double extraction of oil from olives by the centrifugation system [12-18] and the obtained results have generally indicated that the amount of the recovered oil was small (0.3-0.7 kg/100 kg olives) and that a possible anomaly in the percentage of triterpene dialcohols and in the content of waxes of oil might occur. The average quantitative results of some tests, carried out by processing many and big batches of olives in three different large size oil mills (Oil Mill A, B and C), where the double extraction of oil was carried out by two and three-phase centrifugal decanter are (in short) reported in Table V [13, 15, 18]. The data indicate that the total extracted oil varied between 87.0 and 87.5% of the oil content of olives and the oil lost in the by-products varied between 2.4 and 2.6 kg/100 kg of olives. The average oil yield of the first extraction was 19.3 kg/100 kg of olives, whereas the average recovered oil of the second extraction was 0.54 kg/100 kg of olives. However, that last value is an indication only and refers to the mentioned specific test, because it depends, above all, on the oil yield obtained in the first extraction, that, when high, causes, of course, a lower value of the oil yield of the second extraction. Anyhow, the recoverd oil, even if small in quantity, represents an important income for the oil mill, and for the olive grower, when the total amount of olives daily processed is very high. With reference to the chemical-physical characteristics of the oils extracted in the mentioned test, Table VI [13, 15, 18] reports the average values of some parameters

Table IV - Phenolic composition (mg/kg) of oils (cv Coratina) obtained by the centrifugal decanter at 2 and 3-phases in an industrial oil mill

Determinations	Centrifugal decanter working at	
	2-phases	3-phases
3,4-DHPEA	0.87	0.58
p-HPEA	3.7	2.3
3,4-DHPEA-EDA	522	427
p-HPEA-EDA	78.2	67.3
3,4-DHPEA-EA	352	245
Total phenols (mg/kg, as 3,4-DHPEA) *	673	585
Induction time (hours)	17.8	15.5

* Evaluated by the colorimetric method

Table V - Results obtained in three oil mills of Puglia region where the double extraction of oil, from cv. Coratina olives, was carried out by the decanter at three-phases (Oil Mill A), by the decanter at two-phases (Oil Mill B) and by the decanter at two-phases (1st extraction) and at three-phases (2nd extraction) (Oil Mill C)

Oil Mill A	Olives		Oil Yield %		Olive pomace		Oil Mill Wastewater		Oil lost in the by-products *	
	H_2O %	Oil %	1st Extract.	2nd Extract.	H_2O %	Oil %	Dry Matter	Oil g/L		
	45.2	23.2	85.3	2.2	47.6	2.29	9.6	12.2	2.62	
Oil Mill B	Olives		Oil Yield %		Olive pomace 1st Extract.		Olive pomace 2nd Extract.		Oil lost in the by-products *	
	H_2O %	Oil %	1st Extract.	2nd Extract.	H_2O %	Oil %	H_2O %	Oil %		
	48.4	24.0	83.7	3.4	62.8	4.00	68.7	2.8	2.50	
Oil Mill C	Olives		Oil Yield %		Olive Pomace		Oil Mill Wastewater		Oil lost *	Recoverd Stone *
	H_2O %	Oil %	1st Extract	2nd Extract.	H_2O %	Oil %	Dry Matter %	Oil g/L		
	50.5	21.2	85.6	1.4	59.5	3.00	10.4	18.5	2.4	12.6

*Value calculated and expressed as kg/100 kg olives

Table VI - Average characteristics of virgin olive oils of first and second extraction obtained by processing olives (cv Coratina) in oil mills A, B and C located in Puglia region (I)

Oil Mill	Extraction	Free fatty Acids (%)	Peroxide Value (meq/kg)	K_{232}	K_{270}	Total Phenols (mg/kg)	Total Sterols (mg/kg)	Waxes (mg/kg)	Erythrodiol + Uvaol (%)
A	First	0.23	3.1	1.29	0.070	136	---	---	1.7
	Second	0.29	5.3	1.40	0.103	116	---	---	5.2
B	First	0.62	7.8	1.38	0.121	310	1204	45	3.9
	Second	0.86	10.6	1.64	0.197	420	1995	95	9.8
C	First	0.18[a]	5.4[a]	1.60[a]	0.14[a]	306[a]	1060[a]	31.6[a]	3.1[a]
	Second	0.26[b]	6.5[b]	1.78[b]	0.18[b]	366[b]	1590[b]	53.4[b]	8.5[b]

Different letters along the same row indicate significant differences (P≤0.05)

generally controlled to know the qualitative characteristics of oils and, therefore, their commercial category. Moreover, only to ascertain other possible effects of the technical conditions adopted in the second extraction, Table V also reports the average values of the total content of phenols, sterols, waxes and percentage of erythrodiol + uvaol of oils. The data indicate that the values of the qualitative commercial parameters of oils of second extraction were significantly higher than those of oils of the first extraction. However, they were consistent with the limit values established for olive oil of extra virgin category. The average values of the content of total phenols, sterols and waxes and the percentage of the triterpene di-alcohols of the second extraction oils were also significantly higher than those of the first extracted oils. However, only the percentage of erythtrodiol + uvaol was higher than the legal limit (4.5%) established for virgin olive oil. At this point, it needs to highlight that the oil obtained in the second extraction is a virgin olive oil because it is extracted in the oil mill from olive paste by mechanical means only, and, therefore, it can be mixed with other virgin olive oils, per choice of the oil mill manager. To verify the characteristics of the mixture of oils extracted in the first and second centrifugation, the oils, obtained in oil mill C were mixed in the same percentage obtained in the mechanical industrial process. The analytical results of the final mixture are reported in Table VII [19]. The data indicate that the blend of oils obtained from the first and second extraction, has the caracterstics of an extra virgin olive oil, and it couldn't be otherwise because of the very small amount of oil obtained in the second centrifugation. Therefore, the oil of the second mechanical extraction may be mixed with that of the first extraction if the obtained blend does not cause an alteration of the commercial category. Of course, it is interest of the oil mill responsible to avoid a worsening of the commercial quality of virgin olive oil because it would lead to a reduction of its economic value.

Table VII - Average characteristics of virgin olive oils obtained in the first and second centrifugation of olive pastes and those of oil obtained by blending the two oils in the same ratio obtained in oil mill

Determinations	Oil of 1st extraction	Oil of 2nd extraction	Blend of oils of 1st and 2nd extraction
Free fatty acids (%)	0.18[a]	0.26[b]	0.20
Peroxide value (meq/kg)	5.4[a]	6.5[b]	5.4
K_{232}	1.60[a]	1.78[b]	1.60
K_{270}	0.14[a]	0.18[b]	0.14
Total phenols (mg/kg)	306[a]	366[b]	307
Chlorophyll pigments (mg/kg)	39[a]	217[b]	41
Total sterols (mg/kg)	1060[a]	1590[b]	1065
Erythrodiol + Uvaol (%)	3.1[a]	8.5[b]	3.2
Waxes (mg/kg)	31.6[a]	53.4[b]	32.0

Different letters along the same row indicate significant differences (P≤0.05)

Table VIII - Characteristics of olive pomace obtained by the different olive processing systems

Determinations	Pressing	3-phases Centrifugation	2-phases Centrifugation
Amount (kg/t olives)	250-350	450-550	800-850
Moisture (%)	22-35	45-55	65-75
Oil (% on fresh matter)	6-8	3.5-4.5	3.0-4.0
Fiber (%)	20-35	15-25	10-15
Stone fragments (%)	30-45	20-28	15-18
Ash (%)	3-4	2-4	3-4
Nitrogen (mg/100 g)	250-350	200-300	250-350
Phosphorus (mg/100 g)	40-60	30-40	40-50
Potassium (mg/100 g)	150-200	100-150	150-250
Total phenols (mg/100 g)	200-300	150-250	400-600

UTILIZATION OF OLIVE POMACE

Olive pomace is the solid by-product obtained in the oil mill when olives are processed using various systems, the pressing or two- or three-phase centrifugation. The chemical composition of olive pomace is variable and depends, above all, on olive characteristics and on the mechanical system used to extract virgin olive oil. A possible composition of olive pomace is reported in Table VIII [19]. Until the end of the past century, the olive pomace obtained in the oil mill was sold to the industry of olive pomace to produce pomace oil and the de-oiled and dry residue, useful as a fuel. As said, in Italy the mentioned industry has reduced its activity and the oil mill tried to otherwise utilize olive pomace with the aim to recover the lost income. Today, many oil mills, by a suitable stoner machine, carry out the separation of stone fragments from the flesh of olive pomace, coming from the three- or two-phase decanter, with the aim to use them as fuel. The amount of the recovered stones depends on the size of stone fragments of olive pomace and on the diameter of holes of the stoner machine used. The results obtained in a specific test [20], carried out by taking olive pomace samples from different oil mills, indicated that the amount of recovered stone fragments was 12.5 and 15.3 kg/100 kg olives, when olive pomace was, respectively, obtained by the three- or two-phase decanter. A similar result (12.6 kg/100 kg olives) was obtained in the test carried out in oil mill C (Table V) and reported in a specific paper [18]. Olive stones are constituted of lignin, a complex polymeric chemical compound, having a tridimensional structure and formed from phenolic substances (phenol-alcohols). As reported in other paper [21], the chemical composition (on dry matter) of olive stone fragments is the following: Carbon 49.7%; Hydrogen 7.02%; Oxygen 43.0%; Nitrogen 0.041%; Sulphur 0.020%: Chlorine 0.22%. Other chemical-physical characteristics of stone fragments, freshly obtained from the stoner machine, are the following [20]: moisture 19.5-20.8%; oil 0.27-0.49%; ash 0.31-0.39%. Similar results were obtained and reported in the aforesaid paper [21]: moisture 22%; oil 0.46%; ash 0.20%. Moreover, the most important property of stone fragments is the calorific value, resulting, on average, 4117 kcal/kg [20], close to the value of 18.2 MJ/kg, reported in the mentioned paper [21]. Finally, the olive stone fragments, used as fuel, have the

important characteristic to produce, while burning, a negligible amount of ash, as residue, and traces of sulphured and nitrogenous gas (dioxides) only in the smoke.
Wet olive pomace obtained by the centrifugal decanter at the two- and three-phases can be utilised as an amendment and fertiliser of the agricultural land, by its controlled spreading on the cultivated soil, as permitted by the Italian law n. 574/1996. Some papers have been published on this topic, [22-26], important are the results obtained in a specific test and reported in Table IX [27]. The data indicate that spreading 50 t/ha of wet olive pomace, obtained by a three-phase decanter, on the olive grove allowed to significantly increase, in the fourth year of treatment, the olive production from 17.8 kg/tree (control) to 22.0 kg/tree (treated plots with 50 t/ha of olive pomace). Moreover, the values of other parameters also positively increased, as Table IX shows. In the light of what is reported in this mentioned test [27], it is possible to state that similar or better results could be obtained by spreading, on the olive grove, the wet and stoned olive pomace from the two-phase decanter. This is because it also contains the liquid phase of the olive paste, where a large part of organic water-soluble substances, like sugars, acids, pectins, phenols, mineral salts, etc., very important for the fertility of soil, are solved.
In the last years, it has been suggested to add wet olive pomace to other liquid and solid vegetable waste to treat in the biological purification plants, based on the anaerobic digestion, able to produce bio-methane also. That solution on one hand represents a form of disposal of a waste without any benefit to the agricultural sector and useful only for few oil mills, on the other it takes away to the agriculture a natural resource useful to fertilize the cultivated soil or to utilize as renewable source of thermal energy.

Table IX - Results obtained in olive trees cultivation (cv. Leccino) on soil treated, for 4 consecutive years, with 50 t/ha of fresh olive pomace (3-phases dec.)

Determinations		1° year	2° year	3° year	4° year
Olives production (kg/tree)	Control	5.8	8.0	11.8	17.8
	50 t/ha	7.2	10.0	16.0	22.0
Productive efficiency (kg olives/m^3 foliage)	Control	0.5	0.65	0.82	1.25
	50 t/ha	0.6	0.90	0.95	2.35
Olive dry weight (g)	Control	0.72	0.95	0.65	0.70
	50 t/ha	0.88	1.10	0.80	0.75

UTILISATION OF OIL MILL WASTEWATER

Oil mill wastewater (OMW) is the liquid by-product obtained when oil mill processes olives by the pressing system or by the centrifugal decanter at three-phases. The chemical composition of OMW is variable not only in consequence of the employed mechanical system but also for the influence of olive cv, its ripening degree and the different technical operations adopted in oil mill, in particular the amount of water added to olive paste. A possible composition of OMW is reported in Table X [19]. Many papers were published on the theme of purification and/or utilization of OMW, as reported in a specific book [28], but the proposed solutions, generally based on the concentration or the destruction of the natutral organic substances present in OMW, never had a practical use because the results were

Table X - Amount and characteristics of oil mill wastewater (OMW) obtained in olive processing by the different systems adopted in oil mill

Determinations	Adopted system to process olives		
	Pressing	3-phases Centrifugation	2-phases Centrifugation
Amount (L/t olives)	400-500	600-800	100 *
pH	4.5-5.7	4.5-6.0	4.5-5.0
Dry residue (%)	8-20	4-15	1.4-2.0
Organic matter (%)	6-16	3-12	1.3-1.9
Oil content (%)	0.2-0.8	0.6-2.0	0.5-0.6
C.O.D. (g O_2/L)	60-200	50-170	10-12
Total phenols (g/L) **	2-10	2-8	0.5-1.6
Ash (%)	2-4	1-3	0.1
Nitrogen (%)	0.10-0.15	0.05-0.10	---
Phosphorus (%)	0.05-0.10	0.02-0.06	---
Potassium (%)	0.2-0.5	0.1-0.3	---

* Water used to wash oil in the vertical centrifuge; **Expressed as caffeic acid.

Table XI - Average results obtained by spreading OMW on soil cultivated with olive trees for 9 consecutive years

Determinations	Control plot	Plot treated with 30 L OMW/m^2 *
Olive production (kg/tree)	5.7	6.5
Oil content of olives (%)	17.5	17.7
Phenols content of oil (mg/kg)	196	206
Characteristics of the soil Organic matter (g/100 g)	1.77	2.18
Total nitrogen (g/100 g)	0.11	0.13
Available P (mg/kg, as P_2O_5)	34	53
Exchangeable K (mg/kg, as K_2O)	250	265
Reducing substances (mg/100 g)	0.12	0.42
Total microflora (CFU/g of soil)**	3.0 x 10^8	5.9 x 10^9

*Plot not fertilized; ** CFU: Colonies Forming Units

partial and the cost too high, in particular for the energy requested from the studied processes. Other researches, instead, were carried out with the aim to re-use OMW in agriculture, by its recycling on the soil cultivated with herbaceous [29-32] or arboreal plants [33-41]. Among the test carried out on herbaceous cultivations, very important were the results reported in a paper [29] in which OMW, from a three-phase decanter, was spread on soil cultivated with maize for 5 years. The average maize production was 8301 kg/ha, in the plots treated with 200 L OMW/m^2 and not fertilised, whereas the production of the control plots, normally fertilised by chemical fertilisers, was 7714 kg/ha. Similar results were obtained in a specific test, as reported in another paper [32] and shown in Figure 1, where the significant increase of the biomass production of the plots treated with 50 L OMW/m^2 and not fertilised (on average 40.7 kg/plot) is pointed out, with respect to that of the control plots, normally fertilised by chemical synthetic fertilisers (on average 32.4 kg/plot).

However, more interesting tests were carried out by reusing and spreading OMW on soil cultivated with trees. The results obtained spreading OMW on soil cultivated with old olive trees have indicated, as reported in another paper [36], that the average olive

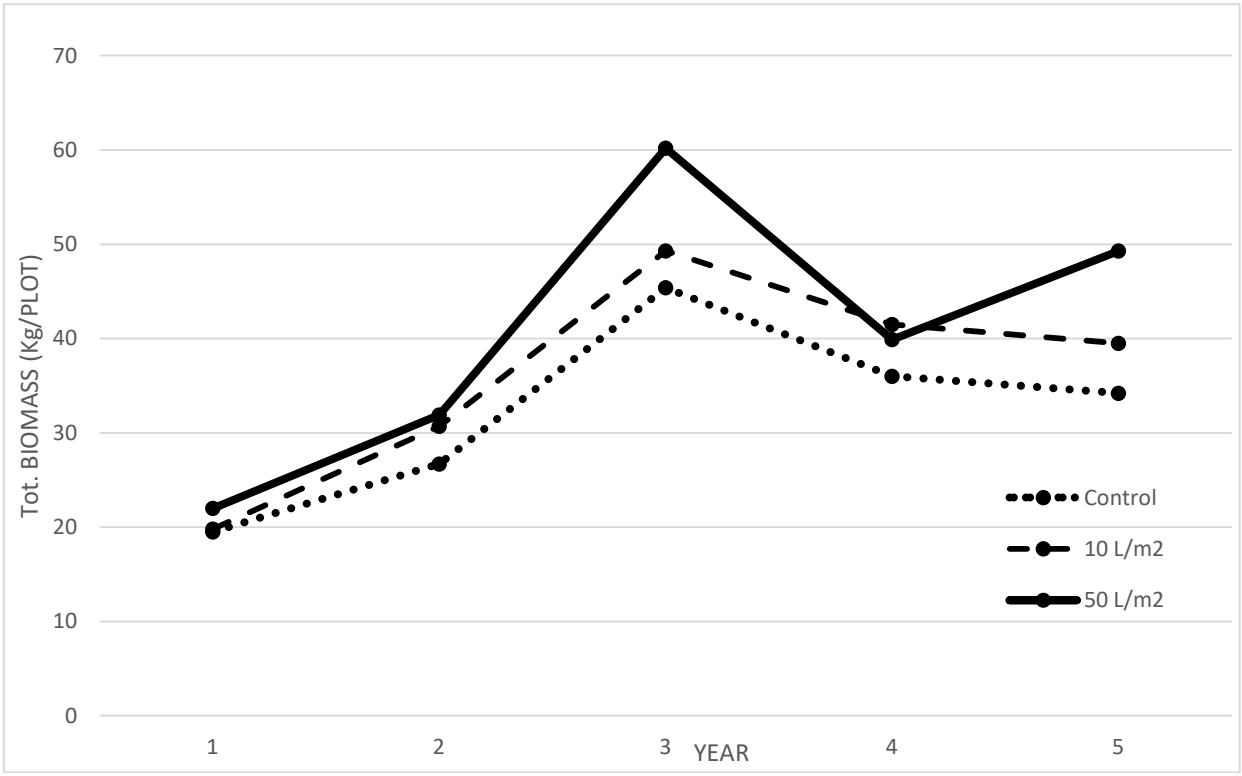


Figure 1 - Results obtained (total biomass) in the maize cultivation (5 consecutive years) on soil treated with different amount of olive mill wastewater (OMW)

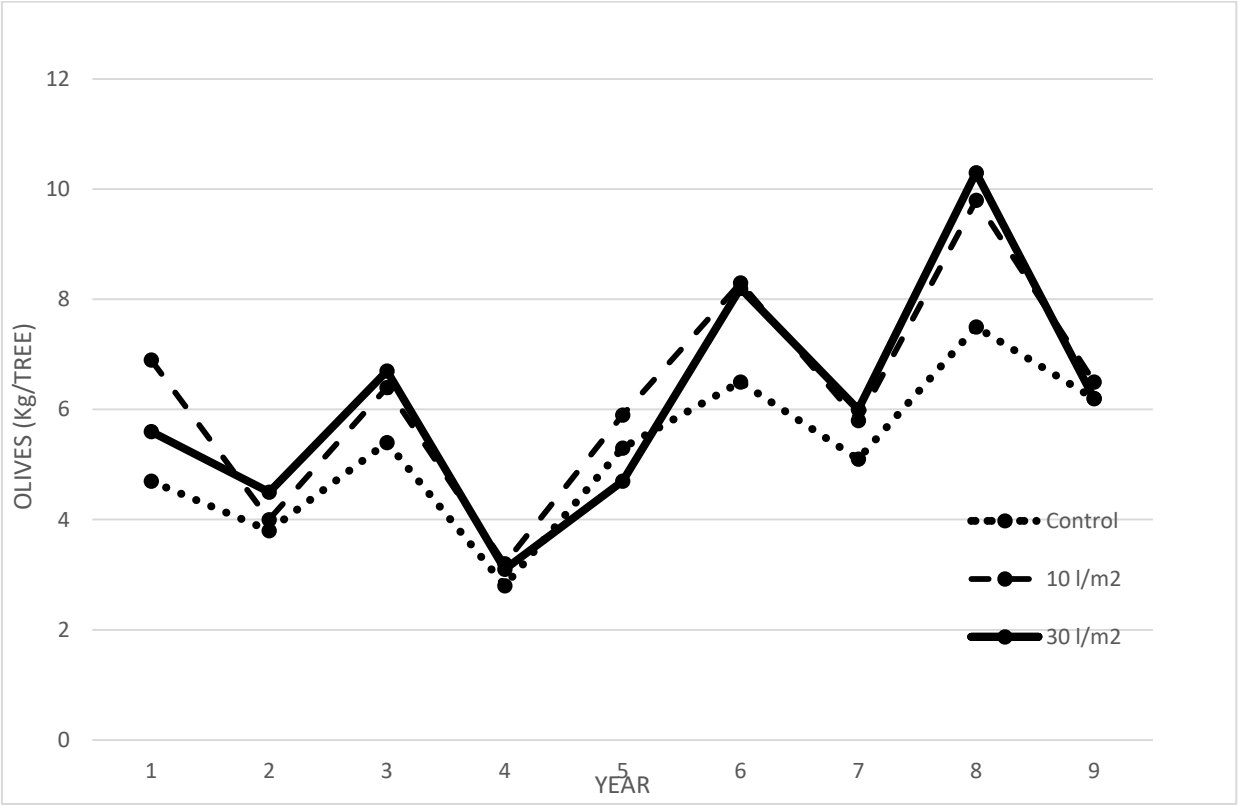


Figure 2 - Olives production in olive trees cultivation (9 consecutive years) on olive orchard treated with different amount of olive mill wastewater (OMW)

production of the plots treated with 100 m^3 OMW/ha was 510 kg/ha, whereas the olive production of the control plots was, on average, 345 kg/ha. On the same subject, the results of a specific test, carried out spreading OMW on soil cultivated with young olive trees and reported in another paper [40] and in Figure 2, have shown that the average olive production (9 consecutive years) of the plots treated with 30 L OMW/m^2 and not fertilised was 6.5 kg/tree, whereas the olive production of the control plots, normally fertilised, was, on average, 5.7 kg/tree, as shown in Table XI. Even though the average olive production wasn't significantly different, it is importanrt to point out that the spreading of OMW allowed avoiding the use of chemical fertilisers. Moreover, the results obtained at the end of the test have shown that the values of some characteristics of the soil of plots treated with OMW were similar or better than those ascertained on the control plots, as reported in the same Table XI [40]. Another interesting test was carried out to ascertain the effects of OMW spread on soil cultivated with a vineyard [41]. The results obtained at the end of the test showed that the average production (11 consecutive years) of grapes for the plots treated with 50 L OMW/m^2 and not fertilised was 30.2 kg/grapevine, whereas the grapes production of the control plots, normally fertilised with chemical fertilisers, was 22.5 kg/grapevine, as reported in the paper [41] and in Figure 3. Moreover, the values of some characteristics of grapes, grape juice and soil were similar or better than those ascertained on the control plots, as reported in Table XII.

