

RISG

2024

VOL. 101

ISSN 2611-9013 RISG on-line 101(3) 141-204 (2024)
Finito di stampare nel mese di novembre 2024



INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

LA RIVISTA ITALIANA DELLE SOSTANZE GRASSE

N. 3 LUGLIO-SETTEMBRE 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)



RISG

LA RIVISTA ITALIANA DELLE SOSTANZE GRASSE

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

Indice

In memoria del Dr. Grieco	143
A systematic review of the essential oils and biological activities of the genus <i>Lindera</i> (Lauraceae) <i>Nur Nabilah Mohd Zaini, Wan Mohd Nuzul Hakimi Wan Salleh</i>	145
Nutritional and phytochemical characterization of Tunisian <i>Moringa oleifera</i> Lam. aerial parts <i>Maha BERGAOUI, Mouna BOULARES</i>	155
Effects of different postharvest storage conditions of black cumin seeds on oxidative stability of cold pressed oil <i>Derya Arslan, Ayşenur Acar</i>	165
General considerations on pesticide residues in olive oils <i>Tiziana Generali</i>	175
FCM: Overall Regulatory Framework <i>Fabiana Vanni, Giorgio Padula, Veruscka Mannoni, Silvia Giamberardini, Marco De Felice, Cinzia Gesumundo</i>	181
Ricerche spettrofotometriche nell'ultravioletto su olii di semi e sulle loro miscele con olio di oliva <i>Un articolo di Giovanni D'Arrigo commentato da Paolo Bondioli e Lanfranco Conte</i>	189
Annunci di Ricerca Partner per Ricerca e Trasferimento tecnologico Enterprise Europe Network	195
Notiziario Recensioni di libri Congressi	199

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

La Direzione e la Redazione della Rivista Italiana delle Sostanze Grasse non si assume alcuna responsabilità per i dati forniti e le opinioni espresse dagli autori, che restano i soli responsabili del contenuto delle loro pubblicazioni.

Copyright:

La riproduzione e/o l'impiego di informazioni pubblicate sulla Rivista è consentita soltanto con l'autorizzazione scritta dell'Editore e a condizione che venga riportata l'indicazione della fonte (Rivista Italiana delle Sostanze Grasse)

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

Tutela dei dati personali: l'indirizzo di Autori e Collaboratori verrà utilizzato per l'invio di iniziative editoriali. Viene garantito il diritto dell'interessato di conoscere, aggiornare, cancellare, rettificare i suoi dati o opporsi all'utilizzo degli stessi, se trattati in violazione del Regolamento (UE) 2016/679 e della normativa vigente in materia di trattamento dei dati personali.

La RIVISTA ITALIANA delle SOSTANZE GRASSE

Comitato di Redazione Innovhub SSI

L. FOLEGATTI *Esperta Oli, Grassi e Derivati*
G. GASPERINI *Responsabile Area Innovazione e Team Prestazioni dei Materiali*
M. MARCHI *Direttore Tecnico Operativo*
D. MARIANI *Esperto Cosmetica e Detergenza*
P. ROVELLINI *Responsabile Team Chimica, Tecnologia e Sicurezza Alimentare*
M. SALA *Esperta Lubrificanti*
S. TAGLIABUE *Esperta Chimica Analitica Strumentale*