The good results obtained by spreading OMW on cultivated soil are due to the supply of mineral and organic matter contained in the liquid by-product of oil mill when olives were processed by pressing and three-phase centrifugation systems. Table XIII reports the amount of chemical and natural (OMW) fertilisers supplied to the soil cultivated with olive grove, previously described [40]. The data indicate that the treatment with 30 L OMW/m^2 supplies to the soil, with respect to the standard fertilisation of control plots, more nitrogen (about twice), the same amount of phosphorus, more potassium (4-5 times) and more than 17 t/ha of dry organic matter. These data explain the same (or higher) olive production of olive trees cultivated in the soil plots treated with the largest amount of OMW and not fertlised. Moreover, it is important to point out that, at the end of the test, after 9 consecutive years of treatment, the chemical and micro-biological characteristics of the soil, treated with 30 L OMW/m^2, were similar or better (significant difference) than those of the control plots. In particular, the increase of the reducing substances of the soil treated with OMW, as shown in Tables XI and XII seems interesting. This is due to the phenols supplied with OMW that, on time, oxidise and polymerise forming substances with similar properties of humic

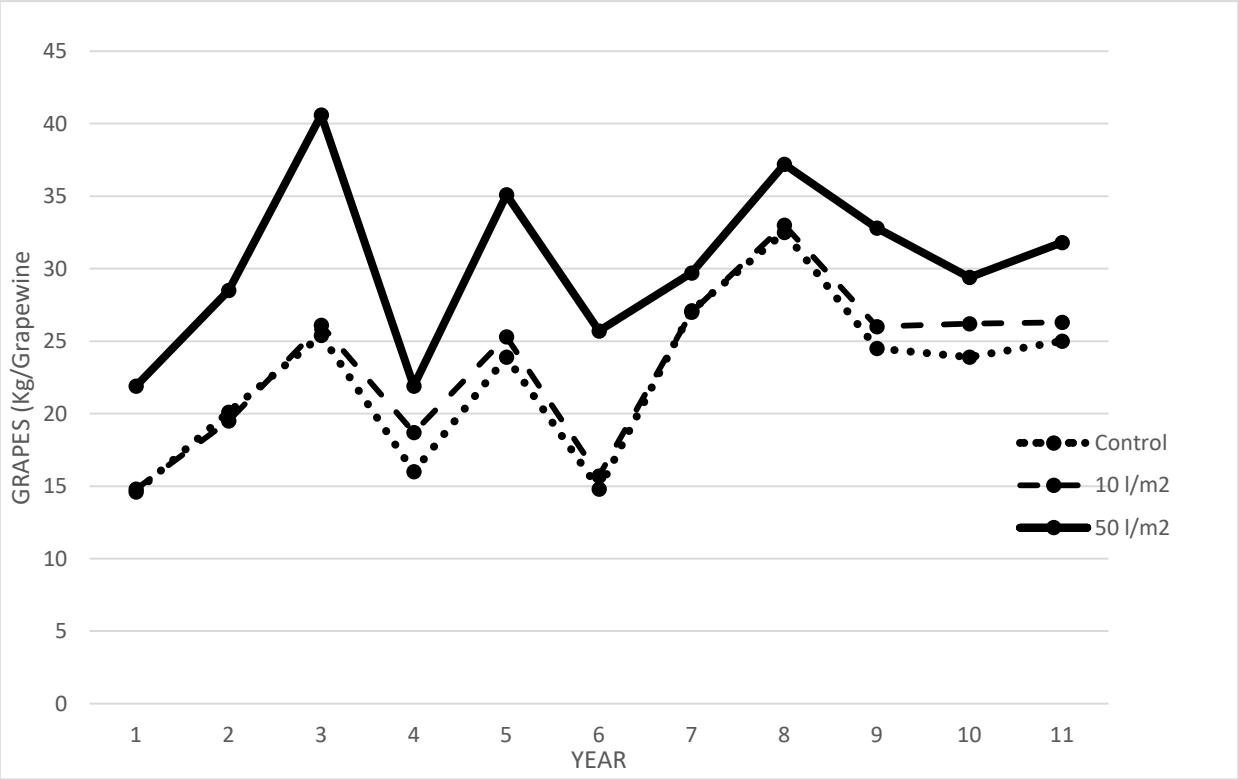


Figure 3 – Grapes production in the grapevine cultivation (11 consecutive years) on soil treated with different amount of olive mill wastewater (OMW)

Table XII - Average results obtained spreading OMW on soil cultivated with grapevine for 11 consecutive years

Determinations	**Control plot**	**Plot treated with 50 L OMW/m² ***
Grapes production (kg/grapevine)	22.5	30.2
Sugars content of grape-juice (%)	15.5	15.7
Organic acidity of grape-juice (g/L)	7.8	8.0
Characteristics of the soil Organic carbon (%)	0.94	1.13
Total nitrogen (g/100 g)	0.10	0.13
Available P (mg/kg, as P_2O_5)	45.4	88.0
Exchangeable K (mg/kg, as K_2O)	244	330
Reducing substances (mg/100 g)	0.17	0.65
Saprobic fungi (CFU/g of soil)**	10^7	10^8

*Plot not fertilized; **CFU: Colonies Forming Units

Table XIII - Dry organic matter and mineral nutritious elements supplied to soil, cultivated with olive grove, by the chemical fertilization and by the controlled spreading of OMW (3-phases decanter)

Parameters	**Control**	**Oil Mill Wastewater (OMW)**		
		5 L / m²	**10 L / m² ***	**30 L / m² ****
Dry organic matter (kg/ ha)	--	2960	5920	17760
Nitrogen (kg/ ha, as element)	46 + 25	71 + 25	35.5 + 50	150
Phosphorus (kg/ ha, as element)	50	50 + 7.5	25 + 15	45
Potassium (kg/ ha, as element)	100	100 + 80	50 + 160	480

*Partially fertlized; **Not fertilized

and fulvic acids [42-43], confirming the results of other studies [44-49] which have ascertained that the phenolic compounds are the precursors in the synthesis of humic substances. These new substances still have a reducing power and are very useful for agriculture because they increase the fertility of soil.

Finally, it is important to point out that the recycling of OMW, and the fresh wet fiber of olive pomace, on the cultivated soil is a practice that accomplishes the concept of sustainable agriculture because it utilises a natural product coming from the agriculture that leads, not only to an increase of the production of arboreal and herbaceous cultivations, but also to an improvement of the chemical and micro-biological properties of the soil. Moreover, the recycling of the by-products of oil mill on cultivated soil, from which they derive, is an example of circular economy because it helps to avoid the use of chemical synthetic fertilisers and, as a consequence, reduces the cost of agricultural activity.

BIBLIOGRAPHY

[1] L. Di Giovacchino, F. Angerosa, L. Di Giacinto, Effect of mixing leaves with olives on the organoleptic quality of oil obtained by centrifugation. J. Am. Oil Chem. Soc. 73, 371-374, (1996)

[2] R. Malheiro, N. Rodrigues, C. Bissaro, F. Leimenn, S. Casal, E. Ramalhosa, J.A. Pereira, Improvement of sensorial and volatile profiles of olive oil by addition of olive leaves. Eur. J. Lipid Sci. Technol. 119 (11), 1700177 (2017)

[3] F. Angerosa, L. Di Giacinto, Caratteristiche di qualità dell'olio di oliva vergine in relazione ai metodi di frangitura. Nota II. Riv. Ital. Sostanze Grasse 72, 1-4, (1995)

[4] F. Caponio, V. Alloggio, T. Gomes, Phenolic compounds of virgin olive oil: influence of paste preparation techniques. Food Chemistry 64, 203-209, (1999)

[5] J. M. Martinez Moreno, C. Gomez Herrera, C. Janer del Valle, Estudios fisico-quimicos sobre las pastas de aceitunas molidas. IV. Las gotas de aceite. Grasas y Aceites 8, 112-118, (1957)

[6] L. Di Giovacchino, N. Costantini, M. L. Ferrante, A. Serraiocco, Influence of malaxation time of olive paste on oil extraction yields and chemical and organoleptic characteristics of virgin olive oil obtained by a centrifugal decanter at water saving. Grasas y Aceites 2, 179-186, (2002)

[7] A.M. Inarejos-Garcia, A. Gomez-Rico, M. Desamparados Salvador, G. Fregapane, Influence of malaxation conditions on virgin olive oil yield, overall quality, and composition. Eur. Food Res. Technol. 228, 671-677, (2009)

[8] E. Boselli, G. Di Lecce, R. Strabbioli, G. Pieralisi, N.G. Frega, Are virgin olive oil obtained below 27 °C better than those produced at higher temperature? Food Science and Technol. 42, 748-757, (2009)

[9] A. Parenti, P. Spugnoli, P. Masella, L. Calamai, Utilizzo dell'anidride carbonica emessa dalle olive durante la trasformazione per migliorare la qualità dell'olio. Primo contributo-Prove di laboratorio, Riv. Ital. Sostanze Grasse 82, 283-290, (2005)

[10] G. De Stefano, P. Piacquadio, M. Servili, L. Di Giovacchino, V. Sciancalepore, Effect of extraction system on the phenolic composition of virgin olive oil. Fett/Lipid 9, 328-332, (1999)

[11] P. Amirante, P. Catalano, Analisi teorica e sperimentale dell'estrazione dell'olio d'oliva per centrifugazione. Riv. Ital. Sostanze Grasse 70, 329-335, (1993)

[12] L. Di Giovacchino, L'estrazione dell'olio dalle olive mediante doppia lavorazione. Nota I: risultati ottenuti con l'abbinamento pressione-centrifugazione. Riv. Ital. Sostanze Grasse 68, 83-90, (1991)

[13] L. Di Giovacchino, N. Costantini, L'estrazione dell'olio dalle olive mediante doppia lavorazione: Nota II: risultati ottenuti con la doppia centrifugazione. Riv. Ital. Sostanze Grasse 68, 519-527, (1991)

[14] J. Alba Mendoza, F. Hidalgo Casado, A. Ruiz Gomez, F. Martinez Roman, J. Moyano Perez, A. Cert Ventulà, C. Perez Camino, V. Ruiz Mendez, Caracteristicas de los aceites de oliva de primera y segunda centrifugacion. Grasas y Aceites 47, 163-181, (1996)

[15] L. Di Giovacchino, M.R. Mucciarella, N. Costantini, G. Surricchio, Double oil extraction from olive paste and olive pomace by centrifugal decanter at 2-phases, type integral. Riv. Ital. Sostanze Grasse 79, 351-355, (2002)

[16] P. Canamasas, L. Ravetti, Evaluation of second extraction of olive oil in Australia. Rural Industries Research and Development Corporation, Pubbl. n. 12/109, (2012)

[17] F. Caponio, G. Squeo, J.I. Monteleone, V.M. Paradiso, A. Pasqualone, C. Summo, First and second centrifugation of olive paste: influence of talc addition on yield, chemical composition and volatile compounds of the oils. Food Sci. and Technol. 64, 439-445, (2015)

[18] L. Di Giovacchino, S.M. Preziuso, M.G. Di Serio, M.R. Mucciarella, G. Di Loreto, B. Lanza, Double extraction of olive oil in large oil mills of Southern Italy: effects on extraction efficiency, oil quality and economy of the process. Eur. J. Lipid Sci. Technol. 119, Special Issue, 1600161, (2017)

[19] L. Di Giovacchino, Tecnologie di lavorazione delle olive in frantoio. Rese di estrazione e qualità dell'olio. Edagricole – Edizioni Agricole di New Business Media. Milano (2021)

[20] M.G. Di Serio, B. Lanza, E. Iannucci, F. Russi, L. Di Giovacchino, Valorization of wet olive pomace produced by 2 and 3-phases centrifugal decanter. Riv. Ital. Sostanze Grasse 88, 111-117, (2011)
[21] A. Leone, R. Romaniello, G. Peri, A. Tamborrino, Development of a new model of olive de-stoner machine: evaluation of electric consumption and kernel characterization. Biomass and Bioenergy 81, 108-116, (2015)
[22] U. Bing, E. Cini, A. Cioni, V. Laurendi, Smaltimento-recupero delle sanse di oliva provenienti da un "due fasi" mediante distribuzione in campo. L'Informatore Agrario 47, 75-78, (1994)
[23] R. Cardelli, C. Benitez, Change of chemical properties in two soils amended with moist olive residues. Agricoltura Mediterranea 128, 171-177, (1998)
[24] L. Di Giovacchino, C. Basti, N. Costantini, G. Surricchio, M.L. Ferrante, Riutilizzo in agricoltura dei sottoprodotti della trasformazione delle olive. In E. Cini, S. Regis, Smaltimento e riutilizzo di reflui dei frantoi, pp. 9-30, ARSIA, Regione Toscana (2000)
[25] E. Bonari, L. Ceccarini, Effetti della distribuzione di dosi crescenti di sanse umide sul terreno agrario. In E. Cini, S. Regis, Smaltimento e riutilizzo di reflui dei frantoi, pp. 31-57, ARSIA, Regione Toscana (2000)
[26] L. Di Giovacchino, C. Basti, L. Seghetti, N. Costantini, G. Surricchio, M.L. Ferrante, Effects of olive pomace spreading on soil cultivated with maize and grapevine. Agricoltura Mediterranea 134, 15-24, (2004)
[27] L. Nasini, G. Gigliotti, E. Federici, G. Cenci, P. Proietti, Effects of solid olive-mill waste amendment on soil fertility and olive (*Olea europea* L.) tree activity. Agriculture, Ecosystem and Environment 164, 292-297, (2013)
[28] M. Niaounakis and C.P. Halvadakis, Olive mill waste management, Literature review and patent survey. Typolito-George Dardanos, Athens, (2004)
[29] A. Garcia-Ortiz Rodriguez, J. Vicente Giraldez Cervera, P. Gonzalez Fernandez, R. Ordonez Fernandez, El riego con alpechin. Agricultura 730, 426-431, (1993)
[30] E. Bonari, M. Macchia, L.G. Angelini, L. Ceccarini, The wastewater from olive oil extraction: their influence on the germinative characteristics of some cultivated and weed species. Agricoltura Mediterranea 123, 273-280, (1993)
[31] F. Caporali, G. Anelli, R. Paolini, E. Campiglia, G. Benedetti, M. Contini, Crop application of treated waste waters from olive oil extraction. Agricoltura Mediterranea 126, 388-395, (1996)
[32] L. Di Giovacchino, C. Basti, N. Costantini, M.L. Ferrante, G. Surricchio, Effects of olive vegetable water spreading on soil cultivated with maize and grapevine. Agricoltura Mediterranea 131, 33-41, (2001)
[33] M. Catalano, T. Gomes, M. De Felice, T. De Leonardis, Smaltimento delle acque di vegetazione dei frantoi oleari. Inquinamento 2, 87-90, (1985)
[34] P. Proietti, A. Cartechini, A. Tombesi, Influenza delle acque reflue di frantoi oleari su olivi in vaso e in campo. L'Informatore Agrario 45, 87-91, (1988)
[35] K. Chartzoulakis, G. Psarras, M. Moutsopoulou, E. Stefanoudaki, Application of olive mill wastewater to a Cretan olive orchard: effects on soil properties, plant performance and the environment. Agriculture, Ecosystem & Environment 138, 293-298, (2010)
[36] S. Magdich, C. Ben Ahmed, M. Boukhris, B. Ben Ruina, E. Ammar, Olive mill wastewater spreading effects on productivity and oil quality of adult Chemlali olive (*Olea europea* L.) in the South of Tunisia. Intern. J. of Agronomy and Agricultural Research 6, 56-67, (2015)
[37] S. Magdich, C. Ben Ahmed, R. Jarboui, B. Ben Ruina, M. Boukhris, E. Ammar, Dose and frequency dependent effects of olive mill wastewater treatment on the chemical and microbial properties of soil. Chemosphere 93, 1896-1903, (2013)
[38] M. Catalano, M. De Felice, Utilizzazione delle acque reflue come fertilizzante. In Atti del Seminario Internazionale "Tecnologie e Impianti per il trattamento dei reflui dei frantloi oleari", pp. 251-262, Lecce, 16-17 novembre 1989
[39] L. Di Giovacchino, C. Basti, N. Costantini, G. Surricchio, M.L. Ferrante, D. Lombardi, Effetti dello spargimento delle acque di vegetazione delle olive sul terreno coltivato con il mais e con la vite. Olivae 91, 37-43, (2002)
[40] B. Lanza, M.G. Di Serio, L. Di Giovacchino, Long-term spreading of olive mill wastewater on olive orchard: effects on olive production, oil quality and soil properties. Communications in Soil Science and Plant Analysis 48(20), 2420-2433, (2017)
[41] B. Lanza, M.G. Di Serio, L. Di Giovacchino, Microbiological and chemical modifications of soil cultivated with grapevine following agronomic application of olive mill wastewater, Water Air Soil Pollution 231:86, (2020)
[42] U. Tomati, E. Medejon, E. Galli, D. Capitani, A. Segre, Structural changes of humic acid during olive mill pomace composting. Comp. Scie. and Util. 9, 134-141, (2001)
[43] F. Alianiello, S. Trinchera, S. Dell'Orco, F. Pinzari, A. Benedetti, Somministrazione al terreno delle acque di vegetazione da frantoio: effetti sulla sostanza organica e sull'attiività microbiologica del suolo. Agricoltura Ricerca 173, 64-74, (1998)
[44] R. Vinken, A. Schaffer, R. Ji, Abiotic association of soil borne monomeric phenols with hu-

mic acids. Organic Geochemistry 36, 583-593. (2005)
[45] M.A. Sanchez-Monedero, A. Roig, J. Cegarra, M.P. Bernal, Relationship between water-soluble carbohydrate and phenol fraction and the humification indices of different organic wastes during composting. Bioresource Technology 70, 193-201, (1999)
[46] A. Baddi, J. Cegarra, J. Merlina, G. Revel, J.C. Hafidi, Qualitative and quantitative evolution of polyphenolic compounds during composting of an olive-mill waste-ws mixture. Journal of Hazardous Materials 165, 1119-1123, (2009)
[47] F.J. Stevenson, Humus, Chemistry, Genesis, Composition, Reaction. Wiley Interscience. New York, (1994)
[48] S. Hachicha, J. Cegarra, F. Sellami, R. Hachicha, N. Drira, K. Medhioub, E. Ammar, Elimination of polyphenols toxicity from olive mill wastewaters sludge by its co-composting with sesame bark. Journal of Hazardous Materials 161, 1131-1139, (2009)
[49] G. Gigliotti, P. Proietti, D. Said-Pullicino, L. Nasini, D. Pezzolla, L. Rosati, P.R. Porceddu, Co-composting of olive husk with high moisture contents: organic matter dynamic and compost quality. International Biodeterioration and Biodegradation 67, 8-14, (2012)

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

FORMAZIONE

PROSSIMI CORSI IN PROGRAMMA

Innovhub SSI organizza corsi rivolti agli operatori del settore oleario, al personale tecnico della Grande Distribuzione Organizzata (GDO), a tecnici specialisti di Università e centri di Ricerca.
È possibile anche definire percorsi di formazione ad hoc sulla base di esigenze specifiche.

Per maggiori informazioni:
pierangela.rovellini@mi.camcom.it

LA QUALITÀ DELLE SOSTANZE GRASSE
20 settembre 2024, Webinar

Analisi dei **parametri** che influenzano la **qualità degli oli di oliva** vergini e di semi e i metodi relativi, degli **aspetti normativi e legislativi** che regolamentano i limiti, i **costituenti principali** degli oli e i loro **componenti minori**.

Docente: Liliana Folegatti
Costo: 200,00 euro + IVA

STRUMENTI E MODELLI PER LA STIMA DELLA SHELF-LIFE
7 ottobre 2024, Webinar

Conoscere le modalità attraverso cui stimare la **shelf-life** di un prodotto, ovvero l'intervallo di tempo fra la sua produzione e il consumo senza che questo possa arrecare danni al consumatore.

Docente: Andrea Milani
Costo: 200,00 euro + IVA

VALUTAZIONE ORGANOLETTICA DEGLI OLI VERGINI DI OLIVA
16 ottobre, via G. Colombo 83

Analisi della **valutazione organolettica** come parametro di qualità distintivo e discriminante, di **fattori** che concorrono alla **qualità del prodotto finale**, del **metodo standard del COI**, della **terminologia** e dell'origine degli **attributi positivi e negativi** riscontrabili nell'olio.

Docenti: Stefania De Cesarei e Liliana Folegatti
Costo: 200,00 euro + IVA

Scopri di più:
www.innovhub-ssi.it | comunicazione.innovhub@mi.camcom.it

Influence of the heating process on the total phenols and fatty acids composition of virgin olive oil originated in Tunisia, Italy, and Spain

Amor Slama[1,2] ✉
Abdelmajid Zouhir[3]
Ammar Cherif[1,4]

[1] University of Tunis El Manar, Faculty of Sciences of Tunis, Laboratory of Neurophysiology, Cellular Physiopathology and Biomolecules Valorisation, Tunis, Tunisia

[2] University of Carthage, Faculty of Sciences of Bizerte, Jarzouna-Bizerte, Tunisia

[3] Research Unit at Institute of Applied Biological Sciences of Tunis (ISSBAT), Tunis, Tunisia

[4] Department of Biology, College of Science, Qassim University, Qassim 5142, Saudi Arabia

✉ CORRESPONDING AUTHOR:
Amor Slama
Email: slamaamor@yahoo.fr
University of Carthage
Faculty of Sciences of Bizerte
Jarzouna-Bizerte 7021, Tunisia
Phone: +21697622401

Received: February 6, 2023
Accepted: December 5, 2023

ABSTRACT

Olive oil is considered a strategic species for human nutrition across the world particularly in the Mediterranean countries, the major producers of this oil. The objective of this research was to determine the influence of the temperature variation and the origin of the oil on the total phenolic contents and fatty acids composition of virgin olive oil originated in Tunisia, Italy, and Spain. The total polyphenols were determined by spectrophotometer and the fatty acids composition was analysed by means of a chromatographic GC-FID. Results showed that the content of polyphenols reduced slightly during the temperature treatment from 60 to 150°C. However, the amount of polyphenols and polyunsaturated fatty acids decreased mainly at high temperatures (180°C), while, saturated and monounsaturated fatty acids increased. The results also show a positive correlation between the polyphenol content and the total polyunsaturated fatty acids during heat treatment. Based on the fatty acids composition and the polyphenols content, which are an essential indicator of the nutritional value of the oil, a decrease in the nutritional quality of the three types of olive oil was recorded. So, it moved on to the ovoid heating process of olive oil, if necessary the temperature should be inferior to 150°C to preserve the quality and the nutritional value of virgin olive oil.

Keywords: polyphenols, fatty acids, virgin olive oil, heating process

1. INTRODUCTION

Polyphenols are important organic molecules of the unsaponifiable fraction found in many plants and many components of the Mediterranean diet, including olive oil, fruit, and vegetables [1]. The polyphenols are subdivided into simple phenols and polymerised forms. These compounds are secondary plant metabolism products [2]. Their structure contains one or more benzene rings carrying one or more hydroxyl functional groups and other constituents [3]. Polyphenols have powerful anti-inflammatory and antioxidant roles; they also have a beneficial role in the prevention of hypertension. They represent an important contribution to the oxidative stability of olive oil. Their presence in the diet would make it possible to fight against cardiovascular diseases, cancers, and osteoporosis [4,5].The results reported by Manna et al. (1997) suggest that olive oil polyphenols could lower the risk of reactive oxygen metabolite–mediated diseases such as some gastrointestinal diseases and atherosclerosis. Other studies mentioned that the protection of olive oil against cardiovascular diseases and cancer was due to its fatty acid profile and the presence of minor amounts of phenolic constituents [7]. These studies have also emphasised the importance of the phenolic compounds of olive oil as modulators of key mechanisms implicated in the development of atherosclerosis. The Antioxidants have an important role in

protecting humans against infections and degenerative diseases [8].
The types of phenols and their concentrations differ greatly among olive oils, depending on fruit varieties and their maturation degree as well as other agronomic and technological factors, such as the extraction procedures [9]. Referring to the total phenol concentrations, olive oils can, therefore, be divided into three groups, containing low (0.05-0.2 g/kg), medium (0.2-0.5 g/kg) and high (0.5-1.0 g/kg) of total phenol concentrations [10]. It is widely known that the quality of virgin olive oil and polyphenol levels in olives are influenced by various agronomic factors such as olive cultivar, climatic conditions, agricultural practices, and ripeness at harvest [11].
Olive oil is characterised by a high content of monounsaturated fatty acids, in particular, oleic acid; moreover, olive oil is a valid source of essential fatty acids: αlinolenic acid and linoleic acid. This composition has contributed to the reduction of LDL-cholesterol levels and the increase of HDL-cholesterol content in plasma.
The method used in oil's processing has a direct impact on its antioxidant capability [12]. During heat treatment, oil is subjected to hydrolysis, oxidation, and polymerisation. The mechanism of the oxidation processes is the same for different fats and oils, the reaction rates vary for different types of fats and oils [13].The changes in lipids after heat treatment, influence their nutritive value which are in agreement with the results of Gharby et al. (2016b).
The objective of this study was to describe and compare the influence of heating on the content of saturated and unsaturated fatty acids and the total polyphenols of Tunisian, Italian, and Spanish virgin olive oil and then identify the possible correlations between the total polyphenols and fatty acids composition.

2. MATERIALS AND METHODS

2.1 SAMPLES

The virgin olive oil of Tunisia was purchased from the local market, the Italian and Spanish virgin olive oils were purchased from the Quebec market (Canada).

2.2 HEATING CONDITIONS

To study the effect of the increased temperature and duration on the olive oil composition, a heat treatment was carried out as below: 150 ml of each oil sample of the three edible oils was heated to 60, 90, 120, 150 and 180°C for 12 hours each one.

2.3 EXTRACTION AND DETERMINATION OF PHENOLIC COMPOUNDS

The Total phenolic composition was determined by using the Folin–Ciocalteu reagent [15]. A 1 ml from each sample was solubilized in 1 ml of hexane, then 3 ml of the mixture methanol/water (60/40, v/v) were added and shaken vigorously for 1 minute. Then 0.5 ml of solution were mixed with 0.5ml of Folin-Ciocalteu phenol reagent, 3 min later, 0.5 ml of Na2CO3 was added, then completed until 5 ml with deionized water. The mixture has left in the darkness for 2 hours; the optical density is measured at 760 nm against water in the UV-Vis spectrophotometer (Biotek, Power XS2, Logiciel Gen 5) [16]. The assay was calibrated against gallic acid in the 0.3125-5.0mg/100 ml range. Polyphenol concentrations were expressed as mg gallic acid equivalents Kg-1 (mgGAE, Kg-1 oil). Each sample was duplicated three times, and the mean was generated as the result.

2.4 METHYLATION OF FATTY ACIDS

The fatty acid methylation procedure was performed in a standardised way to ensure good accuracy and repeatability of the fatty acids analysis. The derivation of the fatty acid methyl esters was the most used technique for lipid analysis by GC-FID, the most based catalysed trans-esterification agents are sodium or potassium methoxide in anhydrous methanol [17]. Indeed, to the 10 mg of oil sample was added 1 ml of hexane and 500 µl of sodium methoxide, after vortexing, the whole is brought to heating between 40 and 50°C for 15 min. Then 4 ml of hexane were added and 5 ml of water saturated with NaCl was used to wash the sample. Stirred and allowed to stand until the separation of two phases. The organic phase was drawn into a Pasteur pipette containing delipidated cotton and approximately 1 cm of dry magnesium sulphate, the filtrate was collected in a test tube.

2.5 CHROMATOGRAPHIC ANALYSIS

The chromatographic separation was performed in an Auto system gas chromatograph with a split/splitless injector and an FID detector, equipped with a BPX 70 capillary column of 60 m length, 0,25 mm i.d., and 0,25 μm film thicknesses (made in the USA) [18]. The oven temperature was held at 60°C for 1 min then ramped to 190°C at the rate of 10°C/min and maintained for 15 min, before the second ramp at the rate of 5°C/min to 200°C. This was then held isothermally for 14 min. Hydrogen was used as carrier gas with a flow rate of 40 ml/min. The temperature of the injector and detector injector was set at 250°C. The total flow was 68,7 ml/min and the pressure was at 125,5/97,6 Kpa. The volume injected was 1 μL, and the time of analysis was 45 min. The results are expressed as peak area percent. All the measurements were carries out with three independent replicates.

3. RESULTS AND DISCUSSION

Olive oil is one of the Mediterranean countries' important crops and it has an integral part of the Mediterranean diet's economic role [19]. The chemical composition of the fresh virgin olive oils (organic oil) of three geographical provenances (Tunisia, Italy, and Spain) was compared with those subjected to the

various heat treatments. The results provided by this study contribute to the understanding the effect of the increased temperature and duration of treatment on the stability and integrity of the biochemical composition of the studied oils (chemical transformation).

3.1 TOTAL FATTY ACIDS COMPOSITION BEFORE HEAT TREATMENT

All studied oils contain palmitic acid and stearic acid as major saturated fatty acids and oleic and linoleic acids as major unsaturated fatty acids (Table I). The Tunisian oil is the richest in saturated fatty acids, whereas the Italian and Spanish oils are the richest in total unsaturated fatty acids. However, Tunisian oil is richer in polyunsaturated fatty acids (C18: 2 and C18: 3) than other oils, the content of these fatty acids is 12.73%, whereas it is only 7.61 and 4.04% respectively for oils from Italy and Spain.

Only the Italian virgin oil contains a trans fatty acid (C18: 1 t) (Fig. 1), the Spanish oil does not contain the C17: 0. The sum of the non-identified fatty acids (NI FA) are 1.48, 1.92, and 1.53% respectively for the oils of Tunisia, Italy, and Spain.

3.2 THE EFFECT OF HEAT TREATMENT ON THE FATTY ACIDS COMPOSITION

The fatty acid composition is an essential indicator of the nutritional value of the oil (Harhar et al., 2011). The

Table I - Fatty acids composition of Tunisian, Italian and Spanish virgin olive oil before treatment.

	Country origin		
Fatty acids (%)	**Tunisian**	**Italian**	**Spanish**
C16:0	16,17	11,22	12,07
C16:1 t	0,06	0,07	0,08
C16:1 c	1,38	0,63	0,93
C17:0	0,04	0,04	-
C17:1	0,05	0,05	0,06
C18:0	2,19	2,09	1,25
C18:1 11c	2,06	1,62	2,75
C18:2 n-6	12,07	6,98	3,47
C18:3 n-3	0,66	0,63	0,57
C20:0	0,33	0,32	0,27
C20:1	0,18	0,23	0,27
εNI FA	1,48	2,03	1,53
εSFA	18,73	13,67	13,59
εUFA	79,79	84,42	84,88

εSFA: Saturated Fatty Acids, εUFA: Unsaturated Fatty Acids,
εNI FA: Non Identified Fatty acids

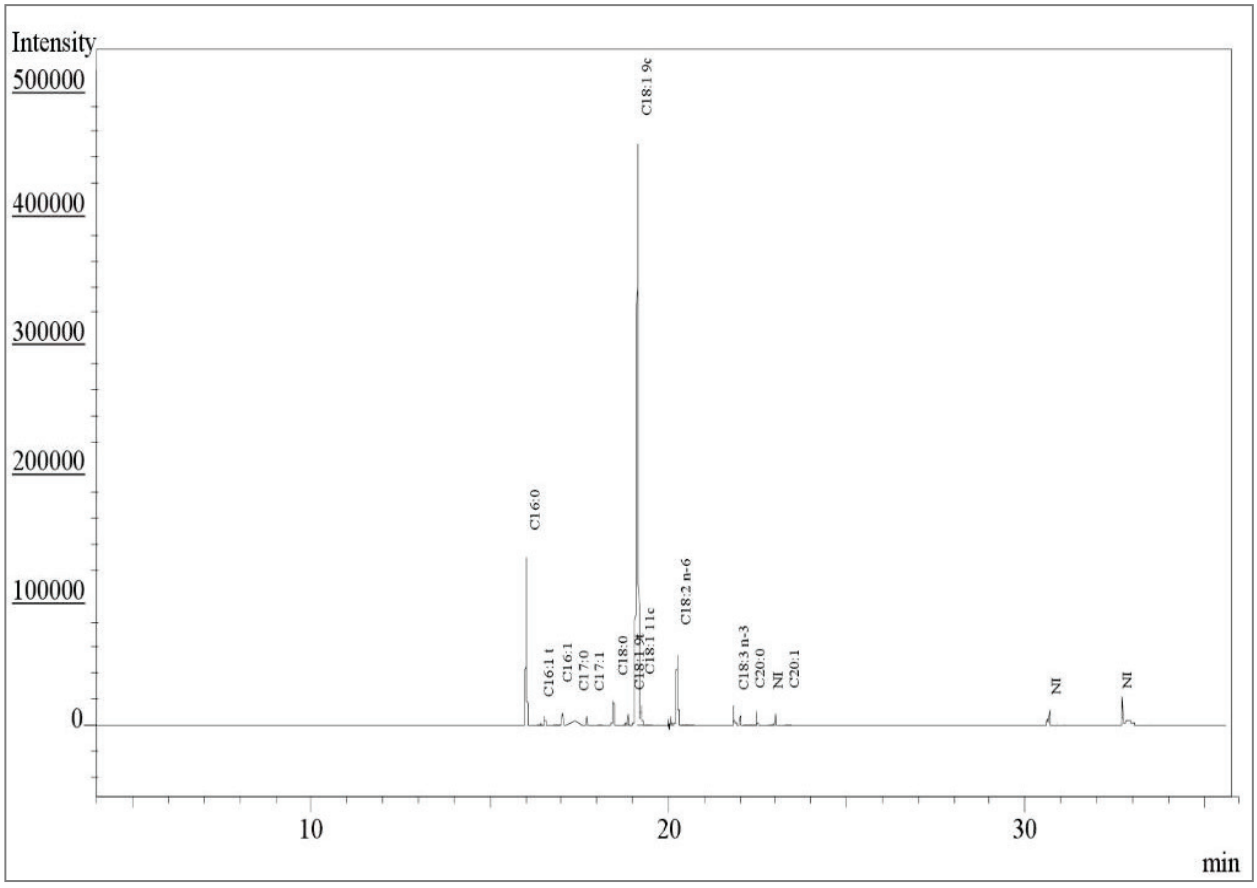


Figure 1 - Chromatogram of methyl esters fatty acids of Italian virgin olive oil obtained. NI: non- identified

heat treatment shows that the virgin olive oils developed a resistance to the increase in temperature; in fact, there is a certain stability of the fatty acid composition of the oils subjected to the temperatures of 60, 90, 120 and 150°C (Table II). Only a slight change in the fatty acid composition was recorded, especially for the total unsaturated fatty acids which decreased from 74.55% to 73.08%. The stability of fatty acid composition from 60 to 150°C suggested that virgin olive oil has a higher content of active antioxidant compounds that protect the unsaturated fatty acids against deterioration during heating.
However, a significant change was recorded at 180°C. An increase in the percentage of saturated fatty acids, notably those of palmitic and stearic acids and monounsaturated fatty acids, and a decrease in the percentage of polyunsaturated fatty acids, in particular those of linoleic and linolenic acids were recorded. These observations are in agreement with a 1H nuclear magnetic resonance study, which confirmed the fact that the fatty acid degradation rate increases with the number of double bonds in the molecule [21]. The study of Gomna et al. (2019) confirmed that the duration of exposure to heat, temperature, oxygen, moisture, and other parameters can affect vegetable oil stability.
The percentage of C16: 1t remained stable throughout the heat treatment time, however, the C18: 1t was detected only at the temperature of 180°C. The evolution of non-identified fatty acids (NI FA) during the heat treatment compared to those of the identified fatty acids could inform about the nature of these fatty acids. These latter which are increased at 180°C can be attributed to saturated and monounsaturated fatty acids.
Overall, the results showed that the composition of the oil changes as a function of duration and of the increase in temperature, could have an impact on its stability and the law of the oxidation mechanism; this is in agreement with the results found by Cao et al. (2020).

Table II - Variation of fatty acids composition during heating of Tunisian virgin olive oil at 60, 90, 120, 150, and 180ºC for 12 hours.

	Heating temperature (ºC)				
Fatty acids (%)	**60**	**90**	**120**	**150**	**180**
C16:0	22,51	22,57	22,66	22,66	23,66
C16:1 t	0,05	0,05	0,05	0,05	0,05
C16:1 c	2,59	2,60	2,58	2,58	2,59
C17:0	0,03	0,03	0,03	0,03	0,04
C17:1	0,04	0,04	0,04	0,04	0,04
C18:0	2,34	2,34	2,35	2,34	2,44
C18:1 9t	-	-	-	-	0,10
C18:1 9c	48,91	48,86	48,99	49,03	49,64
C18:1 11c	2,69	2,69	2,71	2,69	2,72
C18:2 n-6	19,56	19,48	19,33	19,34	17,47
C18:3 n-3	0,59	0,57	0,57	0,57	0,34
C20:0	0,36	0,36	0,36	0,36	0,37
C20:1	0,12	0,12	0,13	0,12	0,13
εSFA	25,24	25,29	25,40	25,40	26,61
εUFA	74,55	74,41	74,40	74,42	73,08
εNI FA	0,21	0,30	0,20	0,18	0,31

εSFA: Saturated Fatty Acids, εUFA: Unsaturated Fatty Acids, εNI FA: Identified Fatty Acids

3.3 THE EFFECT OF HEAT TREATMENT ON THE POLYPHENOLS COMPOSITION

During heat treatment, the total polyphenols ranged from 67.18 to 180.42 mg/kg; from 55.36 to 103.09 mg/kg and from 56.69 to 112.87 mg/kg for Tunisian, Italian and Spain olive oil, respectively. The general trend shows a reduction of total polyphenols content as a function of the increase in heat treatment.
According to Fig. 2, there are three major changes during the heat treatment of these oils: a decrease of the total polyphenols at T = 60°C for the Spanish and Italian oils, this decrease is rather recorded at T = 90°C in Tunisian oil. An increase in total polyphenols is also marked at T = 90°C in Spanish and Italian oils, however, this increase is noted at T = 60°C in Tunisian oil. The third change is similar in the three types of oil, which is a gradual decrease of the total polyphenols from T = 150°C. The decrease becomes considerable from T = 180°C.
This study shows that Tunisian olive oil contains much more polyphenols than the other two types of olive oil. This gives information on the classification in ascending order of the antioxidant capacity of these oils, which can be indicated as follows: Tunisian, Italian, and then Spanish olive oil. The variation in the contents of the total polyphenols depending on the temperature increase represented by the above curves shows ups and downs for the three types of oil. This suggests that during the heat treatment there is a transformation of the minor compounds of the unsaponifiable fraction into polyphenols, however, some phenolic compounds are transformed or degraded under the action of temperature, this is in agreement with the results found by Alean et al. (2016) who reported a reduction of 45% of polyphenol in dried fruit at a temperature of 40°C, while the higher degradation of polyphenols was presented at a temperature of 60°C. It was concluded that the degradation depends on temperature, moisture, and dry times. On the other hand Abhay et al. (2016) proved that high temperature and heating time harm cocoa polyphenols. The studies reported by Hii et al. (2009) and Ndukwu (2009) show that there is a thermal degradation of volatile phenolic constituents due to high temperature.

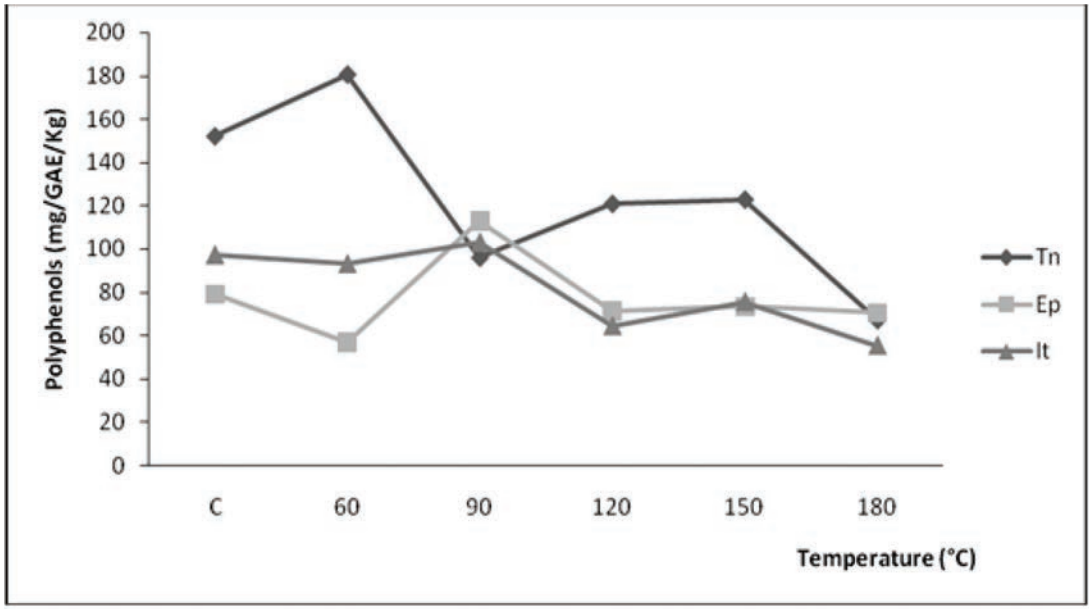


Figure 2 - Variation of total polyphenols during heating processes of Tunisian, Spanish, and Italian virgin olive oil. Tn: Tunisian, Sp: Spanish, It: Italien, C: control

The stability of the oils against oxidation depends not only on the degree of unsaturation but also on the amount of antioxidants present in the unsaponifiable fraction [3]. Other studies Tabee et al., 2008) recorded different results for the stability of oils with similar monounsaturated fatty acids contents during heating depending on the amounts of α-tocopherol and phytosterols present in the oils. The results found by Yi et al. (2020) showed that the antioxidant capacity of corn oils can be increased by enrichment with other tocopherols (γ-oryzanol). According to our results, it can be assumed that the resistance to thermal effects is proportional to the contents of polyphenols, in fact, there is a considerable decline in the amount of total polyphenols when the olive oil was exposed to high temperature (more than 150°C). Therefore, more attention should be paid to the heat treatment used and the heating period to preserve the quality of edible oils [30].

3.4 CORRELATION BETWEEN FATTY ACIDS AND POLYPHENOLS CONTENT

Most results of fatty acids analysis of the Italian olive oil are in agreement with those mentioned by Piscopo et al. (2016); however, the percentages of polyphenols found by those authors were three times higher

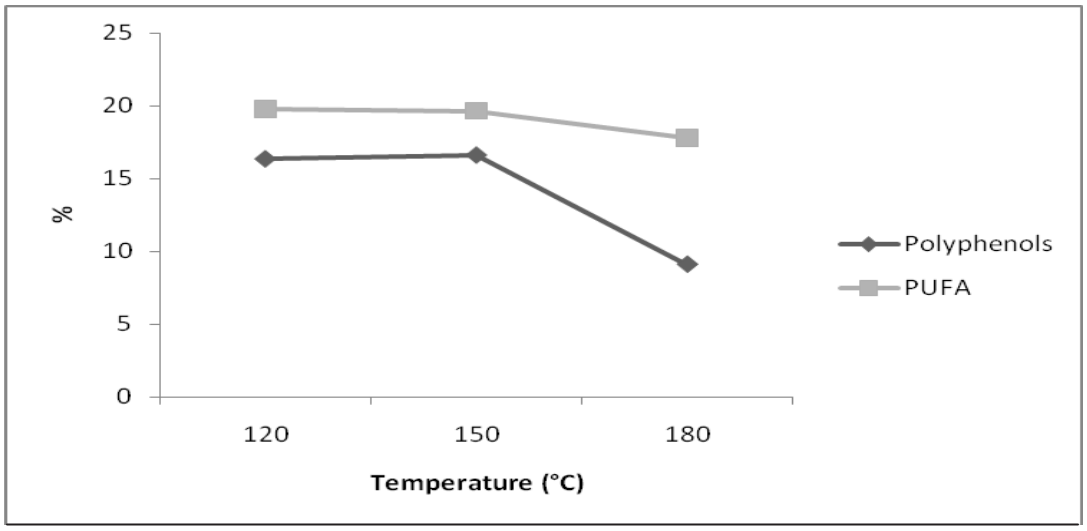


Figure 3 - Relationship between the evolution of polyphenols and polyunsaturated fatty acids (PUFA) of Tunisian virgin olive oil during the heating process. PUFA: Polyunsaturated fatty acids

than those reported by this study.
By examining tables I and II, it can be suggested that the variation in the polyphenol content is proportional to that of the polyunsaturated fatty acids, in particular linoleic and linolenic acid, both of which decrease from T = 150°C (Fig. 3). This could be explained by the sensitivity of these chemical compounds to the temperature increase. Therefore, we should pay more attention to the processing techniques that can be used to preserve the quality of oil and enhance their benefits [32].

4. CONCLUSION

The investigation shows only a slight effect of heating on the fatty acid composition of virgin olive oil at a temperature between 60°C and 150°C; in this condition, the differences between the fatty acid composition before and after heating were not significant. The changes in the fatty acid composition provide insight into the kinetics of the fatty acid oxidation reactions. In fact, the reduction of the content of linoleic and linolenic acid was higher in comparison to the other fatty acids of oil submitted at 180°C.
Olive oil is rich in phenolic compounds, which have a strong antioxidant activity, so, the content and the composition of the polyphenols are further important criteria for the assessment of the quality of the oil.
During heat treatment, there is a slight change in polyphenol content at temperatures of 60°C to 120°C, while the decrease becomes important from 150°C to 180°C, and the effect of the high temperature on polyphenols content was significant. The positive correlation between total polyphenols and polyunsaturated fatty acids, thus begun at 150°C.
The diminution of polyunsaturated fatty acids and polyphenols caused by heat treatment reduces the nutritional value of virgin olive oil; it is advisable to consume this oil in its raw state.

Acknowledgment

We are thankful for the technical support of Mr. Ronan Corcuff

Conflict of interest

The authors confirm that there is no conflicts of interest.

REFERENCES

[1] C.-T. Ho, C.Y. Lee, M.-T. Huang, eds., Phenolic Compounds in Food and Their Effects on Health I: Analysis, Occurrence, and Chemistry, American Chemical Society, Washington, DC, 1992. https://doi.org/10.1021/bk-1992-0506, (1992).
[2] V.R. Gutiérrez, F.J.G. Muriana, J. Villar, Virgin olive oil and cardiovascular diseases. Plasma lipid profile and lipid composition of human erythrocyte membrane., Grasas y Aceites. 49, 9-29, (1998) https://doi.org/10.3989/gya.1998.v49.i1.703.
[3] F. Visioli, G. Bellomo, C. Galli, Free radical-scavenging properties of olive oil polyphenols, Biochem. Biophys. Res. Commun. 247, 60-64, (1998) https://doi.org/10.1006/bbrc.1998.8735.
[4] L. Song, X. Wang, X. Zheng, D. Huang, Polyphenolic antioxidant profiles of yellow camellia, Food Chem. 129, 351-357, (2011). https://doi.org/10.1016/j.foodchem.2011.04.083.
[5] R. Shen, S. Yang, G. Zhao, Q. Shen, X. Diao, Identification of carotenoids in foxtail millet (Setaria italica) and the effects of cooking methods on carotenoid content, Journal of Cereal Science. 86-93, (2015). https://doi.org/10.1016/j.jcs.2014.10.009.
[6] C. Manna, P. Galletti, V. Cucciolla, O. Moltedo, A. Leone, V. Zappia, The Protective Effect of the Olive Oil Polyphenol (3,4-Dihydroxyphenyl)- ethanol Counteracts Reactive Oxygen Metabolite-Induced Cytotoxicity in Caco-2 Cells, The Journal of Nutrition. 127 286-292, (1997). https://doi.org/10.1093/jn/127.2.286.
[7] S. Gharby, H. Harhar, B. Matthäus, Z. Bouzoubaa, Z. Charrouf, The chemical parameters and oxidative resistance to heat treatment of refined and extra virgin Moroccan Picholine olive oil, Journal of Taibah University for Science. 10, 100-106, (2016). https://doi.org/10.1016/j.jtusci.2015.05.004.
[8] I. Kabach, N. Bouchmaa, R. Ben Mrid, Z. Zouaoui, M.E. Maadoudi, A. Kounnoun, F. Asraoui, F. El Mansouri, A. Zyad, F. Cacciola, Y. Oulad El Majdoub, L. Mondello, M. Nhiri, Olea europaea var. Oleaster a promising nutritional food with in vitro antioxidant, antiglycation, antidiabetic and antiproliferative effects, Food Measure, (2022). https://doi.org/10.1007/s11694-022-01655-0.
[9] G. Montedoro, M. Baldioli, M. Servili, Composti fenolici dell'olio d'oliva e la loro importanza sensoriale, nutrizionale e merceologica.Giorn, Ital. Nutr. Clin. Prev. 1, 19-32, (1992a).
[10] G. Montedoro, M. Servili, M. Baldioli, E. Miniati, Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC, J. Agric. Food Chem. 40, 1571-1576, (1992). https://doi.org/10.1021/jf00021a019.
[11] I.M. Abu-Reidah, M. Yasin, S. Urbani, M. Servili, G. Montedoro, Study and characterization of Palestinian monovarietal Nabali virgin olive oils from northern West Bank of Palestine, FOOD RESEARCH INTERNATIONAL. 54, 1959-1964, (2013).
[12] P. Gao, R. Liu, Q. Jin, X. Wang, Effects of processing methods on the chemical composition

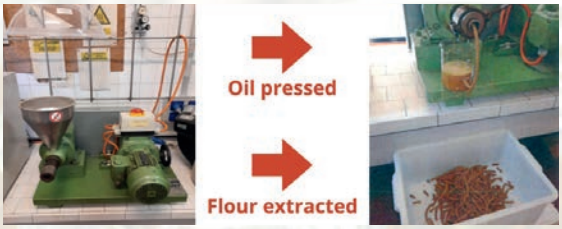
and antioxidant capacity of walnut (Juglans regia L.) oil, LWT 135, 109958.
Https://Pubag.Nal.Usda.Gov/Catalog/7073763. (2021). https://pubag.nal.usda.gov/catalog/7073763
[13] Y.B.C. Man, J.L. Liu, B. Jamilah, R.A. Rahman, Quality Changes of Refined-Bleached-Deodorized (rbd) Palm Olein, Soybean Oil and Their Blends During Deep-Fat Frying, Journal of Food Lipids. 6, 181-193, (1999). https://doi.org/10.1111/j.1745-4522.1999.tb00142.x.
[14] S. Gharby, H. Harhar, B. Matthäus, Z. Bouzoubaa, Z. Charrouf, The chemical parameters and oxidative resistance to heat treatment of refined and extra virgin Moroccan Picholine olive oil, Journal of Taibah University for Science. 10, 100-106, (2016). https://doi.org/10.1016/j.jtusci.2015.05.004.
[15] T. Gutfinger, Polyphenols in olive oils, Journal of the American Oil Chemists' Society. 58, 966-968, (1981). https://doi.org/10.1007/BF02659771.
[16] V.L. Singleton, J.A. Rossi, Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents, Am J Enol Vitic. 16, 144-158, (1965).
[17] W. Christie W, Preparation of ester derivatives of fatty acids for chromatographic analysis, Advances in Lipid Methodology. 69-111, (1993).
[18] N. Vingering, M. Ledoux, Use of BPX-70 60-m GC columns for screening the fatty acid composition of industrial cookies, European Journal of Lipid Science and Technology. 111, 669-677, (2009). https://doi.org/10.1002/ejlt.200800289.
[19] S. Mkaouar, B. Charfi, L. Tounsi, N. Bahloul, K. Allaf, N. Kechaou, Instant controlled pressure drop (DIC) effect on compositional analysis of olive leaves (Olea europaea L.), Food Measure. 16, 1494-1501, (2022). https://doi.org/10.1007/s11694-021-01257-2.
[20] H. Harhar, S. Gharby, B. Kartah, H. El Monfalouti, D. Guillaume, Z. Charrouf, Influence of argan kernel roasting-time on virgin argan oil composition and oxidative stability, Plant Foods Hum Nutr. 66, 163-168, (2011). https://doi.org/10.1007/s11130-011-0220-x.
[21] M. Guillen, A. Ruiz, Study by means of 1H nuclear magnetic resonance of the oxidation process undergone by edible oils of different natures submitted to microwave action, Food Chemistry, (2006). https://eurekamag.com/research/004/477/004477158.php
[22] A. Gomna, K.E. N'Tsoukpoe, N. Le Pierrès, Y. Coulibaly, Review of vegetable oils behaviour at high temperature for solar plants: Stability, properties and current applications, Solar Energy Materials and Solar Cells. 200, 109956, (2019). https://doi.org/10.1016/j.solmat.2019.109956.
[23] J. Cao, X. Jiang, Q. Chen, H. Zhang, H. Sun, W.-M. Zhang, C. Li, Oxidative stabilities of olive and camellia oils: Possible mechanism of aldehydes formation in oleic acid triglyceride at high temperature, LWT. 118 108858, (2020). https://doi.org/10.1016/j.lwt.2019.108858.
[24] J. Alean, F. Chejne, B. Rojano, Degradation of polyphenols during the cocoa drying process, Journal of Food Engineering. 189, 99-105, (2016). https://doi.org/10.1016/j.jfoodeng.2016.05.026.
[25] S.M. Abhay, C.L. Hii, C.L. Law, S. Suzannah, M. Djaeni, Effect of hot-air drying temperature on the polyphenol content and the sensory properties of cocoa beans, International Food Research Journal. 23, 1479-1484, (2016).
[26] C.L. Hii, C.L. Law, S. Suzannah, Misnawi, M. Cloke, Polyphenols in cocoa (Theobroma cacao L.)., Asian Journal of Food and Agro-Industry. 2, 702-722, (2009).
[27] M.C. Ndukwu, Effect of Drying Temperature and Drying Air Velocity on the Drying Rate and Drying Constant of Cocoa Bean, Agricultural Engineering International: CIGR Journal. 9, 1091-1094, (2009).
[28] E. Tabee, S. Azadmard-Damirchi, M. Jägerstad, P.C. Dutta, Effects of α-Tocopherol on Oxidative Stability and Phytosterol Oxidation During Heating in Some Regular and High-Oleic Vegetable Oils, Journal of the American Oil Chemists' Society. 85, 857-867, (2008). https://doi.org/10.1007/s11746-008-1274-2.
[29] B. Yi, J. Lee, M.-J. Kim, Increasing oxidative stability in corn oils through extraction of γ-oryzanol from heat treated rice bran, Journal of Cereal Science. 91, 102880, (2020). https://doi.org/10.1016/j.jcs.2019.102880.
[30] A. Cherif, A. Slama, Stability and Change in Fatty Acids Composition of Soybean, Corn, and Sunflower Oils during the Heating Process, Journal of Food Quality. e6761029, (2020). https://doi.org/10.1155/2022/6761029.
[31] A. Piscopo, A. De Bruno, A. Zappia, C. Ventre, M. Poiana, Characterization of monovarietal olive oils obtained from mills of Calabria region (Southern Italy), Food Chem. 213, 313-318, (2016). https://doi.org/10.1016/j.foodchem.2016.06.080.
[32] A. Slama, A. Cherif, S. Boukhchina, Importance of New Edible Oil Extracted from Seeds of Seven Cereals Species, Journal of Food Quality. 5531414, (2021). https://doi.org/10.1155/2021/5531414.