Comitato Scientifico

M. ÁLVAREZ-ORTÍ - *Escuela Tecnica Superior de Ingenieros Agrónomos, Universidad de Castilla - La Mancha, Albacete (E)*
E. V. ARANITI - *Laboratorio Sensorial "Panel de Cata Mendoza de Aceite de Oliva", Univ. Nacional De Cuyo - Mendoza, Argentina*
R. BARBANI - *Azienda Unità Sanitaria Locale (AUSL), Ambito Veterinario - Casalecchio di Reno (BO)*
E. BERNARDI - *Specialista in Scienza dell'alimentazione, biologa e nutrizionista. Membro EFSA (European Food Safety Authority)*
P. BONDIOLI - *Società Italiana per lo Studio delle Sostanze Grasse SSSG - Milano*
Á. A. CARBONELL BARRACHINA - *AgroFood Technology Dep., Universidad Miguel Hernández de Elche (UMH) – Alicante (E)*
M. L. CLODOVEO - *Dipartimento Interdisciplinare di Medicina, Università degli Studi di Bari*
L. CONTE - *Società Italiana per lo Studio delle Sostanze Grasse SSSG - Milano*
A. FABERI - *Ministero delle Politiche Agricole Alimentari e Forestali - Roma*
T. GALLINA TOSCHI - *Dipartimento di Scienze e Tecnologie Agro-Alimentari, Università di Bologna*
L. GASCO - *Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino*
T. GENERALI - *Dipartimento Ambiente e Salute, Istituto Superiore Sanità - Roma*
C. GESUMUNDO - *Dip. DAMSA - Rep. Esposizione a Contaminanti in Aria, Suolo e da Stili di Vita, Istituto Superiore di Sanità - Roma*
R. GUIDETTI - *Dipartimento di Scienze Agrarie e Ambientali, Produzione Territorio, Agroenergia, Università degli Studi di Milano*
B.A. KAMEL - *Office National de l'Huile de Tunisie - Tunis, Tunisie*
S. KOSTADINOVIĆ VELIČKOVSKA - *Faculty of Agriculture, University "Goce Delčev" - Štip, Republic of North Macedonia*
R. LEARDI - *Dipartimento di Farmacia, Università degli Studi di Genova*
G. LITWINIENKO - *Faculty of Chemistry, University of Warsaw - Poland*
L. MANNINA - *Dipartimento di Chimica e Tecnologie del Farmaco, Fac. Farmacia e Medicina Università Sapienza di Roma*
L. A. MEDINA JUAREZ - *Departamento de Investigaciones Científicas y Tecnológicas, Universidad de Sonora - Mexico*
W. MOREDA - *Instituto de la Grasa (CSIC) - Siviglia (E)*
B. PEJIN - *Institute for Multidisciplinary Research - IMSI, Department of Life Sciences, University of Belgrade - Rep. of Serbia*
N. RIZZI - *Esperto tecnico in Commissioni e Sottocommissioni UNI, ISO e CEN settore agroalimentare*
M. C. SARAI AGUSTIN SALAZAR - *Departamento de Investigaciones Científicas y Tecnológicas, Universidad de Sonora - Mexico*
M. SERVILI - *Dipartimento di Scienze Agrarie, Alimentari e Ambientali - Università di Perugia*
F. VANNI - *Dipartimento Ambiente e Salute, Istituto Superiore di Sanità - Roma*
F. VISIOLI - *Dipartimento di Medicina Molecolare dell'Università di Padova*

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)

In memoria del dr. Domenico Grieco

A Settembre di quest'anno ci ha lasciati il dr. Domenico Grieco, "Mimi" per chi lo conosceva da tanto tempo, laureato in chimica industriale presso l'Università di Bologna nel 1955.

Mimi Grieco era nato a Cerignola, in provincia di Foggia e conservava un grande affetto per la sua terra di origine, quando si trovava a Roma acquistava "La Gazzetta del Mezzogiorno" che a Milano, dove viveva da molto tempo, non arrivava.

È stato per tanti anni direttore del Laboratorio dell'Associazione Granaria di Milano e per molti altri membro del Consiglio Direttivo della Società Italiana per lo Studio delle Sostanze Grasse, nonché della Commissione Tecnica Governativa per gli Oli ed i Grassi e, più recentemente, del Gruppo di lavoro UNI GL 18 "Oli, grassi animali e vegetali e loro sottoprodotti, semi e frutti oleaginosi".