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

FOOD AND FATS TECHNOLOGY

COLD PRESSED SEED OIL EXTRACTION

- Oil extraction from seeds and insects
- Oil and cake characterization



SHORT PATH VACUUM DISTILLATION

- Reduction of cholesterol in butter and lard
- Squalene recovery from olive oil deodorizer distillates
- Enrichment of hemp inflorescence extracts
- Extraction of carotenoids
- Distillation and recovery of high value molecules
- Physical neutralization

LAB REACTOR

- Extraction of nutraceutical compounds from production waste of food industries (e.g grape pomace, olive leaves and citrus fruits)
- Recovery of polyphenols and bioactive com-pounds (e.g. olive pomace and red fruits)
- Esterification reactionof oils and fats
- Preparation of biofuels(biodiesel)
- Refining process of oils and fats (bleaching, neutralization, deodorization)

For further information:
andrea.milani@mi.camcom.it
pierangela.rovellini@mi.camcom.it

www.innovhub-ssi.it

Systematic Review on Chemical Diversity and Biological Activities of Essential Oils from the Genus *Blumea* (Asteraceae)

Wan Mohd Nuzul Hakimi Wan Salleh[1,2] ✉
Abubakar Siddiq Salihu[1,3]

[1] Department of Chemistry
Faculty of Science and Mathematics
Universiti Pendidikan Sultan Idris
Tanjong Malim, Perak, Malaysia

[2] Center of Biodiversity and Conservation
Faculty of Science and Mathematics
Universiti Pendidikan Sultan Idris
Tanjong Malim, Perak, Malaysia

[3] Department of Pure and Industrial Chemistry
Faculty of Natural and Applied Sciences
Umaru Musa Yar'adua University
Katsina, Nigeria

✉ CORRESPONDING AUTHOR:
E-mail: wmnhakimi@fsmt.upsi.edu.my
Phone: +605-4507123

Received: December 24, 2022
Accepted: July 17, 2023

Essential oils from plants have been widely used in the prevention and treatment of numerous human diseases in many parts of the world for thousands of years.. Plants of the genus *Blumea* comprise about 80 species and are distributed in tropical and subtropical Asia, Africa, and Oceania; thirty of which are distributed in the southern provinces of China. The genus has been used locally in the treatment of bronchitis, blood diseases, fevers, and to alleviate a burning sensation. Over the last years, *Blumea* species have attracted great interest due to the variety of their essential oils, bioactive compounds, and pharmacological activities. This work provides comprehensive information regarding the essential oils from *Blumea* species concerning their medicinal uses, chemical composition, and bioactivities. The relevant information about the genus *Blumea* was gathered through electronic databases from 1989 to 2022, including Pubmed, SciFinder, Scopus, Google Scholar, and Web of Science. Based on existing studies of the genus *Blumea*, a total of eighteen species have been reported on the composition of the essential oil. The essential oils are mainly composed of β-caryophyllene, germacrene D, and borneol as the most abundant components. Moreover, essential oils also possess a wide spectrum of pharmacology such as larvicidal, antimicrobial, anti-inflammatory, cytotoxicity, and fumigant toxicity. As a source of traditional folk medicine, the *Blumea* genus has high medicinal value, and they are widely used in medicine. Therefore, further systematic, and comprehensive research on the genus *Blumea* is still required to provide a scientific basis for its clinical applications.

Keywords: Asteraceae, *Blumea*, essential oil, caryophyllene, larvicidal

1. INTRODUCTION

The Asteraceae is the largest family of flora in the world, comprising about 1550 genera and about 23,000 species. It comprises 10% of all flowering plant species. Most species of Asteraceae are annual, biennial, or perennial herbaceous plants, but there are also shrubs, vines, and trees [1]. The family has a widespread distribution, from subpolar to tropical regions in a wide variety of habitats. The largest proportion of the species occurs in the arid and semi-arid regions of subtropical and lower temperate latitudes. Besides, Asteraceae is an economically important family, providing food staples, garden plants, and herbal medicines [2].

Blumea is a genus of flowering plants of the family Asteraceae. *Blumea* is a genus with ca. 80 species and is classified in the subtribe Matricariinae of Anthemideae. It is mainly found in tropical and subtropical Asia, Africa, and Oceania. It is a small tree and shrub, characterised by disciform capitula with outer filiform female florets and inner tubular bisexual florets, tailed anthers, and cypsela wall epidermis with one large oxalate crystal in each cell [3]. This genus includes some important medicinal plants largely used

in traditional medicine. Some members of this genus are also used as herbal teas and condiments both in fresh and dried form due to their distinct aroma [4]. In the southern regions of China, thirty different species were reported, among which two of them, *B. balsamifera* and *B. riparia,* are utilised as conventional herbal remedies [5].

Since 1900s, more than 70 constituents have been isolated from the genus *Blumea*, including flavonoids, monoterpenes, sesquiterpenes, acetylenic thiophenes, triterpenoids, xanthenes, and diterpenes, and successfully exhibited significant bioactivities such as insecticidal, hepatoprotective, antitumor, antifungal, and antioxidant activities [6].

Essential oils from *Blumea* plants have shown a wide range of pharmacological activities, such as antimicrobial, antioxidant, and insecticidal. Recently, the volatile oil from *B. balsamifera* has been used to make over-the-counter medications, such as Jinhoujian spray, and used in China to treat throat sores and canker sores for decades. Besides those with throat sores, some patients in China who had larynx and hypopharynx cancer have taken Jinhoujian spraying as a part of their treatment. In addition, due to its unique scent, the *B. balsamifera* oil has been used as a cosmetics additive. For example, gynaecological lotions and shampoo liquids containing essential oils have been selling well in Southeast Asia for the last few years [7-8]. Also, the essential oil from *B. membranacea* produces a marked and long-lasting fall in the blood pressure of anaesthetised dogs, exerts a direct depressant action on frog hearts, and has a spasmolytic effect on rabbit ilea [9].

Recently, essential oils and other aromatic compounds sourced from plants and used as alternative medicine are gaining interest. Hence, the review regarding *Blumea* essential oils needs to be carried out to simplify and compile the information. The information was collected via electronic searches in databases such as Scopus, PubMed, Science Direct, SciFinder, and Google Scholar. This review aims to give an overview of all published reports on the chemical composition, biological activities, and medicinal uses of *Blumea* essential oils.

2. SEARCH STRATEGY

The protocol for performing this study was developed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) [10] (a) the first step was to exclude duplicate articles, (b) titles and abstracts were then read and the inclusion and exclusion criteria were applied and (c) all articles resulting from this stage were read in full, and the inclusion and exclusion criteria were applied again.

Figure 1 shows the flow diagram of the identification and selection of articles. Following this step, we reached the articles chosen for this study. This systematic review was conducted through searches using Scopus, PubMed, Science Direct, SciFinder, and Google Scholar. The keywords used were '*Blumea*', 'essential oil', and 'biological activity' articles over the period from the beginning of the database until December 2022.

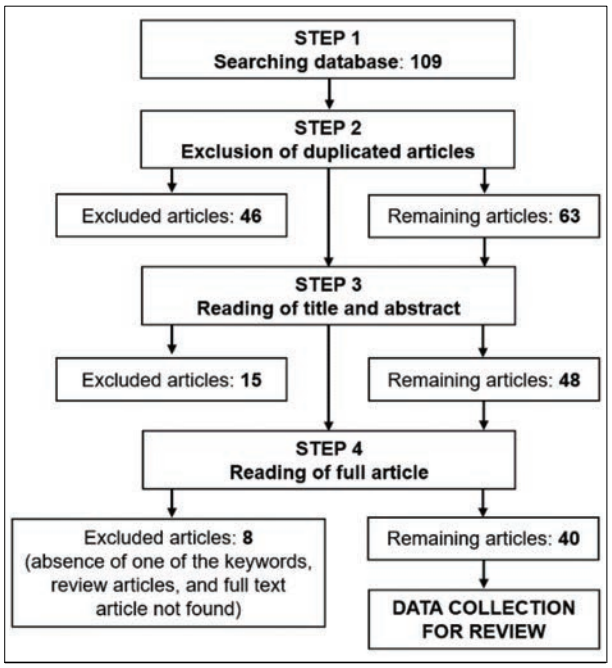


Figure 1 - PRISMA flow diagram of included studies

The inclusion of articles considered the following criteria: (1) type of publication - original research articles, (2) only articles in English, (3) articles must have the chemical composition of *Blumea* essential oils, (4) articles must discuss the bioactivity of the essential oils. As exclusion criteria, the following were used: (1) articles that did not have the search terms in the title and abstract; (2) review articles, (3) full-text articles not found, (4) articles without one of the keywords and (5) articles that did not have the essential oil composition.

3. MEDICINAL USES

The *Blumea* genus includes some key medicinal plants largely used in traditional medicine. Among the *Blumea* species, *B. balsamifera* is one of the best-known species and it is very often cultivated locally where it is known as 'sambong'. It has been widely used as medicine for thousands of years in Southeast Asia countries, such as China, Malaysia, Thailand, Vietnam, and the Philippines. In China, it is generally used as incense due to its richness of essential oils. Besides, the leaves were used as a crude Chinese traditional medicinal material to treat eczema, dermatitis, beriberi, lumbago, menorrhagia, rheumatism, and skin injury, and as an insecticide [6]. Table I shows the medicinal uses of several *Blumea* species [11-28].

4. ESSENTIAL OILS ANALYSIS

Generally, the volatile components of *Blumea* spp. were identified by Gas Chromatography (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS). The plant parts, studies focused on many materials such as leaves, flowers, stems, twigs as well as aerial part. Data analysis of the GC-FID results were conducted using the calculation of Kovats index of homologous alkane (e.g., C7 to C20, while GC-MS mass spectrum was identified by comparison with integrated NIST library and Adams.

In previous studies, eighteen *Blumea* species were described on the composition of the essential oils [29-53]. These were *B. lacera* [12, 29-31], *B. sinuata* [12], *B. riparia* [13], *B. paniculata* [32], *B. balsamifera* [33-40], *B. lanceolaria* [41], *B. eriantha* [17, 42], *B. malcolmii* [43], *B. mollis* [44, 45], *B. densiflora* [46], *B. perrottetiana* [47], *B. megacephala* [48], *B. martiniana* [20], *B. gariepina* [49], *B. brevipes* [50], *B. lanceolaria* [51], *B. virens* [52], and *B. oxyodonta* [53].

Most of the species were reported from China, India, and Vietnam, in addition to Bangladesh, Kenya, Nepal, Nigeria, and Zambia. The highest yield was shown by the *B. balsamifera* leaf oil from China which gave 3.25-3.42% [35, 38].

5. ESSENTIAL OILS COMPOSITION

Investigation of chemical components identified in *Blumea* essential oils shows that the oil consists of several groups of components, which are monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, ester, and ketone. Besides, *B. lacera* essential oil from Nepal [30] reported the highest chemical components, consisting in 72 components. Table II shows the major components identified in *Blumea* essential oils from various localities.

Table I - Medicinal uses of several *Blumea* species

Species	Medicinal uses	References
B. lacera	Used as an expectorant, diuretic, astringent, antispasmodic, antipyretic, antioxidant, antidiarrheal, liver tonic, and stimulant.	[11]
B. sinuata	Its leaves and stems are used to treat boils, remove toxins from the body, and stop bleeding.	[12]
B. riparia	In Chinese traditional medicine, the plant is used to treat headaches, hypertension, colic, and gynecological diseases. In Malaysia, root decoction is used to treat cough, stomachache, and edema.	[13]
B. paniculata	The whole plant is used for the treatment of diarrhea, fever, and parasites, while the roots are used to control stomachache. In veterinary medicine, leave and fruit paste is used as a wound healing of cattle.	[14]
B. balsamifera	The leaves have been employed for the treatment of eczema, beriberi, menorrhagia, dermatitis, lumbago, rheumatism, and skin injury.	[15]
B. lanceolaria	The leaves are used for the treatment of bronchitis, aphthae, and asthma. In India, the plant is used as an anti-cancer agent, and leaves juice is useful for wound healing, chronic ulcers, and infusion of leaves to control dysentery.	[16]
B. membranacea	The essential oil led to blood pressure reduction.	[9]
B. eriantha	The juice extracted from this herb has been reported as a carminative, while the warm leaf infusion is used as sudorific, and the cold infusion is considered as a diuretic and herbal emmenagogue.	[17]
B. densiflora	This plant is used to drive away mosquitoes by burning, and its juice used to prevent mosquito bites in South China because of its light, borneol-like odor	[18]
B. megacephala	The plant is edible and used for malaria, bronchitis, puerperal metrorrhagia, puerperal edema, and barrenness.	[19]
B. martiniana	It has been used for the treatment of parasites and rheumatism in folklore medicine of Yunnan, China.	[20]
B. perrottetiana	It is a common aromatic weed of savannah farms and has been used as an insect repellent and as an anthelmintic.	[21]
B. mollis	The leaf of the plant is traditionally used for skin diseases and the boiled herb is used to treat diarrhea.	[22]
B. brevipes	The plant is traditionally administered after birth and in the treatment of the sexual disorder.	[23]
B. laciniata	In Bangladesh, this herb is used as a folk medicine to treat a range of ailments, including respiratory and blood diseases, fevers, ulcers, and burning sensations. It is also used in Indian and Chinese traditional medicine systems.	[24]
B. aromatica	It has been widely used as traditional Chinese medicine for the treatment of rheumatism, arthralgia, and eczema.	[25]
B. axillaris	The plant is used in the treatment of skin diseases, wounds, external parasites, diarrhea, asthma, and dropsy.	[26]
B. oxyodonta	The decoction of the roots is used for the treatment of impotency and spermatorrhea.	[14]
B. obliqua	It is used as remedies for malaria, influenza, bronchitis, and asthma.	[27]
B. arfakiana	The leaves are used in the traditional medicine of Papua New Guinea for the treatment of stomach pains, insect bites, and anemia.	[28]

Table II - Major chemical components identified from *Blumea* essential oils

Species	Locality	Part	Yield (%)	Identification (No. %)	Major components	References
B. lacera	Vietnam	Floral	1.10	32, 97.7	(*E*)-Caryophyllene (23.8%), germacrene D (18.5%), thymohydroquinone dimethyl ether (5.0%), γ-curcumene (5.9%), ar-curcumene (8.0%), and α-zingiberene (4.7%)	[12]
		Leaf	1.56	36, 97.3	(*E*)-Caryophyllene (27.2%), germacrene D (21.0%), thymohydroquinone dimethyl ether (4.1%), γ-curcumene (7.7%), ar-curcumene (3.7%), and α-zingiberene (7.1%)	[12]
		Stem	0.35	38, 94.7	(*E*)-Caryophyllene (11.7%), germacrene D (11.2%), thymohydroquinone dimethyl ether (28.4%), γ-curcumene (4.7%), ar-curcumene (1.9%), and α-zingiberene (4.6%)	[12]
		Aerial parts	0.06-0.5	49, 99.0	β-Caryophyllene (8.3-12.0%), thymolhydroquinon-dimethylether (6.6-11.9%), and caryophyllene oxide (11.9-21.7%)	[29]
	Nepal	Aerial parts	1.00	77, 98.2	(*Z*)-Lachnophyllum ester (25.5%), (*Z*)-lachnophyllic acid (17.0%), germacrene D (11.0%), (*E*)-β-farnesene (10.1%), bicyclogermacrene (5.2%), (*E*)-caryophyllene (4.8%), and (*E*)-nerolidol (4.2%)	[30]
	Nigeria	Leaf	NM	13, 63.0	Thymoquinol dimethyl ether (33.9%), β-caryophyllene (10.7%), α-humulene (4.6%)	[31]
B. sinuata	Vietnam	Aerial parts	0.16	65, 97.8	Thymohydroquinone dimethyl ether (29.4%), (*E*)-caryophyllene (19.7%), α-pinene (8.8%), germacrene D (7.8%), and α-humulene (4.3%)	[12]
B. riparia	Vietnam	Leaf	0.1-0.2	36, 91.8	Germacrene D (33.6%), (*E*)-β-caryophyllene (11.2%), bicyclogermacrene (9.3%), and caryophyllene oxide (5.9%)	[13]
		Twig	0.1-0.2	30, 94.2	germacrene D (42.6%), bicyclogermacrene (12.1%), and (*E*)-β-caryophyllene (11.6%)	[13]
B. paniculata	India	Leaf	0.09	39, 95.8	Germacrene D (46.9%), β-caryophyllene (5.9%), caryophyllene oxide (3.9%), and hexadecanoic acid (3.5%)	[32]
		Stem	0.50	46, 96.3	Germacrene D (48.1%), α-humulene (4.9%), β-caryophyllene (4.8%), *epi*-α-cadinol (4.7%), α-cadinol (4.3%), and α-pinene (4.2%)	
		Flower	0.80	40, 95.1	Germacrene D (39.6%), α-humulene (8.9%), β-caryophyllene (7.7%), and *epi*-α-cadinol (4.7%)	
B. balsamifera	China	Leaf	NM	39, 86.0	Borneol (25.3%), *trans*-caryophyllene (24.4%), camphor (8.9%), and caryophyllene oxide (5.8%)	[33]
				35, 85.0	Caryophyllene (18.54%), borneol (18.33%), (+)-2-bornanone (11.28%), and α-gurjunene (6.73%)	[34]
		Leaf	3.42	51, 86.3	Caryophyllene (22.5%), xanthoxylin (20.2%), and γ-eudesmol (12.2%)	[35]
		Leaf	0.88	27, 99.23	1,8-Cineole (20.98%), borneol (11.98%), β-caryophyllene (10.38%), camphor (8.06%), terpinen-4-ol (6.49%), α-terpineol (5.91%), and caryophyllene oxide (5.35%)	[36]
		Leaf	NM	49, 97.59	Borneol (43.55%), camphor (9.54%), and β-caryophyllene (6.51%)	[37]

Table II - Continue

Species	Locality	Part	Yield (%)	Identification (No. %)	Major components	References
		Leaf	3.25	42, 97.65	Caryophyllene (19.28%), 1,7,7-trimethyl-(1S-endo)-bicyclo[2.2.1] heptan-2-ol (15.54%), caryophyllene oxide (11.20%), thujopsene (10.36%), 3-t-butyl-4-methoxyphenol methyl derivative (6.04%), and guaiol (5.03%)	[38]
		Leaf	NM	67, 98.56	Caryophyllene (26.47%), thujopsene-l3 (14.45%), 1,7,7-trimethyl-(1*S*-endo)-bicyclo[2.2.1]heptan-2-ol (9.07%), 3-t-butyl-4-methoxyphenol methyl derivative (6.91%), 1*R*,4*R*,7*R*,11*R*-1,3,4,7-tetramethyl-tricyclo[5.3.1.0(4,11)]undec-2-ene (6.03%), and caryophyllene oxide (5.38%)	[39]
	Bangladesh	Leaf	0.40	50, 99.07	Borneol (33.22%), caryophyllene (8.24%), ledol (7.12%), tetracyclo[6,3,2,0,(2.5).0(1,8) tridecan-9-ol, 4,4-dimethyl (5.18%), phytol(4.63%), caryophyllene oxide(4.07%), and thujopsene-13 (4.42%)	[40]
B. lanceolaria	India	Leaf	0.02	36, 97.3	β-Pinene (82.3%), terpinen-4-ol (4.1%), γ-terpinene (2.5%), and sabinene (2.2%)	[41]
B. eriantha	India	Leaf	NM	34, 94.2	(4*E*,6*Z*)-*allo*-Ocimene (12.8%), carvotanacetone (10.6%), and dodecyl acetate (8.9%)	[17]
	India	Leaf	NM	72, 96.83	(4*E*,6*Z*)-Ocimene (13.72%), caryophyllene (9.71%), caryophyllene oxide (5.76%), and carvotanacetone (5.36%)	[42]
B. malcolmii	India	Aerial parts	0.27	18, 99.2	Carvotanacetone (92.1%), carvomenthone (2.3%), and β-caryophyllene (1.1%)	[43]
B. mollis	India	Leaf	NM	22, 92.3	β-Caryophyllene (24.54%), γ-cadinene (16.29%), α-zingiberene (9.34%), β-sesquiphellandrene (7.64%), and α-curcumene (7.49%)	[44]
	India	Leaf	0.50	39, 99.96	Linalool (19.43%), γ-elemene (12.19%), copaene (10.93%), estragole (10.81%), *allo*-ocimene (10.03%), γ-terpinene (8.28%), and *allo*-aromadendrene (7.44%)	[45]
B. densiflora	China	Aerial parts	NM	46, 98.63	Borneol (11.43%), germacrene D (8.66%), β-caryophyllene (6.68%), γ-terpinene (4.35%), sabinene (4.34%), and β-bisabolene (4.24%)	[46]
B. perrottetiana	Nigeria	Aerial parts	0.27	34, 99.9	2,5-Dimethoxy-p-cymene (30.0%), 1,8-cineole (11.0%), sabinene (8.1%), δ-cadinene (5.3%), and β-caryophyllene (3.9%)	[47]
B. megacephala	China	Aerial parts	0.72	65, 94.86	Borneol (13.6 %), β-caryophyllene (9.56 %), germacrene D (9.09 %), sabinene (6.37 %), and α-humulene (4.78 %).	[48]
B. martiniana	China	Aerial parts	0.64	68, 98.55	Linalool (10.36%), germacrene D (9.09%), borneol (6.24%), and γ-terpinene (5.38%)	[20]
B. gariepina	Zambia	Leaf	0.70	8, 97.7	Thymyl acetate (85.4%) and thymol (6.9%)	[49]
B. brevipes	Kenya	Flower	0.70	68, 94.0	Terpinen-4-ol (27.6%), germacrene-D (15.4%), sabinene (8.0%), and γ-terpinene (5.5%)	[50]
B. lanceolaria	Vietnam	Leaf	0.24	12, 99.9	Methyl thymol (94.9%) and *p*-cymene (3.28%)	[51]
B. virens	India	Aerial parts	0.20	52, 97.8	2,5-Dimethoxy-p-cymene (27.6%), hinesol (20.2%), α-pinene (6.9%), and hexadecanoic acid (4.3%)	[52]
B. oxyodonta	India	Aerial parts	0.02	61, 98.8	β-Caryophyllene (23.5%), 2,5-dimethoxy-p-cymene (14.7%), and germacrene D (13.2%)	[53]

β-Caryophyllene has been indicated as a major component in most *Blumea* essential oils. Its abundance was recognised in the essential oils of *B. lacera* [12, 29], *B. riparia* [13], *B. paniculata* [32], *B. balsamifera* [35, 38, 39], *B. mollis* [44], and *B. oxyodonta* [53]. The highest amount of β-caryophyllene was reported from the leaf oil of *B. lacera* from Vietnam which contributed with 27.2% [12]. β-Caryophyllene is found in numerous edible plants that are ingested daily, and it is approved as a food additive by the Food and Drug Administration. This compound can change the inflammatory processes in humans through the endocannabinoid system [54]. Furthermore, this compound could increase the intracellular accumulation of anticancer agents, thereby potentiating their cytotoxicity due to the absorption of 5-fluorouracil across human skin. β-Caryophyllene facilitates the passage of paclitaxel through membranes and thus potentiates its anticancer activity [55].

Germacrene D has been reported to be the major component in several *Blumea* essential oils such as *B. riparia* [12] from Vietnam and *B. paniculata* [32] from India. Both species presented in high amounts which is more than 30%. Germacrene D is one of the most common plant volatiles considered to be a biogenetic precursor of many sesquiterpenes such as cadinane, muurolane and amorphane derivatives. This metabolite is involved in plant-insect interaction acting as a pheromone on receptor neurons ones [56]. It was also shown as an important deterrent and insecticidal agent against different parasites such as mosquitos, aphids, and ticks [57].

In another study, the borneol richness was also found in *B. balsamifera* [37] from China and Bangladesh [40], as well as *B. densiflora* [46] and *B. megacephala* [48], both from China. Borneol was recently reported to exhibit an antibacterial activity [58]. It was also reported to have neuroprotective effects, as well as vasorelaxant properties [59].

Carvotanacetone was also found as a major component in *B. malcolmii* [43] and *B. eriantha* [17] essential oils from India. Previously, the essential oil containing carvotanacetone has been reported to possess a strong bactericidal activity, moderate cytotoxic activity, and an acetylcholinesterase inhibitory effect [60].

Furthermore, oxygenated monoterpenes were found in several *Blumea* essential oils. 1,8-Cineole was identified dominantly in the leaf oil of *B. balsamifera* [36], β-pinene from the leaf oil of *B. lanceolaria* [41], as well as terpinen-4-ol from the flower oil of *B. brevipes* [50]. In addition, linalool was also found in a high percentage in the leaf oil of *B. mollis* [45] and the aerial parts oil of *B. martiniana* [20].

Generally, the chemical variations discovered between the *Blumea* species might have possibly relied on the environment in which the plant existed and was collected in addition to seasonal variations at the time. These factors influence the plant's biosynthetic pathways and consequently, the relative proportion of the main characteristic components [61].

6. BIOLOGICAL ACTIVITIES

The literature study reveals that *Blumea* essential oils have been reported in various biological activities mainly for larvicidal, antimicrobial, anti-inflammatory, cytotoxicity, and fumigant toxicity. The biological activities of *Blumea* essential oils are illustrated in Table III.

Most of the studies focused on the larvicidal activity of *Blumea* essential oils. A larvicide is a type of insecticide used to control mosquitoes, by killing mosquito larvae before they can grow into adults. Among them, *B. sinuata* essential oil [12] showed strong larvicidal activities with 24-h LC_{50} values of 23.4 and 29.1 µg/mL against *Aedes aegypti* and *Aedes albopictus*, respectively, as well as 48-h LC_{50} values of 17.4 and 12.4 µg/mL. In addition, *B. eriantha* essential oil [17] also gave high toxicity against *Anopheles stephensi* (LC_{50} 41.61 µg/mL), *Aedes aegypti* (LC_{50} 44.82 µg/mL), *Culex quinquefasciatus* (LC_{50} 48.92 µg/mL), *Anopheles subpictus* (LC_{50} 51.21 µg/mL), *Aedes albopictus* (LC_{50} 56.33 µg/mL) and *Culex tritaeniorhynchus* (LC_{50} 61.33 µg/mL). Meanwhile, the essential oils of *B. lacera* [12], *B. densiflora* [46], *B. martiniana* [20], *B. mollis* [45], and *B. perrottetiana* [47] also exerted significant activity against various mosquito species.

For the antimicrobial activity, the essential oils act to inhibit the growth of bacterial cells and also inhibit the production of toxic bacterial metabolites. The *Blumea* essential oil were found to display antimicrobial activity against various microbial strains, which includes food spoilage and common human/plant pathogenic bacterial and yeast strains. The most known and basic methods are the disk-diffusion and broth or agar dilution methods. The *B. balsamifera* essential oil [64] appeared to impose an extremely strong inhibition on *Haemophilus parasuis* with MIC and MBC values found to be 0.625 and 1.25 µg/mL, respectively. In addition, *B. mollis* essential oil [66] also showed a strong activity against *Bacillus pumilus*, *Staphylococcus aureus*, and *Streptococcus pyogenes*, each with MIC value of 62.5 µg/mL. In another study, *B. balsamifera* essential oil [36] possessed a strong fumigant toxicity against *Sitophilus zeamais* with LC_{50} value 10.71 mg/L air.

7. CONCLUSION

This article's objective was to provide significant literature on the medicinal uses, chemical compositions, and biological activity evidence of the *Blumea* essential oils. Read together, the results above indicate that the chemical composition of the essential oils of *Blumea* species comprises a diverse group of components that could be considered promising

Table III - Biological activities from *Blumea* essential oils.

Bioactivities	Species	Description	References
Larvicidal	*B. lacera*	The essential oil showed moderate *Aedes* larvicidal activities with 24-h LC_{50} values of 64.7 and 116.7 μg/mL against *Aedes aegypti* and *Aedes albopictus*, respectively, as well as 48-h LC_{50} values of 55.1 and 99.4 μg/mL.	[12]
	B. sinuata	The essential oil showed very good *Aedes* larvicidal activities with 24-h LC_{50} values of 23.4 and 29.1 μg/mL against *Aedes aegypti* and *Aedes albopictus*, respectively, as well as 48-h LC_{50} values of 17.4 and 12.4 μg/mL.	[12]
	B. eriantha	The essential oil showed high toxicity against 3rd instar larvae of six important mosquito species: *Anopheles stephensi* (LC_{50} 41.61 μg/mL), *Aedes aegypti* (LC_{50} 44.82 μg/mL), *Culex quinquefasciatus* (LC_{50} 48.92 μg/mL), *Anopheles subpictus* (LC_{50} 51.21 μg/mL), *Aedes albopictus* (LC_{50} 56.33 μg/mL) and *Culex tritaeniorhynchus* (LC_{50} 61.33 μg/mL).	[17]
	B. densiflora	Toxic effect against fourth-instar larvae of *Anopheles anthropophagus* (LC_{50} 22.32 ppm and LC_{90} 54.04 ppm, 12 h; LC_{50} 10.55 ppm and LC_{90} 33.56 ppm, 24 h).	[46]
	B. martiniana	The essential oil exerted significant activity against *Anopheles anthropophagus* with LC_{50} values of 46.86, 35.87, 44.61, 35.89, and 29.21 mg/L, respectively.	[20]
	B. mollis	The essential oil showed significant toxic effect against early fourth-instar larvae of *Culex quinquefasciatus* with LC_{50} value 71.71 ppm and LC_{90} value 143.41 ppm, after 24 h.	[45]
	B. perrottetiana	The essential oil showed notable activity against the red flour beetle, *Tribolium Castaneum* (73.3% mortality, 12 h and 93.3% mortality, 24 h).	[47]
Antimicrobial	*B. riparia*	The twig oil possessed an MIC value of 100 μg/mL against *Bacillus subtilis*, whereas the leaf oil had MIC values of 50 and 100 μg/mL against *Fusarium oxysporum* and *Saccharomyces cerevisiae.*	[13]
	B. lacera	The essential oil showed potential activity against *Erwinia herbicola* and *Pseudomonas putida* with MIC values of 4.0 and 8.0 μg/mL, respectively.	[62]
	B. eriantha	The essential oil showed a significant activity against *Streptococcus pyogenes* with MIC and MBC values 0.09% and 0.39%, respectively.	[63]
	B. megacephala	The essential oil (1000 μg/disc) has promising effects against several pathogens, giving inhibition zone diameter values (21.5, 21.6, 23.4, 23.8, 21.9) and MIC values (125, 125, 62.5, 125, 125 μg/mL) against *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Hansenula anomala*, respectively.	[48]
	B. balsamifera	The essential oil appeared to impose extremely strong inhibition on *Haemophilus parasuis* in vitro and the MIC was found to be 0.625 μg/mL, and the MBC was 1.25 μg/mL.	[64]
		The essential oil was the most potent with a MIC of 150 μg/mL against *Bacillus cereus* and an MIC of 1.2 μg/mL against *Staphylococcus aureus* and *Candida albicans.*	[65]
	B. mollis	The essential oil showed strong activity against *Bacillus pumilus*, *Staphylococcus aureus*, and *Streptococcus pyogenes*, each with MIC value of 62.5 μg/mL.	[66]
Anti-inflammatory	*B. balsamifera*	The essential oil can significantly reduce the LPS-induced pro-inflammatory elements TNF-α, IL-1β, IL-6, and inflammatory mediator COX-2 in RAW264.7 cells ($p<0.01$). Additionally, it can significantly inhibit the expression of proteins in the NF-κB signaling pathway, such as CD14, TLR4, MyD88, TAK-1, p-IκBα, and NLRP3 inflammasome ($p<0.05$, $p<0.01$).	[33]
		The essential oil can effectively alleviate the skin erythema and epidermal thickening caused by UV-B radiation, via the down-regulation of TNF-a, IL-6 and IL-10, thereby alleviating the damage of UV-B radiation to cells and tissues, and promoting the healing of injury skin.	[34]
Cytotoxicity	*B. lacera*	The essential oil showed promising activity against MDA-MB-231, MCF-7, and 5637 cell lines, with IC_{50} values of 11.2, 27.7, and 22.0 μg/mL.	[30]
Fumigant toxicity	*B. balsamifera*	The essential oil possessed strong fumigant toxicity against *Sitophilus zeamais* with LC_{50} value 10.71 mg/L air.	[36]

for applications in different industries, including the food and pharmaceutical industries. In addition, the essential oil isolated from *Blumea* species may bear the potential for a drug development due to its high concentration of β-caryophyllene, germacrene D, and borneol. Considering the great diversity of chemical components and functional properties of these essential oils, further investigations should focus on the effect of the extraction technique on the quality and extraction yield of the essential oils from *Blumea* species and the use of these oils in various applications such as food products.

REFERENCES

[1] E. Piątkowska, W. Biel, R. Witkowicz, J. Kępińska-Pacelik, Chemical composition and antioxidant activity of Asteraceae family Plants. Appl. Sci. 12(23), 12293 (2022).

[2] M. Jayasundera, S. Florentine, K.U Tennakoon, B.S. Chauhan, Medicinal value of three agricultural weed species of the Asteraceae family: A review. Pharmacogn. J. 13(1), 264-277 (2021).

[3] P. Pornpongrungrueng, F. Borchsenius, M. Englund, A.A. Anderberg, M.H.G. Gustafsson, Phylogenetic relationships in *Blumea* (Asteraceae: Inuleae) as evidenced by molecular and morphological data. Syst. Evol. 269, 223-243 (2007).

[4] I.H. Burkill, A Dictionary of the Economic Products of the Malay Peninsula, Vol. 1, Ministry of Agriculture and Cooperatives, Kuala Lumpur (1966).

[5] L.M. Perry, Medicinal plants of East and Southeast Asia, Harvard University, Cambridge, MA (1980).

[6] M. Chen, H.Z. Jin, W.D. Zhang, S.K. Yan, Y.H. Shen, Chemical constituents of plants from the genus *Blumea*. Chem. Biodiver. 6, 809-817 (2009).

[7] Y.X. Pang, Z.W. Fan, D. Wang, Q. Yang, K. Wang, X.L. Chen, X. Hu, F.L. Yu, Z.X. Chen, External application of the volatile oil from *Blumea balsamifera* may be safe for liver - a study on its chemical composition and hepatotoxicity. Molecules 19, 18479-18492 (2014).

[8] Y. Pang, D. Wang, Z. Fan, X. Chen, F. Yu, X. Hu, K. Wang, L. Yuan, *Blumea balsamifera* - a phytochemical and pharmacological review. Molecules 19(7), 9453-9477 (2014).

[9] S.C. Mehta, H. Vardhan, S.P. Saxena, Some pharmacological actions of the essential oil of *Blumea membranacea*. Indian J. Physiol. Pharmacol. 30, 149-154 (1986).

[10] D. Moher, A. Liberati, J. Tetzlaff, G.A. Douglas, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. Ann. Intern. Med. 151, 264-269, (2009)

[11] M.R. Habib, Y. Igarashi, M.A. Rabbi, Phytochemical composition of *Blumea lacera* leaf and its inhibitory effects on the activity of enzymes related to metabolic diseases. Pharm. Sci. Asia 49, 265-272 (2022).

[12] T.M. Hoi, P. Satyal, L.T. Huong, D.V. Hau, T.D. Binh, D.T.H. Duyen, D.N. Dai, N.G. Huy, H.V. Chinh, V.V. Hoa, N.H. Hung, W.N. Setzer, Essential oils from Vietnamese Asteraceae for environmentally friendly control of *Aedes mosquitoes*. Molecules, 27(22), 7961 (2022).

[13] S.N. The, A.L. Tuan, T.D.T. Thu, N.D. Luyen, T.T. Thi, Essential oils of the Asteraceae plants *Blumea riparia* DC. and *Pluchea pteropoda* Hemsl. ex Hemsl. growing in Vietnam. Nat. Prod. Commun. 17(6), 1-6 (2022).

[14] U. Quattrocchi, CRC world dictionary of medicinal and poisonous plants, common names, scientific names, eponyms, synonyms, and etymology. Boca Raton (FL): CRC Press (2012).

[15] N. Ruangrungsi, P. Tantivatana, P. Tappayuthpijarn, R.P. Borris, G.A. Cordell, Traditional medicinal plants of Thailand VI: isolation of cryptomeridiol from *Blumea balsamifera* (Compositae). Sci. Asia, 11, 47-50 (1985).

[16] K.C.T. Phi, G.N. Kim, H.D. Jang, In vitro and intracellular antioxidant capacity of thymyl methyl ether as a major component in *Blumea lanceolaria* (Roxb.) Druce leaf oil. Food Chem. Toxicol. 50(5), 1583-1588 (2012).

[17] G. Benelli, M. Govindarajan, M. Rajeswary, S. Senthilmurugan, P. Vijayan, N.S. Alharbi, S. Kadaikunnan, J.M. Khaled, Larvicidal activity of *Blumea eriantha* essential oil and its components against six mosquito species, including Zikavirus vectors: the promising potential of (4*E*,6*Z*)-allo-ocimene, carvotanacetone and dodecyl acetate. Parasitol. Res. 116(4), 1175-1188 (2017).

[18] S.C. Gupta, U.M. Khanolkar, O. Koul, B.P. Saxena, Pyrethrin synergistic activity by the essential oils of a few *Blumea* species. Curr. Sci. 46, 304-305 (1977).

[19] D. Tan, Q. Yan, Flavonoids from the aerial parts of *Blumea megacephala*. Chem. Nat. Compd. 55(5), 927-928 (2019).

[20] L. Zhu, Y.J. Tian, Chemical composition and larvicidal effects of essential oil of *Blumea martiniana* against *Anopheles anthropophagus*. Asian Pacific J. Trop. Med. 4(5), 371-374 (2011).

[21] J.C.T. Uphof, Dictionary of Economic Plants, 2nd Ed. Verlag von J. Cramer. Lehre, Germany (1968).

[22] D.N. Guha Bakshi, P. Sensarma, D.C. Pal, A lexicon of medicinal plants in India, Vol. 1. Naya Prokash, Calcutta (1999).

[23] A.D.Q. Agnew, Upland Kenya Wild Flowers, Oxford University Press, London (1974).

[24] A.H.M.M. Rahman, Assessment of angiosperm

weeds of Rajshahi, Bangladesh with emphasis on medicinal plants. Res. Plant Sci. 1, 62-67 (2013).
[25] M.S. Lan, C. Luo, C.H. Tan, L. Chen, S. Wei, D.Y. Zhu, Study on chemical constituents of the ethyl acetate extract from *Blumea aromatica*. J. Chin. Med. Mater. 35, 229-231 (2012).
[26] K.P. Sreelekha, T.P. Ajeesh Krishna, T.P. Adarsh Krishna, P.E. Deepa, D. Udayan, S. Juliet, S.N. Nair, R. Ravindran, Pharmaco-chemical characterization of leaves of *Blumea mollis* (D.Don) Merr. from Western Ghats of Wayanad region of Kerala, India. J. Pharmacogn. Phytochem. 6(4), 319-323 (2017).
[27] V.U. Ahmad, N. Alam, New antifungal bithienylacetylenes from *Blumea obliqua*. J. Nat. Prod. 58(9), 1426-1429 (1995).
[28] G. Rucker, G. Paulini, H. Sakulas, B. Lawong, F. Goeltenboth, Medicinal plants from Papua New Guinea: Graniline-esters and germacranolides from *Blumea arfakiana*. Planta Med. 57(3), 278-281 (1991).
[29] L.V. Hac, T.T. Muoi, N.X. Dung, Essential oils of *Blumea lacera* (Burm. f) DC. (asteraceae) produced from aerial parts of plants grown in central of Vietnam. J. Essent. Oil-Bearing Pl. 6(1), 36-40 (2003).
[30] P. Satyal, B.K. Chhetri, N.S. Dosoky, S. Shrestha, A. Poudel, W.N. Setzer, Chemical composition of *Blumea lacera* essential oil from Nepal. Biological activities of the essential oil and (*Z*)-lachnophyllum ester. Nat. Prod. Commun. 10 (10), pp. 1749-1750 (2015).
[31] I. Laakso, T. Seppänen-Laakso, R. Hiltunen, O. Ekundayo, Composition of the essential oil of *Blumea lacera* DC. (Asteraceae) leaves from Nigeria. Flav. Fragr. J. 4(2), 73-75 (1989).
[32] R.K. Joshi, Sesquiterpenoid-rich leaf, stem, and flower essential oil of the traditional herb *Blumea paniculata* (Willd.) M.R. Almeida from India. Nat. Prod. Res. *in press* (2021).
[33] J. Liao, X. Xie, W. Wang, Y. Gao, Y. Cai, J. Peng, T. Li, Q. Yi, C. He, L. Wang, Anti-inflammatory activity of essential oil from leaves of *Blumea balsamifera* (L.) DC through Inhibiting TLR4/NF-kB signalling pathways and NLRP3 inflammasome activation in LPS-induced RAW264.7 macrophage cells. J. Essent. Oil-Bearing Pl. 24(2), 160-176 (2021).
[34] B. Zhang, M. Tang, W. Zhang, C. Zhang, Y. Ai, X. Liang, Y. Shi, Y. Chen, L. Zhang, T. He, Chemical composition of *Blumea balsamifera* and *Magnolia sieboldii* essential oils and prevention of UV-bradiation-induced skin photoaging. Nat. Prod. Res. 35(24), 5977-5980 (2021).
[35] Y.H. Wang, X.Y. Yu, Biological activities and chemical compositions of volatile oil and essential oil from the leaves of *Blumea balsamifera*. J. Essent. Oil-Bearing Pl. 21(6), 1511-1531 (2018).
[36] S.S. Chu, S.S. Du, Z.L. Liu, Fumigant compounds from the essential oil of Chinese *Blumea balsamifera* leaves against the maize weevil (Sitophilus zeamais). J. Chem. 289874 (2013).
[37] Y. Yuan, M. Huang, Y.X. Pang, F.L. Yu, C. Chen, L.W. Liu, Z.X. Chen, Y.B. Zhang, X.L. Chen, X. Hu, Variations in essential oil yield, composition, and antioxidant activity of different plant organs from *Blumea balsamifera* (L.) DC. at different growth times. Molecules 21(8), 1024 (2016).
[38] Z.L. Jiang, Y. Zhou, W.C. Ge, K. Yuan, Phytochemical compositions of volatile oil from *Blumea balsamifera* and their biological activities. Phcog. Mag. 10(30), 346-352 (2014).
[39] Y.X. Li, X.H. Pei, D.Q. Jiang, K. Yuan, Phytochemical compositions of the essential oil from *Blumea balsamifera*. Asian J. Chem. 25(11), 6361-6365 (2013).
[40] N.I. Bhuiyan, J.U. Chowdhury, J. Begum, Chemical components in volatile oil from *Blumea balsamifera* (L.) DC. Bangladesh J. Bot. 38(1), 107-109 (2009).
[41] R.K. Joshi, GC-MS analysis of volatile organic constituents of traditionally used medicinal plants from the Western Ghats of India: *Blumea lanceolaria* (Roxb.) Druce. *Heliotropium indicum* L. and *Triumfetta rhomboidea* Jacq. J. Mex. Chem. Soc. 64(2), 1-9 (2020).
[42] P.P. Pednekar, R.V. Dhumal, A.G. Datar, G.R. Vanage, In vivo dermal absorption and sub-acute toxicity studies of essential oil from *Blumea eriantha* DC. Int. J. Pharm. Pharm. Sci. 5(3), 351-358 (2013).
[43] R.K. Joshi, S.R. Pai, Reinvestigation of carvotanacetone after 100 years along with minor terpenoid constituents of *Blumea malcolmii* Hook. F. essential oil. Nat. Prod. Res. 30(20), 2368-2371 (2016).
[44] T. Tamilarasi, R. Thirugnanasampandan, Antioxidant activity evaluation of essential oil and RAPD analysis of in vitro regenerated *Blumea mollis* (D. Don) Merr. Acta Physiol. Plant. 36(6), 1593-1598 (2014).
[45] A. Senthilkumar, K. Kannathasan, V. Venkatesalu, Chemical constituents and larvicidal property of the essential oil of *Blumea mollis* (D. Don) Merr. against *Culex quinquefasciatus*. Parasitol. Res. 103(4), 959-962 (2008).
[46] L. Zhu, Y. Tian, Chemical composition and larvicidal activity of *Blumea densiflora* essential oils against *Anopheles anthropophagus*: A malarial vector mosquito. Parasitol. Res. 109(5), 1417-1422 (2011).
[47] M.S. Owolabi, L. Lajide, H.E. Villanueva, W.N. Setzer, Essential oil composition and insecticidal activity of *Blumea perrottetiana* growing in southwestern Nigeria. Nat. Prod. Commun. 5(7), 1135-1138 (2010).
[48] L. Zhu, Y.J. Tian, L. Yang, J.G. Jiang, Chemical

composition and antimicrobial activities of essential oil of *Blumea megacephala*. EXCLI J. 10, 62-68 (2011).
[49] E.H. Chisowa, D.R. Hall, D.I. Farman, Chemical composition of the essential oil of *Blumea gariepina* DC. J. Essent. Oil Res. 10(3), 337-338 (1998).
[50] J.W. Mwangi, K.J. Achola, W. Lwande, A. Hassanali, R. Laurent, Constituents of the essential oil of *Blumea brevipes* (Oliv. & Hiern) willd. Flav. Fragr. J. 9(5), 233-235 (1994).
[51] N.X. Dung, D.T. Loi, D.T. Hung, P.A. Leclercq, Chemical composition of the oil of *Blumea lanceolaria* (Roxb.) Druce from Vietnam. J. Essent. Oil Res. 3(4), 285-286 (1991).
[52] R.K. Joshi, Chemical composition of *Blumea virens* roots from India. Chem. Nat. Compd. 54(3), 584-585 (2018).
[53] R.K. Joshi, Terpenoids of *Blumea oxyodonta* essential oil. Chem. Nat. Compd. 54(2), 377-379 (2018).
[54] E.J. Corey, R.B. Mitra, H. Uda, Total synthesis of d,l-caryophyllene and d,l-isocaryophyllene. J. Am. Chem. Soc. 86(3), 485-492 (1964).
[55] J. Legault, A. Pichette A, Potentiating effect of beta-caryophyllene on anticancer activity of alpha-humulene, isocaryophyllene and paclitaxel. J. Pharm. Pharmacol. 59(12), 1643-1647 (2007).
[56] M. Stranden, A.K. Borg-Karlson, H. Mustaparta, Receptor neuron discrimination of the germacrene D enantiomers in the moth helicoverpa armigera. Chem. Senses 27(2), 143-152 (2002).
[57] M.A. Birkett, S. Al Abassi, T. Krober, K. Chamberlain, A.M. Hooper, P.M. Guerin, J. Pettersson, J.A. Pickett, R. Slade, L.J. Wadhams, Antiectoparasitic activity of the gum resin, gum haggar, from the East African plant, *Commiphora holtziana*. Phytochemistry 69(8), 1710-1715 (2008).
[58] N.F. Leite-Sampaio, C. Gondim, R.A.A. Martins, A. Siyadatpanah, R. Norouzi, B. Kim, C.E. Sobral-Souza, G.E.C. Gondim, J. Ribeiro-Filho, H.D.M. Coutinho, Potentiation of the activity of antibiotics against ATCC and MDR bacterial strains with (+)-α-pinene and (–)-borneol. Biomed. Res. Int. 8217380 (2022).
[59] S.E. Santos, F. Ribeiro, P.M.N. Menezes, L.A.M. Duarte-Filho, J.S.S. Quintans, L.J. Quintans-Junior, F.S. Silva, L.A.A. Ribeiro, New insights on relaxant effects of (–)-borneol monoterpene in rat aortic rings. Fundam. Clin. Pharmacol. 33, 148-158 (2019).
[60] N.A. Ali, F.S. Sharopov, M. Alhaj, G.M. Hill, A. Porzel, N. Arnold, W.N. Setzer, J. Schmidt, L. Wessjohann, Chemical composition and biological activity of essential oil from *Pulicaria undulata* from Yemen. Nat. Prod. Commun. 7, 257-260 (2012).
[61] N.M. Shakri, W.M.N.H.W. Salleh, N.A.M. Ali, Chemical composition and biological activities of the essential oils of genus *Xylopia* L. (Annonaceae) - a review. Riv. Ital. Sostanze Grasse 97(4), 25-34 (2020).
[62] A.K. Pandey, M. Mohan, P. Singh, U.T. Palni, N.N. Tripathi, Chemical composition, antibacterial and antioxidant activity of essential oil of *Eupatorium adenophorum* Spreng. from Eastern Uttar Pradesh, India. Food Biosci. 7, 80-87 (2014).
[63] P.P. Pednekar, B.V. Vakil, R.T. Sane, A.G. Datar, Antimicrobial activity of essential oil of *Blumea eriantha* against skin pathogens. Int. J. Pharm. Pharm. Sci. 4(4), 296-299 (2012).
[64] C. He, P. Yang, L. Wang, X. Jiang, W. Zhang, X. Liang, L. Yin, Z. Yin, Y. Geng, Z. Zhong, X. Song, Y. Zou, L. Li, C. Lv, Antibacterial effect of *Blumea balsamifera* DC. essential oil against *Haemophilus parasuis*. Arch. Microbiol. 202(9), 2499-2508 (2020).
[65] U. Sakee, S. Maneerat, T.P.T. Cushnie, W. De-Eknamkul, Antimicrobial activity of *Blumea balsamifera* (Lin.) DC. extracts and essential oil. Nat. Prod. Res. 25(19), 1849-1856 (2011).
[66] A. Senthilkumar, K. Kannathasan, V. Venkatesalu, Antibacterial activity of the leaf essential oil of *Blumea mollis* (D. Don) Merr. World J. Microbiol. Biotech. 25(7), 1297-1300 (2009).

Correlation between chemical composition and topical safety and efficacy of *Alchemilla vulgaris* L. extract in emulsion vehicle

Marija Tasić-Kostov[1] ✉
Ivana Nešić [1]
VanjaTadić [2]
Dragana Pavlović [1]

[1] Faculty of Medicine
University of Niš
Department of Pharmacy
Niš, Serbia

[2] Institute for Medicinal Plant Research
"Dr Josif Pančić"
Department of pharmaceutical research
and development
Belgrade, Serbia

✉ CORRESPONDING AUTHOR:
Marija Tasić-Kostov, PhD,
Associate professor
Department of Pharmacy,
University of Niš-Faculty of Medicine
81 Blvd. Dr Zoran Djindjić,
18000 Niš, Serbia
Tel: (+38163) 1045488
Fax: (+38118) 4238770
e-mail: marijatk@medfak.ni.ac.rs

Received: February 2, 2023
Accepted: December 14, 2023

Medicinal plant extracts represent one of the largest categories of topical actives in the marketplace today due to the increasing consumer demand for natural products. Regarding the traditional application of Lady's mantle (*Alchemilla vulgaris* L., Rosaceae) in dermatological disorders, the aim of this study was to investigate its skin safety and efficacy as anti-irritant topical active incorporated into emulsion vehicle, and possible connection to the chemical composition of the extract. The total phenols, tannins and flavonoids content of leaf ethanol extract were determined. HPLC analysis revealed the significant presence of ellagic acid and its derivatives and quercetin derivatives. Further, we investigated the effects of creams formulated with chemically characterised *A. vulgaris* extract containing the natural alkyl polyglucoside (APG) emulsifier on skin. The safety and efficacy of creams was assessed estimating their antioxidant activity *in vitro* and *in vivo* safety. Then, *in vivo* sodium lauryl sulfate test was applied on artificially irritated skin of human volunteers. Lady's mantle extract *per se* showed satisfying lipid peroxidation inhibition potential. Creams based on APG emulsifiers with investigated extract showed significant anti-irritant effect in an *in vivo* double blind randomised study, and safety, suggesting that it could be used in anti-irritant topical phytopharmaceutical emulsions which protect skin against damage caused by free radicals and reactive oxygen species.
Keywords: Lady's mantle ethanol extract; quercetin derivatives; ellagic acid derivatives; antioxidant potential; safety and efficacy of topical phytopreparation.