La sua partecipazione al Consiglio è sempre stata estremamente attiva, nonché preziosa sia nel proporre iniziative, sia per la sua capacità di "ammorbidire" situazioni di tensione che si potevano presentare, come in ogni consesso in cui differenti approcci a problemi comuni si palesino.

Mimi era attivissimo ogni qual volta ci fosse da organizzare un'iniziativa della Società, in cui si gettava a capofitto con l'entusiasmo e l'energia di un ventenne. Alcuni di noi lo ricordano impegnato ad aiutare nella preparazione del materiale congressuale, piegare cartelline, riempirle e quant'altro servisse.

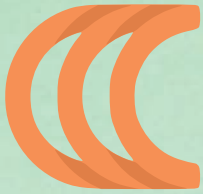
La sua lunga "militanza" nel Consiglio direttivo della SISSG non lo aveva però sclerotizzato nel culto del passato: quando negli anni 2000 si intraprese un'operazione di rilancio della Società fu tra i più entusiasti e attivi attori e non solo sostenitori di questo processo che ha portato la SISSG a ciò che è oggi.

Il Dr. Grieco è stato anche per lunghi anni il Tesoriere della Società, ruolo che ha sempre ricoperto con precisione, costanza e buon garbo nel sollecitare i Soci ritardatari. La sua funzione si è poi conclusa passando il testimone nel 2016, alla bella età di 86 anni.

Attentissimo alle problematiche del comparto produttivo delle sostanze grasse e in particolare degli oli d'oliva, ne conosceva la storia nei più intimi dettagli, spesso non solo tecnici, ma anche intrecciati alle vicende personali dei protagonisti.

Sempre signorile e col sorriso sulle labbra, ironico e gentile, ci mancherai, Mimi!

Il Consiglio Direttivo SISSG



INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

GUIDA TECNICA ALLA SOSTENIBILITÀ IN COSMESI



Normative e
regulatory



Tendenze in formulazioni,
tecnologie e packaging



Le opportunità della
sostenibilità



SCARICA LA GUIDA

A systematic review of the essential oils and biological activities of the genus *Lindera* (Lauraceae)

Nur Nabilah Mohd Zaini
Wan Mohd Nuzul Hakimi Wan
Salleh ✉

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

The genus *Lindera* consists of approximately 100 species that are widely distributed in tropical and subtropical areas throughout the world. It is represented by widely well-known medicinal and aromatic plants that produce essential oil. This review attempts to summarise the information on the essential oils of *Lindera* species together with their chemical composition and biological properties. The data and information were collected via an electronic search engine, namely: Scopus, ScienceDirect, Google Scholar, PubMed, and SciFinder. A total of thirteen *Lindera* species have been reported for their essential oils and biological activities. Sesquiterpenes were identified as the major group components in *Lindera* species with the presence mainly of β -caryophyllene, as well as monoterpenes which were dominated by limonene, α -copaene, α -pinene, and 1,8-cineole. In addition, the *Lindera* essential oils also displayed various biological activities including anti-allergic, anti-arthritic, antiviral, antibacterial, anticancer, anti-inflammatory, antitumor, and cytotoxicity. The outcome of these studies will further support the therapeutic potential of the genus *Lindera* and provide convincing evidence for its future clinical applications in modern medicine.

Keywords: Essential oil; *Lindera*; β -caryophyllene; limonene; anti-inflammatory; antibacterial

1. INTRODUCTION

Lauraceae, one of the most primitive families of plants, belongs to the Magnoliidae subclass, a family of pantropical plants that includes trees and shrubs. It is composed of approximately 55 subgenera totalling over 3,000 species. The family is highly diversified in Southeast Asia, Madagascar, Northern South America, and the east coast of Brazil [1-3]. The genus *Cinnamomum*, *Litsea*, *Lindera*, *Neolindera*, and *Parabenzoin* are the example of well-known subgenera in Lauraceae family. In China, there are about 25 genera and 445 species that distribute over the low and moderate altitude ranges from the southwest to the south. The genera *Sinosassafras* and *Sinopora* are two of them that are native to China, while *Laurus* and *Persea* are the commercially grown genera [4]. Besides, in Malaysia, there are about 213 species from 16 genera and locally known as 'medang' [5].

Lindera is a genus of about 100 species of flowering plants in the family Lauraceae. It can be found all over the world in tropical, subtropical climates, and temperate zones of Asia and Midwestern America [6]. The most common *Lindera* species are *L. aggregata*, *L. chunii merr*, *L. communis*, *L. erythrocarpa*, *L. fragrans*, *L. glauca*, *L. glucida*, *L. megaphylla*, *L. melissifolia*, *L. nacusua*, *L. neesiana*, *L. obtusiloba*, *L. pipericarpa*, *L. pulcherrima*, *L. radix*, *L. strychnifolia*, and *L. umbellata*. The specific floral morphology of the Lauraceae family can be used to identify it. The bark has many lenticels and is smooth and leathery. After being cut, the inner bark emits sap that ranges in colour from pale yellow to pale brown that is fragrant, yellow, orange, reddish, and

✉ CORRESPONDING AUTHOR:
E-mail: wmnhakimi@fsm.ups.edu.my
Tel.: +605-4507123
<https://orcid.org/0000-0003-1408-229X>

Received: June 21, 2023
Accepted: October 27, 2023

pinkish. The leaves are plain, stipule-free, opposite, spiral, whorled, and alternate. The flower is differently accrescent and are bisexual, actinomorphic, tiny, regular, greenish-white, or yellow in colour, aromatic, and trimerous [7]. Most of the flowers have six sepals that are arranged in two cycles. The fruits, which are baccate or drupaceous and frequently seated or surrounded by a persistent and cup-shaped corolla, have taxonomic significance. Many tropical species in the Lauraceae family have persistent leaves, which stay on the plant even when they are no longer useful [8].

Essential oils have been utilised for thousands of years for their therapeutic and medical effects. Ancient civilizations like Egypt, Greece, and Rome are known using essential oils in their religious rituals. Essential oils as secondary metabolites involve complex mixtures of natural compounds with versatile organic structures representing useful medicinal properties. Essential oils are important natural sources and are used as raw materials to produce fragrance compounds in cosmetics, as flavouring additives for food and beverages, as scenting agents in a variety of household products, and as intermediates in the synthesis of other perfume chemicals. Meanwhile, essential oils from aromatic and medicinal plants have been known since antiquity to possess biological activities, most notably antibacterial, antifungal, and antioxidant properties [9-15].

The essential oils from *Lindera* species have been broadly studied and investigated, the most reported species are *L. aggregata*, *L. obtusiloba*, and *L. glauca*. Thus, the current reviews of the essential oils were aimed to simplify and compile the information available. The information was obtained via electronic searches in Scopus, PubMed, ScienceDirect, and Google Scholar. Furthermore, this review will provide an overview of the chemical compositions, biological activities, and some of the medicinal uses of previously published reports on essential oils of *Lindera* species.

2. SEARCH STRATEGY

Searches on Scopus, PubMed, ScienceDirect, and Google Scholar were used to carry out the systematic review. "*Lindera*," "essential oil," and "biological activity" were the search terms used. All articles from the start of the database up through June 2023 have been viewed. The protocol for performing the current study was developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) [16]. The flowchart for article identification and selection is shown in Figure 1.

Titles and abstracts were reviewed after duplicate articles were removed, and the inclusion and exclusion criteria were used. After thoroughly reading each article that resulted from the earlier stages, the inclusion and exclusion criteria were once more applied. The

articles that met all criteria at the end of the last stage were chosen for the current study. In addition, as a second search approach, we added studies found by a manual search of the included studies' reference lists. Included are articles on the genus *Lindera* that discuss traditional applications, essential oils, and biological activities. Articles that were discussed about traditional uses, essential oils, and their biological activities of *Lindera* were included together.