INTRODUCTION

Due to the rising demands of modern consumers for natural products, plant extracts have become one of the largest categories of cosmetic actives found in the marketplace today [1]. At the same time, one of the most important goals of modern dermatology is to improve and strengthen prevention, diagnosis, and treatment of diseases, which could be accomplished by taking advantage of specific properties of the substances originated from plants. Aside consumers' requests, the popularity of phytopreparations for topical use (both cosmetics and dermopharmaceutics) probably lies in the fact that naturally occurring mixtures of active compounds in plants might be more effective than individual molecules and manufactured combinations of synthetic products [2, 3].

In recent years, an increasing scientific attention has been given to investigations of antioxidant activity of plant extracts [4], which might be closely related to their polyphenol content. Apart of the scavenging UV-induced radicals and inhibiting propagation of lipid peroxidative chain reactions, flavonoids, as a class of polyphenol compounds, might provide the protective effect against UV radiation by acting as strong UV-absorbing screens [5]. On the other hand, skin aging (both intrinsic and extrinsic), and some systemic diseases result in dry skin characterised by the inflammation and loss of elasticity.

These manifestations might be closely connected to oxidative stress and a skin-barrier defect which leads to a consequent loss of water from the *stratum corneum* (SC) [5, 6]. Treatment with moisturising emulsions (creams) and antioxidant agents is normally of great benefit to dry skin, helping to achieve a healthy SC which then is capable to form an effective permeability barrier, restricting the water loss from the body and blocking the penetration of harmful irritants and allergens [6, 7].

It has been widely accepted that topically applied antioxidants may be effective in the treatment of photo-aged skin [7]. However, in contrast to extensive scientific data on plant antioxidants' activity *per se*, there are only few studies dealing with the efficacy of these actives after they have been incorporated into emulsion vehicles. Most of these studies pointed out some problematic issues, such as potential loss of antioxidant activity which seemed to be dependent on the composition of the vehicle [8].

Skin irritation is one of the most common adverse effects of topicals [9]. Regulation (EC) 1223/2009 on cosmetics, which fully came into force in July 2013, requires evidence of safety profile of a cosmetic product when applied under normal and foreseeable conditions of use, as one of the main demands for such product to be placed onto the market. Hence, the importance of investigations of the potential for causing local (skin and eyes) adverse effects is emphasised [10, 11]. In the case of dermopharmaceutics, tests performed to predict skin irritation are regularly included during the new drug development and application process [12]. It is important to stress that mildness has become one of the most important features claimed for topical products [13]. Earlier, skin irritation profile of cosmetic actives, including plant extracts, and final products was assessed routinely and reliably using *in vivo* animal testing. But the ethical concerns prohibited the sale on the EU market of any cosmetic product that has been tested on animals or using any alternative methods other than validated ones [9]. *In vivo* tests on humans were used in our study [12].

Common Lady's mantle, *Alchemilla vulgaris* L., Rosaceae*,* is a perennial herbaceous plant widespread throughout Europe [14]. It is known for its astringent and anti-inflammatory properties and traditionally it has been used to treat ulcers, eczema, and skin rashes [15]. Published data indicated that the aerial part of the *A. vulgaris* contained the complex of diverse biologically active substances, dominated by phenolic substances (flavonoids, coumarols and polyphenols, including phenylcarbonic acids, up to 9.6%), depending on the developmental phase and site of the plant collection [15].

Taking into account the abovementioned challenges, the current study had the following objectives: a) chemical characterisation of *A. vulgaris* extract used in the investigation employing HPLC method, giving the insight in its total phenols, flavonoids and tannins content and b) *in vivo* assessment of skin irritation profile and *in vivo/in vitro* assessment of efficacy of the extract *per se* and after incorporation into emulsion vehicle based on natural alkyl polyglucoside (APG) emulsifier, as a starting point in evaluation of the potential protective effect of this extract in oxidative stress-mediated skin disorders [16]. To investigate *A. vulgaris* extract for cosmetic applications, we have followed the demands of new Regulation (EC) 1223/2009 on cosmetics. The efficacy of *A. vulgaris* extract in maintaining the normal skin properties was assessed as *in vivo* effects of O/W emulsions containing investigated extracts in two concentrations, 1 and 2% on the sodium lauryl sulphate (SLS)-irritated human skin [17, 18]. The potential of the investigated extract to affect the dry skin, i.e. to reverse skin barrier function and regulate disturbed skin's pH alongside with its hydration skin effects were accomplished by means of non-invasive biophysical measurements assessing the following parameters: transepidermal water loss (TEWL), pH of the skin and electrical capacitance (EC) as a measure of skin hydration level. We aimed to estimate whether the investigated plant extract in emulsion vehicles could be promoted as a safe and effective antioxidant topical active, taking into account the aspects of the skin safety of the final formulation and the influence of the emulsion vehicle on its topical activity, with regard to investigated chemical composition of the extract.

EXPERIMENTAL PART

GENERAL

Sodium bicarbonate (analytical grade), DPPH (1,1'-diphenyl-2-picrylhydrazyl) (analytical grade), trolox (analytical grade) and Folin-Ciocalteu reagent were purchased from Sigma Aldrich, Saint Luis, MO, USA. Analytical grade reagents 2,6-di-*tert*-butyl-4-methylphenol (BHT), absolute ethanol (96%, v/v) were purchased from Merck (Darmstadt, Germany). Acetonitrile (MeCN), water and methanol were of HPLC grade, and they were purchased from Merck (Darmstadt, Germany). Reference HPLC standards isoquercitrin, rutin, hyperoside, apigenin-7-*O*-glucoside, quercetin and ellagic acid were purchased from Sigma Aldrich, St. Louis, MO, or from Extrasynthese (Genay, France). Their purity was declared as >98%, based on the manufacturer's internal high-precision HPLC method. Linoleic acid, ammonium thiocyanate, Tween 20, SLS and phosphate buffer were purchased from Sigma-Aldrich.

Preparation of A. vulgaris extract

The extraction was performed in the Soxhlet apparatus, until the dried and powdered plant drug of *A. vulgaris folium* was completely exhausted, using 70 vol% of ethanol. Extraction was performed at the temperature of 60°C, with the continuous work of circulatory and uniform flow of the solvent. The extract

was put in the receiving container and the solvent was evaporated at the temperature of 65°C, under vacuum, to a volume giving the extract with dry residue with the value of more than 60%.

DETERMINATION OF TOTAL PHENOLS CONTENT (TPC)

The TPC was determined by the Folin-Ciocalteu method [29]. One hundred microliters of MeOH solution of the investigated sample (prepared concentration of stock solution was 2.5 mg/mL) was mixed with 0.75 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22°C for 5 min; 0.75 mL of sodium bicarbonate (60 g/L) solution was added to mixture. After 90 min at 22°C, absorbance was measured at 725 nm. Gallic acid (0-100mg/L) was used for calibration of a standard curve. The calibration curve showed the linear regression at $r > 0.99$, and the results are expressed as milligrams of gallic acid equivalents per gram of the extract dry weight (mg GAE/g DW). Triplicate measurements were taken, and data were presented as mean ± standard deviation (SD).

TOTAL FLAVONOIDS CONTENT (TFC)

The percentage of TFC was estimated using the method described in DAB 10 [30]. Briefly, the sample (502.7mg) was extracted with acetone/HCl under a reflux condenser; the absorbance of $AlCl_3$ complex of flavonoid fraction extracted by ethyl acetate was measured by UV-VIS Spectrophotometer HP 8453 at 425 nm. The content of flavonoid, expressed as hyperoside percentage, presented the mean ± standard deviation of three determinations.

TANNINS CONTENT (TC)

The percentage of TC was achieved using the method described in the European Pharmacopoeia, Ph. Eur. 9.0 [31]. Shortly, decoctions, prepared from the investigated samples, were treated with phosphomolybdotungstic reagent in alkaline medium after and without treatment with hide powder. The absorbance was measured by UV-VIS Spectrophotometer HP 8453 (Agilent Technologies, USA), at λ_{max} 760 nm. From the difference in absorbance of total polyphenols and polyphenols not adsorbed by hide powder, the percentage content of tannins expressed as pyrogallol (%, w/w), were calculated from the expression:

$$\frac{62.5\,(A^1 - A_2) \times m_2}{A_3 \times m_1}$$

where m_1 represents mass of the sample to be examined, in grams; and m_2 is mass of pyrogallol, in grams. The results represent the mean ± SD of three determinations.

HPLC FINGERPRINT

HPLC fingerprint of the investigated sample was achieved by HPLC (Agilent Technologies 1200). Detection was performed using a Diode Array Detector (DAD), and the chromatograms were recorded at λ = 360 nm (isoquercitrin, rutin, hyperoside, apigenin-7-*O*-glucoside, quercetin and ellagic acid). HPLC separation of components was achieved using a LiChrospher 100 RP 18e (5 μm), 250 × 4 mm i.d. column, with a flow rate of 0.8 mL/min and mobile phase, A [1% H_3PO_4 (w/w) in water)], B (MeCN), elution, combination of gradient mode: 89-75% A, 0-35 min; 75-60% A, 35-55 min; 60-35% A, 55-60 min; 35-0% A, 60-70 min). A portion of the sample solution (15.9 mg/mL EtOH, DW = 63.2%), previously prepared as described, was filtered through 0.2 μm PTFE filters (Fisher, Pittsburgh, PA) prior to HPLC analysis. The injected volume was 4 μL for all prepared solutions of extract and the standards. Standard solutions for the determination of polyphenolic compounds were prepared in EtOH, with the concentrations for isoquercitrin 0.22 mg/mL, hyperoside 0.25 mg/mL, rutin 0.45 mg/mL, apigenin-7-*O*-glucoside 0.18 mg/mL, quercetin 0.1 mg/mL and ellagic acid 0.1 mg/mL. The identification was carried out thanks to retention time and spectra matching. Once spectra matching succeeded, results were confirmed by means of the so-called peak purity test, meaning that each peak was tested for purity by a three-point purity test and for the similarity by a library search comparing the peak spectrum to that of the standards. High similarity index and a common retention time with the standard were considered a positive identification. Those peaks not fulfilling the requirements were not quantified; a similar UV/VIS absorption spectrum but different retention time was considered as a partial identification (derivative of the phenolic compound with the similar absorption spectrum). Under the conditions employed in this study, the relative standard deviation for the retention times in three repetitive runs was in the range of 0.18 - 1.79%. Quantification was performed by external calibration with corresponding standards. The results represent the mean ± SD of three determinations.

PREPARATION OF EMULSION SAMPLES

Emulsion samples based on C14-22 Alcohols & C12-20 Alkyl Glucoside (Montanov L®, Seppic, France) as an emulsifier in a concentration of 8% (w/w) and Cocoglucoside & Coconut Alcohol (Montanov S®, Seppic, France) as a coemulsifier in a concentration of 2% (w/w). Emulsion samples were labelled as P-placebo (cream without extract), C1-cream containing 1% of *A. vulgaris* extract and C2- cream containing 2% of *A. vulgaris* extract. The samples also contained 18.5% (w/w) of caprylic-capric triglycerides and 1.5% of cetearyl alcohol as an oil phase, purified water, glycerol as a humectant in the concentration of 5% (w/w). Samples were adequately preserved using Dekaben C (Jan Dekker International, Netherlands) preservative blend.

The samples were manufactured in a manner that the

oil phase (which was heated to 70°C) was added to the water phase (75°C) and mixed with the laboratory stirrer (stirrer RW16 basic, IKA®WERKE, Germany) at 800 rpm for 3 min. Then, the mixing continued at a lower speed - 500 rpm for 3 min. During this process the temperature was constantly maintained at 70°C. After that, samples were mixed at 500 rpm for 1 min and at 400 rpm until they were cooled down, at room temperature.

A. vulgaris extract was mixed with glycerine and the mixture was incorporated into the cooled emulsions. Then, the samples were well homogenised and allowed to equilibrate for 24 h prior to use in the study.

DETERMINATION OF ANTIOXIDANT CAPACITY OF A. VULGARIS EXTRACT PER SE AND IN EMULSION SAMPLES

The antioxidant activity was estimated by determination of the inhibitory activity towards lipid peroxidation using the thiocyanate method [32]. The study was carried out with the stock solution (20 mg/ml) of the extract. The cream formulations containing 1.0 and 2.0% of *A. vulgaris* extract were first diluted 1:3 with the extraction solution (Tween 20/water 1:5, w/w) and mixed for 15 min at 400 rpm using the laboratory stirrer, at the temperature of 50°C. The obtained samples were used as stock solutions for further investigation and were kept for 20 min prior to the measurement of their antioxidant activity [25].

A 0.5 mL aliquot of stock solution was added to linoleic acid emulsion (2.5 mL, 40 mM, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2804 g linoleic acid, 0.2804 g Tween 20 as emulsifier in 50mL 40mM phosphate buffer and the mixture was then homogenised. The final volume was adjusted to 5mL with 40mM phosphate buffer, pH 7.0.

After incubation at 37°C in the dark for 72 h, a 0.1 mL aliquot of the reaction solution was mixed with 4.7 mL of ethanol (75%), 0.1 mL $FeCl_2$ (20 mM), and 0.1 mL ammonium thiocyanate (30%). The absorbance of this mixture was measured at 500 nm, after it was stirred for 3 min. BHT was used as a reference compound. To eliminate the solvent effect, the control sample, which contained the same amount of solvent added to the linoleic acid emulsion [8].

The inhibition percent of linoleic acid peroxidation was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\Delta Ac - \Delta As}{\Delta Ac} \times 100$$

where ΔAc represented the difference between absorbance values of the control before and after incubation, and ΔAs represented the difference between absorbance values of the sample before and after incubation.

IN VIVO SKIN PERFORMANCE

Two different *in vivo* studies were conducted – safety and efficacy study. *In vivo* skin effects of the samples P, C1 and C2 were assessed via three skin parameters - TEWL, pH and *stratum corneum* hydration (SCH) in both studies. SCH was measured as EC (electrical capacitance).

Since the increase in TEWL is observed after the application of skin irritants, TEWL measurements are often used to support the cosmetic claims of product mildness [27]. In the safety study, parameters were measured prior to (baseline values on the first day of the experiment) and 60 min upon cessation of 24h occlusive treatment (second day of the experiment). As many as 21 healthy female volunteers (28.4±5.9 years) were recruited. The flexor side of their left forearm was treated with the placebo (P sample) while the right forearm was treated with the C1 and C2 using a precisely delineated and marked cardboard ruler (with two empty spaces in the form of rectangles, 16 cm^2 each). Two additional sites were left as non-treated control under occlusion (UCO) on the right and without occlusion (UC) on the left forearm. Samples were applied in quantities of 0.016 g/cm^2, spread vigorously with a rubber glove, and immediately covered with Parafilm® and then with cotton adhesive tapes. All parameters were measured according to the published guidelines and documents [27]

To estimate the efficacy of the same samples in terms of their effects on biophysical parameters on artificially dried skin (i.e. on skin pretreated with SLS), an additional group of 16 healthy volunteers (24.2±1.6 years) was recruited. SLS in a closed patch test is frequently used for the experimental induction of skin inflammation and dryness which can be evaluated using non-invasive biophysical measurements [17]. Namely, 75µL of the irritant (aqueous SLS, 12%, purity of SLS >99%, Merck, Darmstadt, Germany) was applied under the patch occlusion for 6h on four places of both forearms. pplication was performed using filter paper (9 cm^2), covered with Parafilm® and then fixed with cotton adhesive Sensifix® tapes. Baseline values were taken prior to the sample application and the outcome was removed 24 hours after the occlusion.

Then, 24 hours after an exposure to SLS, the volunteers started to treat exposed skin sites with test samples to study and compare the modification of provoked dryness by different samples. The samples were marked with differently coloured labels, and the volunteers were given clear instructions regarding the amount, type of samples and frequency of applications (morning and evening). One site on each arm in both groups was left as an untreated control, whereby it was SLS-treated on the left forearm (UCO), and without treatment on the right (UC).

The measurements of EC, TEWL and pH were performed before and 24h after SLS irritation, as well as after 2 and 5 days of the treatment.

In vivo measurements were performed in accordance with the Declaration of Helsinki, and the volunteers signed a written consent. They were thoroughly in-

formed of the study and instructed not to use any skin cleansing or skin care products on the test sites the week prior to the study as well as throughout the experiment. The study was approved by the local Ethical Committee on Human Research (No 12-6316-2/7 from 16.6.2016.). All subjects had healthy skin and no known allergy to any ingredient of the samples. Before any measurements were taken, the subjects were asked to acclimatise for 30 min under controlled conditions (21±1°C and 50±5% RH). TEWL was measured using Tewameter®TM 210, pH using pHmeter®900 and EC by means of Corneometer®CM 825; all probes are part of Multi Probe Adapter MPA®9 (Courage & Khazaka Electronic GmbH, Germany).

STATISTICAL ANALYSIS

All data were presented as means +/- standard error of the means (SEM). When evaluating the safety of the samples, parameters (EC, TEWL, pH) were expressed as absolute changes to baseline (Δ values) second versus first day. *In vivo* effects of the samples were compared mutually and related to untreated controls, under and without occlusion (UC and UCO), using the one-way ANOVA, followed by Tukey's *t*-test, where appropriate. The same test was used for comparison of the antioxidant activities (measured by both methods) of the emulsion samples.

When evaluating the efficacy of the samples on pre-irritated skin, the values of measured parameters after SLS irritation, after 2 and 5 days of application were compared to appropriate baseline values using a paired sample t-test. Data obtained from skin sites treated with different gels were compared mutually, to both untreated controls as well as to the placebo sample using t-test for unpaired data.

The differences were accepted as statistically significant at $p < 0.05$. Statistical analysis was performed with commercial statistical software SPSS for Windows 17.0.

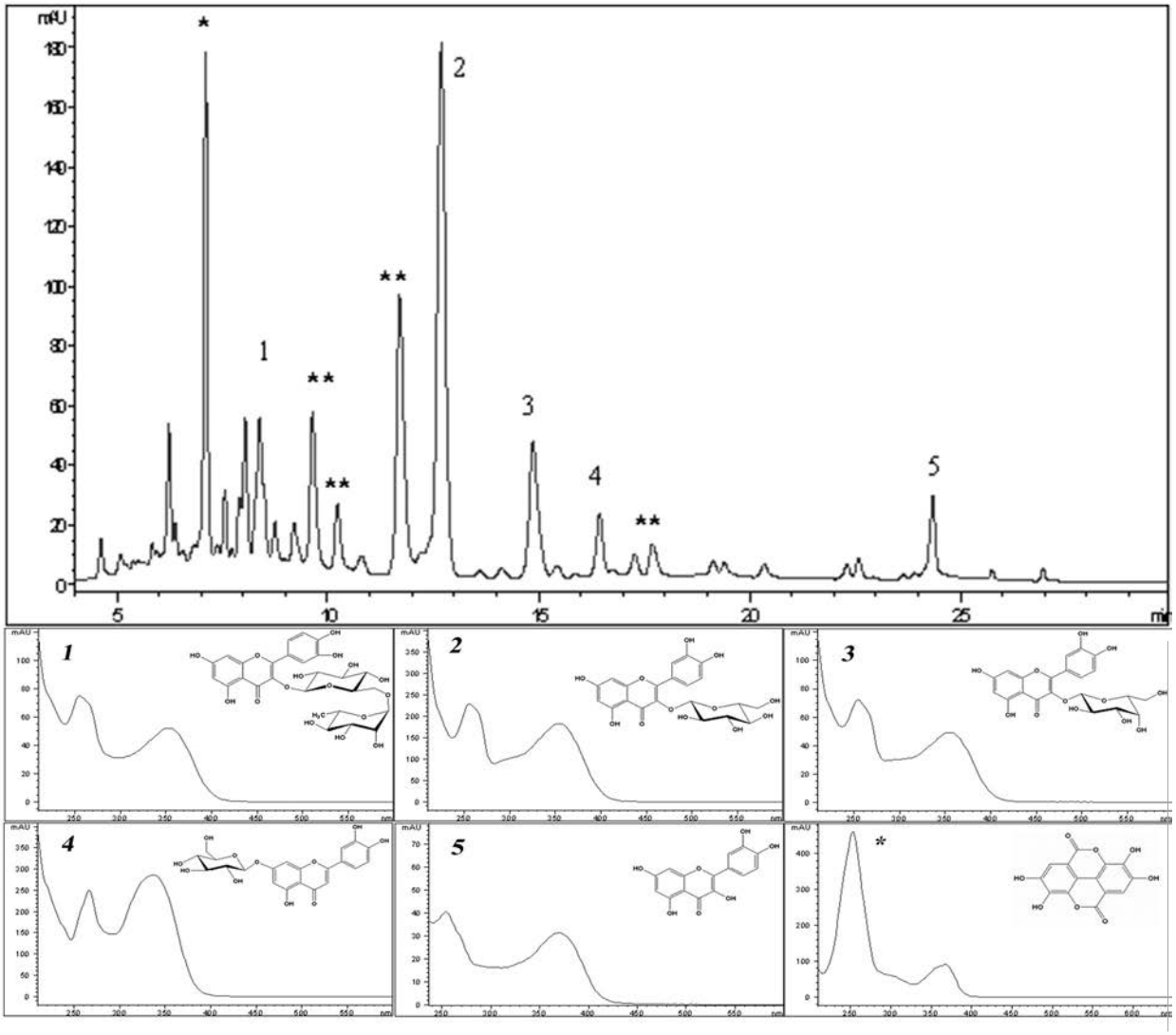


Figure 1 - Chemical profile obtained by HPLC analysis with the identified flavonoids in the lady's mantle (*Alchemilla vulgaris*) investigated extract: 1. rutin; 2. isoquercitrin; 3. hyperoside; 4. apigenin-7-*O*-glucoside; 5. quercetin; * ellagic acid derivatives

RESULTS AND DISCUSSION

HPLC profile of the investigated extract of A.vulgaris is presented in Table I and Figure 1. The following flavonoid compounds were identified: rutin, isoquercitrin, hyperoside, apigenin-7-O-glucoside, quercetin and ellagic acid and its derivatives.

Polyphenolic compounds are an important group of plant bioactives which exhibit various biological activities, including antioxidative and anti-inflammatory [19-21]. The presence of a relatively high content of ellagic acid and its derivatives, polyphenol compounds in the investigated extract of A.vulgaris, (Figure 1) might contribute to the in vivo effects observed in this study. Moreover, the presence of quercetin is of particular importance regarding that flavonoid with its analogue, isoquercitrin, are among the most potent antioxidants [19]. Moreover, it was shown that topical formulations containing quercetin are highly effective in the protection from oxidative stress-induced skin damages [20, 21].

Namely, ellagic acid and its derivatives have recently received attention as the agents that may have potential bioactivities preventing chronic diseases. Bae et al. [22] examined the effects of ellagic acid on collagen breakdown and inflammatory responses in UV (ultraviolet)-B irradiated human skin cells and hairless mice and revealed its photoprotective effects on the skin wrinkle formation. Moreover, ellagic acid may be effective against inflammation, may have a prolonged onset and duration of action, and may interact with cyclooxygenase inhibitors.

The topical application of plant extracts with a strong antioxidant activity could protect the skin against the toxic effects of reactive oxygen species (ROS), chemically reactive species containing oxygen [23]. Regarding that polyunsaturated fatty acids (PUFA) are accessible to peroxidation; it is one of the most investigated consequences of ROS action on cell membrane structure and function [24]. In fact, inhibition of lipid peroxidation seems to be the method of choice to determine the antioxidant capacity of topically applied preparations.

The results obtained for the evaluation of antioxidant activity of the investigated extract and creams C1 (cream containing 1% of *Alchemilla vulgaris* extract) and C2 (cream containing 2% of *Alchemilla vulgaris* extract) were given in Table II. The capacity of the *A. vulgaris* extract to inhibit lipid peroxidation was notable; it remained almost unchanged after its incorporation in APG-based vehicle in both concentrations (Table II), showing that a satisfying efficacy in oxidative stress-mediated skin disorders could be reached at 1% of extract in vehicle, without need for a further increase of concentration. That could be of great benefit, having in mind that even the vitamin E, one of the most effective and frequently used antioxidants active in commercial formulations, often lose its activity after incorporation into an emulsion carrier (cream) [25].

It was previously shown that the emulsions and creams with antioxidants reached a low degree of inhibition of the oxidative reaction in the experimental system if they had not been correctly solubilised in the mixture. Proper homogenisation with an adequate extraction solution is necessary for measuring the antioxidant capacity of emulsion samples [8, 25]. In this study, we used the extraction solution (Tween-20/ H_2O 1:5, w/w) to provide better solubilisation of the actives.

So, the following could be assumed from our antioxidant activity evaluation: cetearyl glucoside and cetearyl alcohol-based emulsion could be an eligible vehicle for *A.vulgaris* in order to reach its antioxidant activity; but also the methodologies in this study (used on *A.vulgaris* extract) are appropriate to analyse the antioxidant activity of herbal extracts when incorporated in the emulsion vehicle. Naturally, interferences with the colloidal structure of the vehicle might occur, but they affected neither the antioxidant activity of extract nor its determination.

Regarding the *In vivo* skin performance study, all participants reported their strict compliance with the instructions given initially.

Irritants have been indicated to induce the release of ROS to the skin even at non-cytotoxic concentrations

Table I - The chemical characterization of the investigated extract of *A.vulgaris*

Extract *Alchemilla vulgaris*		
Total flavonoids (expressed as hyperoside percentage**)**		4.1
Total phenol content (mg GAE/g DW)		314.2
Tannins (%)		4.12
DW (dry weight, %)		63.2
Identified compounds by HPLC (their abundance expressed in mg/g DW of the investigated extract)		
1	Rutin	4.8
2	Isoquercitrin	17.8
3	Hyperoside	4.1
4	Apigenin-7-*O*-glucoside	3.4
5	Quercetin	1.4
*	Ellagic acid an dits derivatives expressed as ellagic acid	21.1

Table II - Antioxidant activities of *Alchemilla vulgaris* L. extract and creams C1 and C2; results are given as means ± S.D.[a]

Sample	Antioxidant activity ±SD (%)
Alchemilla vulgaris L. extract	60,0 ± 3,27
C1	58,82 ± 0,37
C2	58,83 ± 0,50

[26], so it is extremely important to establish the safety profile of formulations intended for an application on skin, particularly if it is already damaged. The *in vivo* skin safety profile was investigated in this study; absolute changes of the *in vivo* measured parameters (TEWL, pH and EC) after 24h occlusion related to the baseline values for the investigated samples, as well as for both controls, were presented in Figure 2.

Creams containing *A. vulgaris* extract 1% and 2% investigated in this study are not expected to affect the skin barrier and cause an immediate irritation of the skin. That is, the application of all test samples (Figure 2) increased the TEWL values after 24h, but no statistically significant change was found. The increase of TEWL, which normally indicate the skin barrier damage, could be here attributed to the actual occlusion [27]. This could support the claim that creams with *Alchemilla vulgaris* L. extract are not expected to affect the skin barrier and cause an immediate irritation of the skin.

Regarding the efficacy investigations, the in vivo effects of the creams on the human skin pretreated with SLS were investigated. The results are shown in Table III.

Bearing in mind that oxidative stress can alter the cellular hydration state [28], it could be a great additional benefit for antioxidant cosmetics to exhibit a satisfying skin moisturising potential. The results obtained for skin hydration (EC) of the investigated samples in the safety study (Figure 2) revealed a good correlation with the EC values obtained for the treatment with investigated samples of previously SLS dried skin (Table III). Skin treated with test sample C2 exhibited a significant increase in EC after 24h in comparison with both controls. Since the increase in this parameter was not significant for skin treated with a sample containing 1% of the extract (sample C1), it could be presumed that the *A. vulgaris* extract beneficially affects the skin moisturising potential of creams containing it in 2%.

TEWL, as a measure of the level of damage to the skin barrier, showed a statistically significant increase at all skin sites treated with SLS. Then, there was a decrease in TEWL after two days on all treated skin sites regardless the treatment (P, C1 or C2) while on

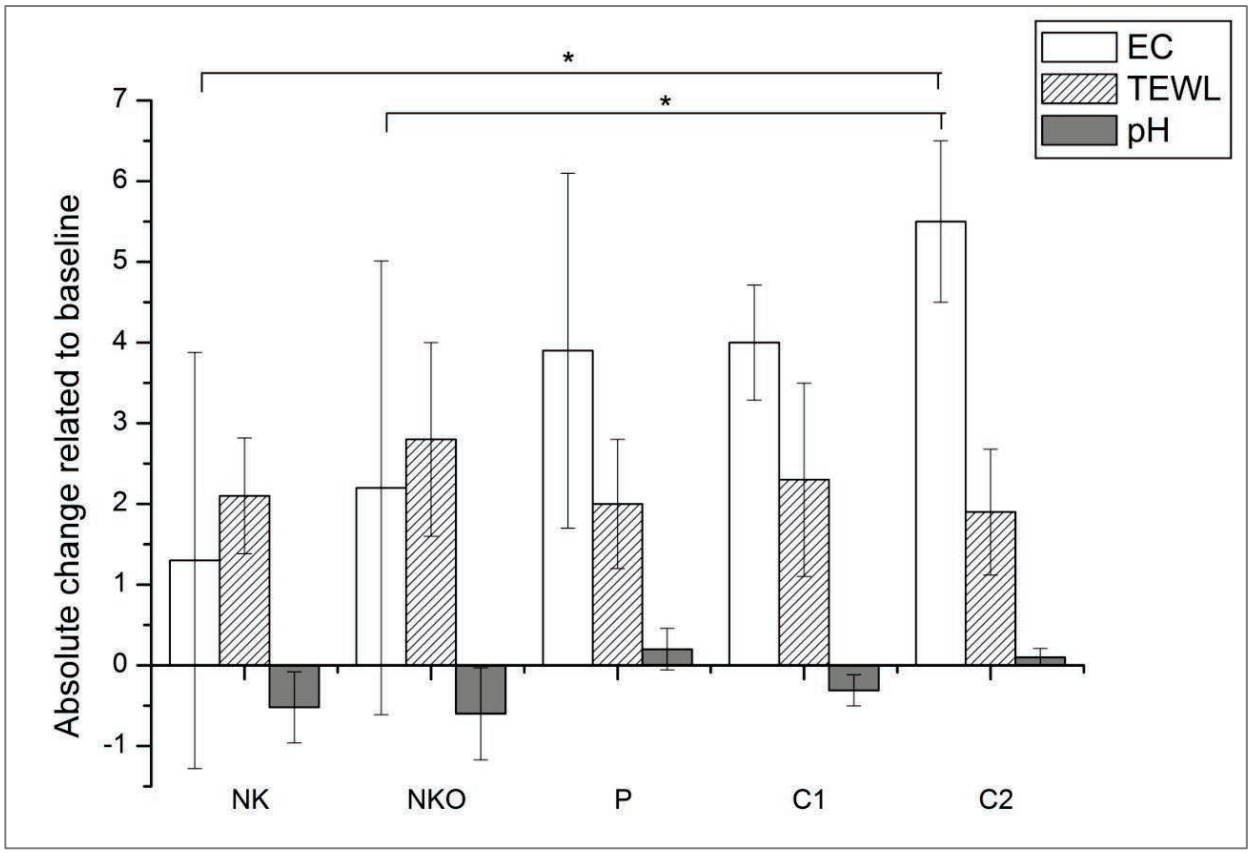


Figure 2 - The influence of the investigated samples on *in vivo* measured skin parameters: EC (as a measure of SCH), TEWL and pH. The effects of different samples were compared mutually and related to baseline as well as to UC and UCO. Significant differences were marked with *p<0.05.

Table III - The influence of the irritation *per se* and investigated samples after irritation on biophysical skin parameters: TEWL, pH and EC

Sample	Baseline(a)	Upon SLS irritation(b)	Upon 2 days treatment(c)	Upon 5 days treatment(d)	p-values (repeated measures ANOVA)
TEWL					
Control (UCO)	7,5±1,5	11,5±3,2^{UC}	10,7±3,2^{UCO}	7,5±1,8^{UC,P,C1,C2}	0,05*;a:b;a:c;b:d;c:d
Control (UC)	6,7±2,5	7,0±1,3^{UCO,C2}	7,2±1,7^{UC}	6,9±2,3	0,841
P	6,7±2,0	9,7±2,9	8,5±2,5	6,8±2,6	0,005*;a:b;b:d
C1	6,4±1,9	10,2±3,1^{UC}	7,8±2,0	6,3±2,5	0,005*;a:b;b:c;b:d
C2	6,6±1,7	9,7±2,8	8,7±2,9	6,7±1,5	0,01*;a:b; b:d
p-values (one-way ANOVA)	0,679	0,004#	0,020#	0,676	-
pH					
Control (UCO)	5,0±0,3	5,3±0,4	5,1±0,3	5,1±0,4	0,404
Control (UC)	4,9±0,4	5,2±0,4	5,1±0,2	5,3±0,6	0,276
P	5,0±0,3	5,3±0,4	5,2±0,3	5,4±0,5	0,084
C1	5,1±0,3	5,4±0,4	5,2±0,2	5,1±0,5	0,03*;a:b
C2	5,1±0,3	5,3±0,4	5,3±0,3	5,4±0,4	0,290
p-values (one-way ANOVA)	0,790	0,777	0,088	0,779	-
EC					
Control (UCO)	29,1±5,1	30,7±7,1	32,1±6,7^{1,3}	32,6±5,$^{P, C1,C2}$	<0,025*; a:d
Control (UC)	28,1±5,6	28,2±5,0	29,9±5,8^{C1}	31,2±6,5$^{P, C1,C2}$	0,054
P	27,5±6,6	26,8±6,9	42,4±9, ^{UC,UCO}	42,6±5,2 ^{UC,UCO}	<0,001*; a:c; a:d;b:c;b:d
C1	26,6±7,9	23,4±6,4	36,6±8,3	41,6±6,2 ^{UC,UCO}	<0,001*; a:c; a:d; b:c;b:d
C2	27,1±7,3	29,6±9,1	41,9±7,8	44,8±6,1 ^{UC,UCO}	<0,001*; a:c; a:d; b:c;b:d
p-values (one-way ANOVA)	0,943	0,103	0,000#	0,000#	-

the skin site that was artificially dried and irritated, but not treated afterwards (UCO), TEWL returned to baseline values only after five days. This indicates that all investigated samples could be effective in the repair of the interrupted skin barrier function which might be essential in achieving properly hydrated skin.

The values of EC measured on the skin before and after the artificially provoked dryness, as well as after the treatment with investigated samples were expressed as absolute changes to the baseline and shown in Table III. It can be concluded that, although SLS treatment significantly interrupted the skin barrier function (which was expressed as TEWL increase after irritation), there was no significant decrease in the value of the electrical capacitance as a reflection of the state of hydration of SLS dried skin. However, all investigated samples significantly increased the degree of hydration of the skin during treatment, and in particular C2 cream which contained 2% of A. vulgaris extract.

The results of the pH measurements of the skin were as expected. The initial upward trend in pH values after irritation was followed by a return to baseline values after two days of treatment by any of the samples. Return to pre-irritation, but slower, was observed in the untreated skin sites, which could be attributed to a certain buffering capacity of the skin itself.

Our study showed that *A. vulgaris* extract in topical products (both cosmetics and dermopharmaceutics) could play an ameliorative role in improving the overall health of the skin barrier. Our goal was to emphasise the fact, confirmed by this study, that topical treatment with herbal antioxidant formulations containing flavonoid compounds (with focus on ellagic acid), alongside the effective moisturising treatment could be an effective way to enhance the skin's own recovery potential.

Besides showing beneficial effects on the skin, the extract provided the fulfilment of the demand for the satisfactory safety profile i.e. succeeded in meeting the safety requirements of the Regulation (EC) 1223/2009 on cosmetics what was of the particular importance. Therefore, the desirable effect of the traditional application of *A. vulgaris* in the treatment of skin disorders alongside its safety was confirmed by this work. The results indicated that *A. vulgaris* might be a candidate as a natural and safe topical active in phytopharmaceuticals and cosmetics as its extracts in a proper natural emulsion vehicle possess a strong antioxidant, skin hydrating and anti-irritant potential.

Acknowledgments

This research was supported by the Ministry of Science, Technological
Development and Innovation of the Republic of Serbia (Grant No. 451-03-65/2024-03/200113 and 451-03-66/2024-03/200113) and the Faculty of Medicine University of Niš Internal Scientific Project No. 15.

Conflict of Interest

The authors have declared that there is no conflict of interest.

References

[1] Stallings, A.F.; Lupo, M.P. Practical uses of botanicals in skin care. J. Clin. Aesthet. Dermatol. 2009, 2(1), 36-40.

[2] Zimmermann, G.R..; Lehár, J.; Keith, C.T. Multi-target therapeutics: when the whole is greater than the sum of the parts. Drug. Discov. Today. 2007, 12, 34-42.

[3] Rathera, M.A.; Bhata, B.A.; Qurishi, M.A. Multicomponent phytotherapeutic approach gaining momentum: Is the “one drug model breaking down? Phytomedicine. 2013, 13, 1-14.

[4] Erdemoglu, N.; Turan, N.; Cakõcõ, I.; Sener, B.; Aydõn, A. Antioxidant activities of some Lamiaceae plant extracts. Phytother. Res. 2006; 20: 9–13.

[5] Bonina, F.; Lanza, M.; Montenegro, L.; Puglisi, C.; Tomaino, A.; Trombetta, D.; Castelli, F.; Saija, A. Flavonoids as potential protective agents against photo-oxidative skin damage. Int. J. Pharm. 1996, 145, 87–94.

[6] Loden M. The clinical benefit of moisturizers. JEADV 2005, 19, 672-688.

[7] Saija, A.; Tomaino, A.; Trombetta, D.; De Pasquale, A.; Uccella, N.; Barbuzzi, T.; Paolino, D.; Bonina, F. In vitro and in vivo evaluation of caffeic and ferulic acids as topical photoprotective agents. Int. J. Pharm. 2000, 199, 39–47.

[8] Tasic-Kostov, M.; Pavlovic, D.; Lukic, M.; Jaksic, I.; Arsic, I.; Savic, S. Lactobionic acid as antioxidant and moisturizing active in alkyl polyglucoside-based topical emulsions: the colloidal structure, stability and efficacy evaluation. Int. J. Cosmetic. Sci. 2012, 34 (5), 424–434.

[9] Vinardell, MP.; Mitjans, M. Alternative methods for eye and skin irritation tests: an overview. J. Pharm. Sci. 2008, 97, 46-59.

[10] Tasić-Kostov M, Reichl S, Lukić M, Jakšić I, Savić S. Two alkyl polyglucoside natural surfactants varying in the chain lenght in stabilization of lactobionic acid containing emulsions: physicochemical characterization and in vitro irritation potential assessment, Riv. Ital. Sostanze Grasse 2011, 88 (4), 256-264.

[11] Regulation (EC) No 1223/2009 of the European parliament and of the Council of 30 November 2009 on cosmetic products (recast) (Text with EEA relevance), OJEU, L 342/59, 2009.

[12] Bárány E. Human in vivo skin irritancy testing. In Dry skin and Moisturizers; Loden, M., Maibach, H.I. Eds.; CRC Press LLC, Boca Raton, 2000; pp. 243–250.

[13] Gabard, B; Chatelain, E.; Bieli, E.; Haas, S. Surfactant irritation: in vitro corneosurfametry and in vivo bioengineering. Skin Res. Technol. 2001, 7, 49–55.

[14] Smolyakova, I.M.; Andreeva, V.Y.; Kalinkina, G.I.; Avdeenko, S.N.; Shchetinin, P.P. Development of extraction techniques and standardization methods for a common lady’s mantle (Alchemilla vulgaris) extract. Pharm. Chem. J. 2012, 45(11), 675-678.

[15] Shrivastava, R.; Cucuat, N.; John, GW. Effects of Alchemilla vulgaris and glycerine on epithelial and myofibroblast cell growth and cutaneous lesion healing in rats. Phytother. Res. 2007, 21, 369–373.

[16] Fitzmaurice, S.D.; Sivamani, R.; Isseroff, R. Antioxidant therapies for wound healing: a clinical guide to currently commercially available products. Skin Pharmacol Physiol. 2011, 24, 113-26.

[17] Tadić, V.; Arsić, I.; Zvezdanović, J.; Zugić, A.; Cvetković, D.; Pavkov, S. The estimation of the traditionally used yarrow (Achillea millefolium L. Asteraceae) oil extracts with anti-inflammatory potential in topical application. J. Ethnopharmacol. 2017, 199, 138-148.

[18] Löffler, H.; Happle, R. Profile of irritant patch testing with detergents: sodium lauryl sulphate, sodium laureth sulphate and alkyl polyglucoside. Contact Dermatitis 2003, 48(1), 26-32.

[19] Ahmad, N.; Abbasi, B.H.; Fazal, H. Evaluation of antioxidant activity and its association with plant development in Silybum marianum L. Ind. Crops Prod. 2013, 49, 164–168.

[20] Casagrande, R.; Georgetti S.R.; Verri, W.A.; Dorta, D.J.; dos Santos, A.C.; Fonseca, M.J.V. Protective effect of topical formulations containing quercetin against UVB-induced oxidative stress in hairless mice. J. Photochem. Photobiol B. 2006, 84 (1), 21-27.

[21] Lachowicz, S.; Oszmiański, J. Profile of Bioactive Compounds in the Morphological Parts of Wild Fallopia japonica (Houtt) and Fallopia sachalinensis (F. Schmidt) and Their Antioxidative Activity. Molecules 2019, 24, 1436.

[22] Bae, Y-I.; Choi1, J-S.; Kang1, S-W.; Lee, Y-J.; Park, J.; Kang Y-H. Dietary compound ellagic acid alleviates skin wrinkle and inflammation induced by UV-B irradiation. Exp. Dermatol. 2010, 19, e182–e190

[23] Korkina, L.G.; Mikhal’chik, E.V.; Suprun, M.V.; Pastore, S.; Dal Toso, R. Molecular mechanisms underlying wound healing and anti-inflammatory properties of naturally occurring biotechnologically produced phenylpropanoid glycosides. Cell. Mol. Biol. 2007, 53 (5), 78–83.

[24] Blokhina, O.; Virolainen, E.; Fagerstedt, K.V. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann. Bot. 2003, 91 Spec No(2),179–194.

[25] Di Mambro, V.I.; Azzolini, A.E.C.S.; Valim, Y.M.L.; Fonseca, M.J.V. Comparison of antioxidant activities of tocopherols alone and in pharmaceutical formulations. Int. J. Pharm. 2003, 262, 93-99.

[26] Mehrota, T.; Mishra, K.P.; Raman, G.; Banerjee,

G. Differential regulation of free radicals (reactive oxygen and nitrogen species) by contact allergens and irritants in human keratinocyte cell line. Toxicol. Mech. Methods 2005, 15, 343–350.
[27] Savic, S.; Weber, C.; Tamburic, S.; Savic, M.; Muller-Goymann, C.C. Topical vehicles based on natural surfactant/fatty alcohols mixed emulsifier: the influence of two polyols on the colloidal structure and in vitro/in vivo skin performance. J. Pharm. Sci. 2009, 98, 2073-2090.
[28] Krutmann, J.; Yarosh, D. Modern photoprotection of human skin. In Skin Aging; Krutmann, J., Gilchrest, B.A. Eds.; Springer-Verlag, Berlin Heidelberg, Germany, 2006; pp. 104-112.
[29] Velioglu, Y.S.; Mazza Gao, L.; Oomah, B.D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 1998, 46, 4113-4117 (1998).
[30] Weißdornblätter mit Blüten. In: Deutsches Arzneibuch, Band 2, Monographien R-Z; Amlitche Ausgabe Deutcher Apotheker Verlag. Stuttgart, Frankfurt: Govi-Verlag GmbH; 1991.
[31] European Pharmacopoeia 9.0, 2017. Council of Europe, Strasbourg Cedex, France.
[32] Manojlović, N.; Ranković, B.; Kosanić, M.; Vasiljević, P.; Stanojković, T. Chemical composition of three Parmelia lichens and antioxidant, antimicrobial and cytotoxic activities of some their major metabolites. Phytomedicine 2012, 19 (13), 1166-1172.

ANNUNCI DI RICERCA PARTNER

per progetti di ricerca e trasferimento tecnologico

Enterprise Europe Network (EEN)

131

Anno 2024
(aggiornato al 30 giugno 2024)

Progetto BOLV20221019007

Combined Processing of Legumes Using Wet, Oil-free Technology

SME from Latvia active in food processing sector has developed combined wet, oil-free roasting technology of legumes. The result of technology application is roasted legumes with an increased plant-based protein content and low moisture content in the final product. Product may be used as a replacement of animal proteins in the daily diet. Company is looking for distributors of healthy own produced products and outsourced production including from grains supplied by customer.
Dead-line for EOIs: 1 Nov 2024

Progetto TOES20231110025

Basque SME offers technology combining photonics and IoT sensors to monitor quality in food production industrial processes

A fast-growing innovative Basque startup founded to achieve more efficient and sustainable production by applying photonic solutions to industrial processes is looking for partners interested in in either adopting the technology for their quality monitoring processes or integrating it into their portfolio as an added-value feature (joint venture). A commercial agreement with technical assistance is envisaged.
Dead-line for EOIs: 09 Nov 2024

Progetto BRSE20231201006

Swedish Company - Global Leader in Dietary Supplements Manufacturing Seeks Contract Manufacturing Partnerships Worldwide

This is a leading player in the food industry, specializing in the manufacture of dietary supplements. With an extensive international presence in countries like Sweden, Norway, China, India, the Middle East, and Africa, The Swedish Company is actively expanding its reach in Europe, Asia, and Africa. The company aims to establish strategic partnerships with contract manufacturers possessing GMP and Halal certifications to enhance its its global market share
Dead-line for EOIs: 17 Nov 2024

Progetto TOES20231204004

Accelerated shelf-life studies in food products

A Spanish technological center offers accelerated shelf-life studies in food products. This method allows to predict the behaviour of the products and anticipate their evolution under the usual storage and distribution conditions. To achieve this, an estimate is made using predictive models in which the parameters that most influence its deterioration are modified, such as temperature, humidity, and light, among others.
They seek commercial agreement and/or R&D agreement.
Dead-line for EOIs: 03 Dec 2024

Progetto BOCZ20221208018

Czech innovative company and producer of vertical hydroponic growing systems for growing herbs, fruits, vegetables and microgreens is looking for distributos for international cooperation

The company, engaged in research, development and production of indoor vertical systems, technologies and elements for growing plants under artificial lighting, is looking for trade intermediaries (distributors/agents/representatives) or franchise partners to distribute, represent or offer its products on external markets.
Dead-line for EOIs: 07 Dec 2024

Progetto TOTR20240105014

Turkish Agricultural Research Institute looking for partnerships for applying under HORIZON-CL6-2024-FARM2FORK-01-2: New healthy and sustainable food products and processes project call

Turkish Agricultural Research Institute has been expertised mainly on the breeding of field crops and horticulture as well as seed production and conservation of these genetic resources, protection of these plants from stressors in soil to provide food safety and nutrition.
Dead-line for EOIs: 04 Jan 2025

Progetto RDRCO20231221024

Colombian foodtech is in search of partners to collaborate in the creation of research, development, and innovation (R&D&I) projects, as well as to identify financing opportunities in the agri-food sector.

The foodtech is an entity with over 21 years of experience in the agro-industrial sector. Seeking to contribute through a comprehensive portfolio of services that encompass research in globally relevant thematic areas, laboratory testing services, knowledge transfer activities, design and development of food products, specialized consulting, as well as the formulation and execution of research, technological development, and innovation (R&D&I) projects.
Dead-line for EOIs: 12 Jan 2025

Progetto BOGR20230113005

Greek SME producing award-winning organic extra virgin olive oil is looking for international partners under distribution services agreement

A sustainable Greek company specialised in processing organic extra virgin olive oil, is interested in finding commercial agents or distributors for its products. Koroneiki olives are considered to be one of the finest varieties in Greece, and the extra virgin olive oil made by the company from these olives is produced from their privately-owned and strictly organic orchard with official recognition for

its label in terms of its balanced and fruity taste.
This Greek company does not focus only on making profits, but actively works to deliver sustainability, food and feed safety, and fight extinction and abuse of animals.
Dead-line for EOIs: 12 Jan 2025

Progetto BOUA20230129001

Ukrainian manufacturer of natural and healthy snacks is looking for reliable partners abroad under a commercial agency agreement or a distribution agreement

Ukrainian producer of natural food products manufactures its products only from plant components - vegetables, fruits, dried fruits, nuts. The products do not undergo heat treatment, so they retain all trace elements, vitamins, antioxidants and enzymes. They do not contain sugar/preservatives and any harmful additives.
The manufacturer is now looking for new reliable commercial partners abroad.
Dead-line for EOIs: 28 Jan 2025

Progetto BOIT20240411010

An Italian producer offers its fish and food preserves under distribution services agreements

This Italian company, established in 1913, offers fish and food preserves under distribution services agreements with international partners. The production cycle is entirely carried out in Italy, starting from “round” yellowfin tunas. Thanks to its dedication to quality and its ability to combine technology and tradition, the company guarantees a premium quality product, appreciated and established in the Italian and international markets.
Dead-line for EOIs: 11 apr 2025

Progetto TOPL20240507014

Polish startup specializes in plant-based fats offering healthy, sustainable alternatives to animal fats with zero trans fats seeking for investors and business partners: producers of confectionery and sport nutrition to further develop their innovative products

The startup company, operating since November 2023, specializes in manufactures of trans-fat free edible fat from vegetable oil. The functional dietary fats are a healthy alternative to animal fats such as butter, lard, and dairy fats. In addition, they develop two innovative products as an alternative to milk fat from raw olive pomace oil: dairy-free butter and cream without palm oil.
Dead-line for EOIs: 8 May 2025

Progetto RDRRO20230526011

A Romanian research institute is looking for partners for Eurostars calls

The Romanian research institute is looking for international partners (SMEs, SME + research organization) involved in the production of food/food supplements or feed additives to form a consortium in the next Eurostars calls (September 2023, March 2024).
The main topics are the valorization of raw materials from natural renewable resources (plant peptide-rich protein hydrolysates) and identification and quantification of chemical compounds by conventional and modern methods; valorization of agri-food waste/by-products; classical and modern methods of extraction of active ingredients from yeasts and medicinal and aromatic plants with applications in the food and feed sectors.
Dead-line for EOIs: 25 May 2025

Progetto TRIT20230512014

Smart packaging solutions for food freshness monitoring: new partners are sought for tailored development and industrial scale-up

A highly scientific Italian start up and academic spin off devoted to transfer chemical sensing knowhow into smart packaging solutions for freshness monitoring of perishable foods, developed and patended several lab-tested prototypes. New partners are sought for tailored development and industrial scale-up under Commercial agreement with technical assistance or Research and development cooperation agreement.
Dead-line for EOIs: 15 May 2025

Progetto TRES20230526017

Spanish Natural ingredients company looks for new scientific evidence technologies to incorporate in its research and manufacturing processes. Collaboration under r&d , commercial with technical assistance or investment agreements is offered

A Madrid based natural ingredients innovative SME, with a well stablished product portfolio, premium brands and presence in more than 40 countries, looks for collaborations in order to incorporate new technologies (extraction, drying, packaging) and/or new products (natural origin) for its sustainable healthy food supplements category . The company, looks for researchers, entrepreneurs or innovative smes willing to collaborate under r&d, investment or technical agreement.
Dead-line for EOIs: 30 May 2025

Per ricevere ulteriori informazioni e per entrare in contatto con i soggetti titolari degli annunci si prega di inviare una mail a:
federico.agostini@mi.camcom.it
specificando il codice progetto di interesse.