The following criteria were taken into consideration when including articles, which are original; journal articles are the only type of publications accepted, only articles written in English are permitted, the chemical composition of essential oils must be presented together, and their biological activity must be discussed in articles. The following were used as the exclusion criteria, which is the publications did not include the search terms in the title and abstract, incomplete article text could not be retrieved, and the papers did not list the essential oils' chemical composition.

3. MEDICINAL USES OF THE GENUS LINDERA

Worldwide, herbal medicines are being used as complementary and alternative medicine to treat various health diseases related. Most of Lauraceae family are used to make timber for export. They are suited for making plywood and decorative projects like interior finishing, panelling, furniture, and cabinets. The bark of many species offers marketable qualities such as

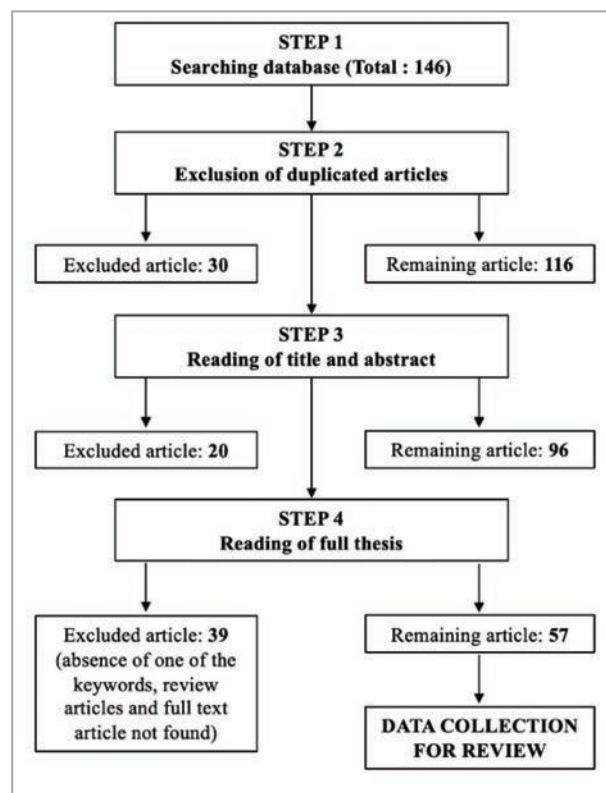


Figure 1 – PRISMA flow diagram of included studies

cinnamon [6]. Earlier studies reported that *Lindera* species are great for ornamental and economical use, as well as for their medicinal and therapeutic benefits. Different species of *Lindera* have been used as medicine to treat a wide range of conditions, such as gastrointestinal disorders, respiratory infections, and menstrual cramps [4]. Table I illustrates the medicinal uses of several *Lindera* species [17-33].

L. aggregata has been used in traditional Chinese medicine to treat a number of conditions, such as pain, inflammation, and gastrointestinal problems, while in Japan, this species has been used to treat cardiac, renal and rheumatic diseases [34]. Moreover, as an herbal medicine, *L. aggregata* is included in 24 formulae in Chinese Pharmacopoeia [35]. Meanwhile, *L. radix* is a very well-known species in Chinese culture, where the roots can be used to warm the kidneys by promoting blood circulation to lessen discomfort and relieve congestion [36]. In addition, in Taiwanese culture, the root parts of *L. akoensis* were used to treat trauma and inflammation [37]. Besides, the leaves of *L. obtusiloba* are traditionally consumed as both tea and food that traditionally used for restoring blood stasis and inflammatory disorders [38], whereas the bark of *L. obtusiloba* is used to treat bruises and throat congestion [39]. In another report, the leaves of *L. obtusiloba* could be used as an agent to suppress mucus hypersecretion, as it contains limonene that was known to be effective in reducing

allergic airway inflammation [40]. Furthermore, the roots, bark, and twigs of *L. umbellata* have beneficial effects on gastric ulcer, abdominal pain, cholera, and beriberi, and its volatile oil was found to have anti-spasmodic effects [36]. Overall, the health benefits of *Lindera* are mainly attributed to its diverse bioactive constituents, which may contribute to its multiple health functions.

4. CHEMICAL COMPOSITIONS OF LINDERA ESSENTIAL OILS

In previous studies, thirteen *Lindera* species were described on the composition of the essential oils [41-57]. These were *L. chunii* [41], *L. communis* [42], *L. erythrocarpa* [23,43], *L. fragrans* [25], *L. glauca* [29, 44-47], *L. melissifolia* [48], *L. nacusua* [49], *L. neesiana* [31], *L. obtusiloba* [50-52], *L. pipericarpa* [53], *L. pulcherrima* [27,54], *L. strychnifolia* [55,56], and *L. umbellata* [33,57]. Most of *Lindera* species were reported mainly from China (13 studies), followed by Korea (5 studies), India (3 studies), whereas Vietnam, Japan, USA, Nepal, and Malaysia each were reported in one study. Table II shows the details of the reported *Lindera* essential oils, comprising various species, localities, plant parts, total components, percentage yield, and several major components.

Analysis of the chemical components identified in *Lindera* essential oils shows that the oil consists of

Table I – Medicinal uses of several *Lindera* species

Species	Traditional uses
<i>L. aggregata</i>	Remedy for rheumatic, cardiac, and renal illnesses [6]
	Treat conditions affecting the digestive system, metabolism, inflammation, and urinary system [17]
	Used as nutritional supplements to prevent liver damage and decrease cholesterol [17]
<i>L. akoensis</i>	Used to treat stomach pain, fever, respiratory infections, headaches, migraines, and reducing inflammation or swelling [18]
	Used in Taiwanese folk therapy for inflammation [18]
<i>L. angustifolia</i>	Relieve swelling caused by contusions, rheumatic pain, and stomachaches [19]
	Used to treat carminative diuretics, and pain relievers especially in nervous headache and migraine [20]
	Used to treat several gastrointestinal, nervous, and rheumatic disorders [21]
<i>L. erythrocarpa</i>	Used to treat multiple cardioprotective and cancerous disorders [22]
	Treating digestive disorders, thirst, pain, and neuralgia [23]
	Treat indigestion in folk medicine [24]
	Used to treat diabetic properties and breast cancer [6]
<i>L. fragrans</i>	Acts as an effective barrier that prohibits mosquitoes from biting [25]
	Used to treat rheumatic numbness and low back pain [25]
	Remedy for bad breath [26]
	Treat depressive-like behaviours that prolonged mild stress caused [24]
<i>L. pulcherrima</i>	Used as spice for the remedy of cold, fever, and cough [27]
	Treat intestinal worms and cure sores [27]
<i>L. radix</i>	Used in pelvic inflammatory disease [28]
<i>L. glauca</i>	Treat several kinds of stomach and heart discomfort problems [29]
	Used to treat rheumatoid arthritis, extravasation, and contusion [29]
<i>L. neesiana</i>	To treat indigestion, gastric disorders, constipation and intestinal issues [30]
	Eliminate intestinal parasitic worms like round and tape worms [31]
<i>L. obtusiloba</i>	Treatment for type II diabetes by lowering blood glucose levels as well as improve blood circulation, inflammation, fever, and abdominal pain [6]
<i>L. umbellata</i>	Effective treatment in relation properties [32]
	To treat neuralgia, stiff neck, and back pain [33]

several groups of components, which are monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. The essential oil of *L. obtusiloba* and *L. glauca* has received the most attention and has been widely investigated. The root oil of *L. glauca* gave the highest total components with 87 components (98.6%) [45], followed by the fruits oil of *L. obtusiloba*, with 78 components (98.8%) [52]. Meanwhile, the highest yield was given by the leaf oil of *L. obtusiloba* which gave 4.23% [51].