Enterprise Europe Network (EEN)

È la rete nata nel 2008 per volontà della Commissione Europea con l'obiettivo di supportare l'innovazione, il trasferimento tecnologico e l'internazionalizzazione di piccole e medie imprese ed enti di ricerca.
Si avvale di oltre 600 organizzazioni presenti in 60 paesi e offre un sistema integrato di servizi gratuiti per aiutare le imprese a individuare nuovi partner commerciali, produttivi e tecnologici all'estero; per promuovere la partecipazione ai programmi Europei per la ricerca, come Horizon Europe, e per fornire gli strumenti utili per essere più competitivi sui mercati internazionali, migliorando la conoscenza dei mercati e della legislazione europea.
In Lombardia i servizi di Enterprise Europe Network sono garantiti da **Simpler** (Support Services to IMProve innovation and competitiveness of businesses in Lombardia and Emilia-Romagna), di cui Innovhub SSI è partner.

Come ti può aiutare la rete EEN?

Far crescere l'azienda e sostenere l'internazionalizzazione:
- Informazioni sulla legislazione EU
- Informazioni e assistenza sul Regolamento REACH
- Ricerca di finanziamenti a supporto delle imprese
- Supporto per l'individuazione di opportunità commerciali all'estero
- Sostegno per lo sviluppo di nuovi prodotti o processi

Sviluppare partneriati:
- Supporto alla partecipazione a brokerage event e company mission e per la conclusione di accordi di trasferimento tecnologico
- Assistenza nella ricerca partner

Implementare processi di innovazione e trasferimento tecnologico:
- Servizio di analisi delle capacità di gestione e miglioramento dell'innovazione
- Supporto al trasferimento tecnologico/open innovation
- Informazione su bandi di finanziamento e supporto alla partecipazione a programmi di ricerca
- Pre-screening delle proposte progettuali EIC Accelerator

I servizi della rete EEN sono gratuiti.

Per cercare il tuo partner in Europa, consulta il nostro database: https://een.ec.europa.eu/partners

Per maggiori informazioni contattare:
Federico Agostini
federico.agostini@mi.camcom.it

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

enterprise europe network

RECENSIONI DI LIBRI

ECOSISTEMA DOP
IL MANAGEMENT PER LE FILIERE A INDICAZIONE GEOGRAFICA

AUTORE:
RICCARDO DESERTI

I Edizione - 2024
€ 29,00 - Edagricole di New Business Media srl
ISBN: 978-88-506-5661-5
Pagine 232 - formato 17 x 24 cm
e-mail: libri.edagricole@newbusinessmedia.it
www.edagricole.it

Sotto il “nome unico” registrato opera un reticolo di iniziative economiche che possiamo identificare come "ecosistema di imprese IG". All'interno di un ciclo economico virtuoso, le IG si possono trasformare in catalizzatori dello sviluppo di un territorio. Il nesso con il territorio di origine distingue un'Indicazione Geografica da qualsiasi altro prodotto alimentare. Lo sviluppo dell'IG si fonda su tre pilastri: vincolo produttivo, dimensione di medio-lungo termine e proprietà diffusa. Le IG sono esempi di valorizzazione delle imprese, dei lavoratori coinvolti e dei territori di origine.

INDICE:
Le Indicazioni Geografiche: un'introduzione - Il modello delle filiere IG - Valutare le filiere Dop e Igp - Costruire il GI-Business Plan - Per una nuova politica del territorio: ecosistema DOP - Suggerimenti per entrare nel mondo IG - Le 10 domande per l'imprenditore IG - Prima di voltare l'ultima pagina.

L’AUTORE:
Riccardo Deserti, laureato in Scienze, ha lavorato presso la Società di studi economici Nomisma Spa di Bologna, di cui ha ricoperto la carica di Amministratore Delegato. Dal 2006 al 2012 ha lavorato presso il Ministero delle Politiche Agricole Alimentari e Forestali, ricoprendo, tra le altre, la carica di Direttore Generale della direzione dei prodotti di qualità e di Capo della Segreteria tecnica del Ministro. Dal 2012 è Direttore generale del Consorzio del Formaggio Parmigiano Reggiano. Nell’ambito del sistema di rappresentanza dei prodotti IG è Vicepresidente di Origin Italia e Presidente “oriGIn”, che riunisce oltre 600 Indicazioni Geografiche di 40 paesi.

COLTIVAZIONI ERBACEE 2
AROMATICHE E OFFICINALI, ORTICOLE AGROINDUSTRIALI, FORAGGERE E COLTURE A FINI ECOSISTEMICI ED ENERGETICI

A CURA DI:
GIULIANO MOSCA, AMEDEO REYNERI

I Edizione - 2024
€ 53,00 - Edagricole di New Business Media srl
ISBN: 978-88-506-5622-6
Pagine 488 - formato 19,5 x 26 cm
e-mail: libri.edagricole@newbusinessmedia.it
www.edagricole.it

Nella seconda metà del secolo le produzioni globali dovrebbero aumentare del 70-100% ma la produzione dovrà avvenire in un’ottica di forte riduzione di superfici e acqua disponibile e di conservazione dell’ambiente per arginare la crisi climatica.
Ciò comporta che le conoscenze relative alle principali specie coltivate debbano essere fortemente innovate.
Nel volume l’argomento è svolto in un’ottica di filiera con attenzione alle destinazioni d’uso e trattando le differenti, possibili tipologie di gestione colturale dall’agricoltura convenzionale a quella biologica e di precisione o conservativa.

Sono trattati anche i principali rischi biotici e abiotici e il quadro complessivo delle destinazioni d'uso delle singole colture.

INDICE:
Menta - Lavanda e lavandino - Stevia - Tabacco - Pomodoro da industria - Carciofo - Fagiolo e fagiolino - Pisello - Spinacio - Patata - Foraggicoltura - Qualità, raccolta e conservazione dei foraggi - Colture di copertura (cover crops) - Tappeti erbosi - Inerbimenti - Piante da fitorimediazione - Colture erbacee a fini ecosistemici - Canna comune - Panico verga - Come coltivare in futuro.

CURATORI:
Giuliano Mosca, già Ordinario di "Agronomia e Coltivazioni erbacee" nell'Università degli Studi di Padova, è stato responsabile di unità di ricerca nell'ambito di progetti nazionali ed europei. Accademico emerito e Presidente della Accademia dei Gergofili sezione Nord-Est, Accademico ordinario dell'Accademia Nazionale di Agricoltura.
Amedeo Reyneri è Ordinario di "Agronomia e Coltivazioni erbacee" nell'Università degli Studi di Torino; ha partecipato a programmi di ricerca nazionali ed europei. Attualmente coordina il gruppo scientifico di studio sulla problematica della coesistenza con le colture geneticamente modificate e il gruppo internazionale di studio sulla sanità dei cereali.

CONGRESSI

PALMEX Thailand 2024 Exhibition

1 - 2 August 2024 | Suratthani, Thailand

PALMEX Thailand 2024 is the only specialized Palm Oil event in Thailand that brings together an international congregation of both upstream and downstream palm oil companies and also its supporting industries gathered in South of Thailand, Thailand to showcase the latest developments in the palm oil industry.
Thailand, currently ranked #3 in the world for CPO is a potential and viable market for palm oil technology companies as the industry is currently honing new palm oil technologies and equipment to help spur its production further.
Fireworks Trade Media Group which have organized successful palm oil events such as PALMEX Indonesia and PALMEX Malaysia is the organizer of this event supported also by the Thai Oil Palm & Palm Oil Associations.
Discover more on the web site:
https://www.thaipalmoil.com/

21st International Sunflower Conference

20 - 24 August 2024 | Bayannur, China

The 21st ISC will be a 5-day conference consisting of plenary talks, scientific and industry workshops, and poster sessions. Topics of interest include Genetics and Breeding, Genomics and Biotechnology, Biotic Stress and Abiotic Stress, Crop Production and other fields of the entire sunflower industry chain. This event will strengthen the global scientific sunflower community, as well as demonstrate the dynamic changes and significant accomplishments of the confection sunflower industry in China.
Although there has been only a two-year window for organizing the conference due to the Covid-19 pandemic, the organizers are continually dedicated to the conference preparation to make the 21st ISC truly successful.
More information and program updates:
http://www.esanrui.com/isc

SCI 2024 - Chemistry Elements of Future XXVIII National Congress of Società Chimica Italiana

26 – 30 August | Milan, Italy

The SCI National Congress represents the main Chemistry event in Italy, a place where discuss about the key role that chemistry plays in facing the challenges posed by sustainable development: circular economy, environmental preservation, climate change mitigation, energy solutions, safeguarding health and guiding the transition towards the economy and society of the future.
The congress will bring together top level chemists from both national and international domains, representing a broad spectrum of sectors including research, teaching, industry, academia and many professional fields.
An exciting new addition to SCI2024 is the large exhibition area, offering the chance to explore innovative technical solutions for our work in the different fields.
More information on https://sci2024.org/

OFI International 2024

9 - 11 September 2024 | Rotterda006D, the Netherlands

OFI International 2024 will bring together major players across the European, Asian and global oils and fats community under one roof, with a focus on 'Solutions for Sustainability, Processing & Trade'.
Program overview:
9 September - Site visits and port of Rotterdam tours
Site visits will be organised to some of the major companies located in the port, giving delegates first-hand experience of their operations.
10-11 September - OFI Technical Commercial

Conference
Organised by OFI with the theme, 'Innovations in Processing & Refining – Addressing Sustainability and Traceability Challenges in the Supply Chain'. With oils and fats processors facing increasing pressure to maximise yields in the most competitive and sustainable way, this two-day conference will focus on the latest refining, and processing technologies, addressing latest industry issues on sustainability, waste feedstocks and contaminants such as MCPDEs, GEs, MOSH/MOAH, chlorinated paraffins and other persistent organic pollutants (POPs).
11 September - OFI Trade Outlook and Logistics Conference
Under the theme 'Managing risks and building supply chain resilience', the conference will focus on current and future price trends impacting the world's major vegetable oils including war and climate impacts on shipping; and what traders and producers must do to meet sustainability regulations such as the EU Deforestation Regulation (EUDR) and the EU Union Database Biofuels (UDB).
10-11 September - OFI International Exhibition 2024
Exhibition of major suppliers to the oils and fats industry.
10 September
3rd Sustainable Vegetable Oil Conference (SVOC)
This annual event organised by the Council of Palm Oil Producing Countries (CPOPC) is a crucial platform aimed at facilitating a comprehensive dialogue among international stakeholders of the vegetable oil industry.
This year's theme of 'Sustainability Transformation Beyond Borders' is centred around implementation of the EU Deforestation Regulation (EUDR), food & energy security, and the role of vegetable oil in meeting rapid growth of the global population. See more information at:
https://www.ofimagazine.com/ofi-international-2024

International Agro-Industrial Exhibition - Oil and Fat Industry

11 - 12 September 2024 | Kyiv, Ukraine
It is a key event for all those interested in the latest technologies and solutions in the production of oils and fats.
Programme:
- Exhibition of products and technologies: View and evaluate new developments in the production of oils and fats from leading companies in the industry.
- Seminars and lectures from experts: Learn about advanced approaches to production, processing and management from our renowned speakers.
- Business forum and networking: Meet industry representatives and discuss potential partnerships and collaborations.

Main topics of the conference «Innovations in the Oil and Fat Industry»:
- Technologies for the production of oils and fats.
- Innovative methods of processing and extraction.
- Quality and standards in oil production.
- Management strategies for the production of oils and fats.
- Use of oils in food and other industrial sectors.
- Waste management and sustainability of production.

The event «Oil and Fat Products Tasting» will give the opportunity to discover the best samples of oils and fat products from leading manufacturers.
More info: https://oil.agroinkom.com.ua/en/

North American SAF Conference & Expo

11 - 13 September 2024 | Saint Paul, Minnesota, USA
The conference will showcase the latest strategies for aviation fuel decarbonization, solutions for key industry challenges, and highlight the current opportunities for airlines, corporations and fuel producers.
The North American SAF Conference & Expo is designed to promote the development and adoption of practical solutions to produce SAF and decarbonize the aviation sector. Exhibitors will connect with attendees and showcase the latest technologies and services currently offered within the industry. During two days of live sessions, attendees will learn from industry experts and gain knowledge to become better informed to guide business decisions as the SAF industry continues to expand.

Main topics:
- Wine and Fermented Beverages quality
- Traceability and Counterfeit in Fermented Beverages
- Metabolomic and Proteomic Profiles in Wine, Beer, and Spirits
- Cutting-Edge MS Techniques Applied to Enology
- Monitoring Oenological Processes and Fermentation
- Identification of Contaminants and Faults in Alcoholic Beverages

More information and program on the web site:
https://saf.bbiconferences.com/ema/DisplayPage.asp?pageId=Home

6th International Symposium on Dietary Fat and Health

16 - 17 September 2024 | Frankfurt, Germany
Many people believe that fat in the diet and the

heart are closely related. This is usually about the connection between fat and the risk factor LDL cholesterol. But is the relationship between heart and dietary fat that simple? To sum it up: no. In order to show the diverse interactions between the different fatty acids in the diet and coronary heart disease, the chosen topic is “Fat in the diet and the heart”.
Among other things, the question of whether a low-fat diet is a heart-healthy diet can be answered. It must also be shown that fatty acids have different effects on different people and patient groups. The correct decision regarding dietary fat must be based on the existing fat metabolism changes.
More info:
https://veranstaltungen.gdch.de/microsite/index.cfm?l=11686&sp_id=1

GERLI Lipidomics Meeting at the End of the World

23 - 26 September 2024 | Plouzané, France

This highly anticipated event will bring together leading researchers to discuss the latest advancements in understanding lipid structure and functions in various organisms inhabiting aquatic and terrestrial environments.
During the meeting, new tools and techniques from disciplines such as biochemistry, cell biology, physiology, ecology, genomics, proteomics and lipidomics will be presented. These cutting-edge approaches will shed light on crucial questions concerning the diversity, functions and metabolism of lipids, as well as their industrial valorization. To this end, 7 sessions will be proposed, from the description of the molecules’ structure to the definition of their role in the physiology of organisms, down to their use to feed populations. Sessions will also focus on analytical preservation and tracability techniques, as well as emerging fluxomics techniques.
More information on:
https://eurofedlipid.org/19th-gerli-lipidomics-meeting-at-the-end-of-the-world/

Canadian Lipids and Proteins Conference

23 - 27 September 2024 | Ottawa, Canada

The meeting is an opportunity for members of the section, students, and the scientific community to highlight new research and technological developments in lipids, proteins, and co-products. There will be an emphasis on sustainability, and green technologies, as well as innovative and integrated approaches for the production and analysis of lipids, proteins, foods, value added products, and bioactive compounds.
The conference aims for a multidisciplinary approach to gather excellent works from scholars at universities, government, and industry.
For more information, visit:
https://payments.carleton.ca/canadian-lipids-and-proteins-conference-2024/

Symposium on Structured Lipid Phases

30 September – 2 October 2024 | Berlin, Germany

Dealing with the different aspects of structured lipid phases the symposium tries to address the need for more room for discussions often sacrificed at the larger congresses with parallel sessions due to tight scheduling.
This is an attempt to stimulate the dialogue also between academia and industry to relate global challenges and future ambitions to goals that are relevant to the community. This could imply sharing long-term ambitions and nagging questions (industry) as much as imperfect but inspiring work (academia).
The symposium is meant to be a stage for exchange, challenges and a stimulus for cooperation while being an inspiration to the junior researchers.
The following subject areas should not be read to tightly in but give guidance:

- fat crystallization
- analytical techniques and structure analysis
- modelling and data processing
- oleogelation
- confectionary
- emulsions, delivery and non-food applications

See more on:
https://www.tu.berlin/en/lvt/events/berlin-symposium-on-structured-lipid-phases

ICIS Pan American Oleochemicals Conference

2 - 3 October 2024 | Miami, Florida, USA

The 4th ICIS Conference connects industry leaders from across the value chain to stay updated, exchange insights, and network. The Pan American market faces new challenges, and a new approach is needed. With the European Union's Deforestation Regulation pending and green policies expected to change post-US elections, adaptation is not just an option – it's a necessity.
Feedstock availability and innovative production methods are reshaping the market landscape. From the future of tallow in the US to the booming palm oil industry in Colombia, the opportunities are endless – for those willing to seize them.
This disruption guarantees that businesses clinging to outdated strategies will struggle. To thrive in this new era, the industry must embrace change. It must reinvent strategies, technology, and operations to not just survive, but to drive unparalleled success through transformation.
The entire oleochemicals value chain reunites on 2-3 October at the 4th ICIS Pan American

Oleochemicals Conference in Miami! Producers, surfactant manufacturers, traders and end users from North, South and Central America as well as other regions, will get together to connect and network, making it a must-attend event.
Program overview:
2 October, 2024

- Outlook on the Pan American oleochemicals market
- Modelling outcomes of the US elections on the oleochemicals industry
- Interview: Global Impacts of the European Union Deforestation Regulation (EUDR)
- Panel discussion: The future of tallow in the US – what happens next?
- Panel discussion: Tapping into sustainable feedstock sources in South America
- Brazilian glycerine and its global reach
- From biofuels to green chemistry: the palm oil journey to decarbonisation
- Panel discussion: The impact of the growing renewable fuels and sustainable aviation fuels (SAF) markets on the oleochemical industry
- Technical replacements for tall oil fatty acids (TOFA) amid shrinking capacity

3 October, 2024

- Panel discussion: Technical innovation to accommodate feedstock changes in the US
- Efficiency of production and logistics of shipping: which hinders industry development more?
- Fireside chat: Navigating sustainability strategies for oleochemical manufacturers
- Panel discussion: Changes in requirements from the FMCG sector
- Looking ahead: what is the future trajectory of the oleochemicals market in the Pan American region?

More information and entire program at:
https://events.icis.com/website/13907/

PALMEX Indonesia

9 - 11 October 2024 | Medan, Indonesia

The 14th PALMEX Indonesia 2024 is the only specialized Palm Oil event in Asia that brings together an international congregation of both upstream and downstream palm oil companies and also its supporting industries gathered in the capital city of North Sumatera, Medan to showcase the latest developments in the palm oil industry. North Sumatera, home to one of Indonesia's largest concentration of oil palm plantations and also the presence of many supporting facilities such as palm oil processing plants making its capital Medan the perfect venue for the show. This unique event seeks to educate the public on the importance of the palm oil industry in Indonesia and the future trends of palm oil in the region. The international character and regional audience of PALMEX Indonesia provides unparalleled marketing, education and networking opportunities.
For more information and update visit:
https://palmoilexpo.com/index.html

Oil and Fats International Congress (OFIC) 2024 (+ Online)

22 – 23 October 2024 | Kuala Lumpur, Malaysia

A hybrid conference platform with the aim to help the oil palm community to keep up to-date with the latest economic, sustainability and technological developments, to network with peers and exhibitors and to share research findings via oral and poster presentations.
The program also includes three Foundations Lectures and One Panel Discussion as follows:
1. Tan Sri B. Bek-Nielsen Foundation Lecture
2. Tun Tan Sri Raja Alias Foundation Lecture
3. Tun Dr Lim Keng Yaik Foundation Lecture
4. Panel Discussion - Evening Forum

Info: https://mosta.org.my/events/ofic-2024/

YABITED Fats and Oils Congress

7-9 November 2024 | Antalya, Turkey

This international congress will give the people from all over the world important opportunities to discuss every aspects of fats and oils during high-quality lecture programme and network with professionals from academia and industry. Fats and oils are probably the most important nutrients among all foods for human health. The title of 5th YABITED Fats and Oils Congress is: *"Bring Your Ideas for Healthier Future"*. Some special sessions such as olive oil, oilseeds, processing, oxidation and refining etc. will also be part of the congress to make discussions on the related scientific and technological developments.
The scientific committee will prepare a unique lecture programme consisting of every aspect of fats and oils.
See more: https://yabited2024.com/EN/

Global Grain Geneva 2024

12-14 November 2024 | Geneva, Switzerland

800+ peers from around the globe will connect, share challenges and trade, as well as millers, buyers, traders, brokers, NGOs, governments and more.
Expert speaker panel - Global Grain attracts senior speakers from across the global supply chain. Hear from leading traders, analysts, brokers, investors, NGOs and more.
Networking opportunities - Meet the who's who of the grain-trading community. With professionals from over 55 countries in attendance, this truly is the global event for grain.
Get ahead of risks - In a volatile market, expert insights are more necessary than ever before. Be where 70+ speakers share the latest data, analy-

sis and forecasts on upcoming harvests and trade routes to inform your strategies.
Key talks - Key sessions include presentations on agriculture price drivers, global logistics trends, and digitalization in grain trading. Panel discussions will cover key grain exporting and importing regions (MENA, EU, Black Sea, South America, etc.).

- The Big Picture: Agriculture Price Drivers into 2025
- Global Grain Market Prospective 2024-25
- Global Oilseeds and Veg Oils Market Updates
- Vegoils Markets Drivers in 2024-2025, Prices Developments
- Global Pulses Market Fundamentals and Trading Trends
- Black Sea Grain Market Updates

See the event page:
https://www.fastmarkets.com/events/global-grain-geneva/

Biofuels Expo 2024

14 – 15 November | London, UK

The 2nd International Conference is organised in coordination with generous support and cooperation from passionate academicians and Organizing Committee members with the theme "Endorsing New Developments in Biofuels and Bioenergy for a Better Environment". Leading delegates, scientist, researchers, scholars, professors, energy experts will take part in this approaching to witness various scientific discussions and bestow future improvement in the field of Biofuels. The intention is to convey the society the most recent research results and advances in the field of Biofuels and Bioenergy. This conference will highlight the information research on its impact on outcomes through oral demonstration and presentation.
More info: https://biofuelsconference.org/about-us

DAIRY EXPO TECH 2024

5-6 December 2024 | Piacenza, Italy

The Piacenza Expo will host the Dairy Expo Tech, a meeting organised by Senaf focused on the machines and the equipments used in the dairy industry. The aim of the convention is to support the growth of the dairy industry displaying innovative solutions to overcome the challenges that this business is facing, such as sustainability, digitalization, staff training and lack of personnel, production efficiency, profitability etc.
Plenty of time will be dedicated to agricultural and economical politics, in order to encourage initiatives for social, environmental and economic sustainability.
Reducing carbon dioxide emissions, developing an increasingly sustainable production system and lowering the energy and water consumption are the targets of today's dairy industry.
These changes can't be applied without technological innovation, which not only provides environmental benefits, but can also help reducing costs and raw material consumption, fastening and automating the production process, exploiting digital opportunities etc.
Among the many events, are to be highlighted the Dairy Summit and Dairy Tech Summit, to stimulate discussions on production, transformation and distribution, the Dairy Overview, focused on new trends, and the Dairy Award, to give credit to the most innovative technological solutions. A panel named Piazza Formazione, in collaboration with the most prestigious universities, will be dedicated to the cooperation between education and business.
See the event page: https://www.dairyexpotech.it/

Convegno SISSG

11-13 December 2024 | Parma, Italy

The Biennal Congress of Società Italiana delle Sostanze Grasse (SISSG) will be held in Parma, from December 11 to 13, at the Starhotel Du Park.
The meeting will be focused on the use of fats as ingredients and will include four different sessions:

- Lipids as Ingredients
- Lipids and Animal Feeding
- Lipids and Nutracetics
- Lipids and Cosmetics

Experts from Universities, Research Centres and Companies will host each session through keynote presentations and thematic reports.

Check updates on the event page:
https://www.sissg.it/2024/06/21/congresso-sissg-2024-parma-11-13-dicembre-2024/

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

www.innovhub-ssi.it

La
Rivista Italiana
delle
Sostanze Grasse

Via Giuseppe Colombo, 79
20133 MILANO

Editorial office:
Chiara Zigliani
risg@mi.camcom.it

Author instructions

La Rivista Italiana delle Sostanze Grasse (RISG) welcomes research, experimental or technological papers, short communications, reviews articles on edible and industrial oils and fats of vegetable and animal origin, soaps, detergents, surfactants, cosmetics and toiletries, mineral oils, lubricants.

The manuscript will be evaluated by a team of referees whose opinion is essential for acceptance for publication. We shall ask you to indicate three names of qualified experts as a referee.

The Authors publishing their manuscripts on our scientific Journal are authorized and encouraged to upload the full text in .pdf format on their page of **ResearchGate**, increasing the diffusion and visibility of the results.

From 2020 papers are available as **Open access** on our website for free download.

In our website you can also find the *guide-lines* for manuscript preparation and submission as well as *Privacy information* notice pursuant to Regulation (EU) 2016/679 and current legislation on personal data processing.

Visit our website:
https://www.innovhub-ssi.it/media/pubblicazioni/risg-rivista-italiana-sostanze-grasse.kl

INNOVHUB - STAZIONI SPERIMENTALI PER L'INDUSTRIA S.r.l.
Sede Legale: Via Meravigli 9/b, 20123 Milano
Capitale sociale: Euro 10.000.000,00 i.v.
C.F. e n. iscr. al R.I. di Milano, Monza Brianza, Lodi: 97425580152 - P. IVA: 05121060965
REA: MI -1798570 - Socio Unico: Camera di Commercio di Milano, Monza-Brianza, Lodi
Via Giuseppe Colombo 79 - 21033 MILANO
www.innovhub-ssi.it