β -Caryophyllene, a sesquiterpene, was found as the

most reported major component and can be found in the leaf oil of *L. communis* [42], *L. erythrocarpa* [43], *L. fragrans* [29], *L. glauca* [45], *L. nacusua* [49], *L. obtusiloba* [51], *L. pipericarpa* [53], and *L. pulcherrima* [54]. Another sesquiterpenes were also presented in high amounts such as viridiflorene, germacrene B [41], β -cadinene [41,50], δ -cadinene [41,50], α -humulene [43], germacrene A [29], aromadendrene, γ -cadinene [46], and β -selinene [55,56]. Meanwhile, oxygenated sesquiterpenes were also documented in high amounts such as α -cadinol, globulol, and t-cadinol, which were reported from the stem and leaf oils of

Table II – Major components identified in *Lindera* essential oils

Species	Locality (Part)	Total, %	Yield, %	Group, %	Major components (%)
<i>L. chunii</i>	China (Flowers)	37, 95.4	0.59	SH, 59.1	Viridiflorene (14.6%), β -cadinene (9.5%), globulol (6.3%), germacrene B (6.2%), t-cadinol (5.4%) [41]
	China (Leaves)	34, 96.2	0.46	SH, 59.1	Germacrene B (6.2%), globulol (11.6%), ledol (10.2%), γ -muurolene (4.4%) [41]
	China (Stems)	37, 96.4	0.06	OS, 52.1	α -Cadinol (8.6%), globulol (7.7%), t-cadinol (7.3%), δ -cadinene (6.5%) [41]
<i>L. communis</i>	China (Leaves)	23, NM	NM	NM	Spathulenol (22.5%), endo-1,3,3-trimethyl-2-norbornanol (10.0%), β -caryophyllene (6.7%) [42]
<i>L. erythrocarpa</i>	Korea (Leaves)	15, 63.7	0.07	NM	Nerolidol (18.7%), β -caryophyllene (14.4%), α -humulene (7.7%), germacrene D (4.8%), α -pinene (4.47%) [23]
	Korea (Leaves)	31, NM	NM	NM	Nerolidol (26.9%), β -caryophyllene (13.2%), methyl cinnamate (8.5%), α -humulene (8.4%), geranyl acetate (7.8%) [43]
<i>L. fragrans</i>	China (Leaves)	62, 76.4	NM	SH, 47.8	Spathulenol (27.6%), ledol (6.8%), β -caryophyllene (4.0%) [29]
<i>L. glauca</i>	China (Fruit)	48, 95.7	NM	MH, 56.9	(<i>E</i>)- β -Ocimene (41.5%), α -copaene (13.1%), δ -cadinene (6.2%) [29]
	China (Fruit)	70, 98.6	1.9	MH, 56.6	(<i>E</i>)- β -Ocimene (30.5%), β -caryophyllene (4.8%), δ -guaiene (4.7%) [44]
	China (fruit)	87, 73.9	0.6	SH, 40.0	(<i>E</i>)- β -Ocimene (12.9%), α -pinene (4.0%), β -caryophyllene (3.7%), cadin-1(10),4-diene (3.4%), camphene (2.5%) [45]
	China (fruit)	72, 88.6	1.2	MH, 57.7	(<i>E</i>)- β -Ocimene (37.4%), β -caryophyllene (3.7%), myrcene (3.5%) [45]
	China (fruit)	74, 87.2	1.4	MH, 44.7	(<i>E</i>)- β -Ocimene (30.3%), α -copaene (12.7%) [45]
	China (Fruits)	54	NM	NM	<i>N</i> -Carproic acid (25.3%), germacrene A (10.7%), <i>n</i> -dodecanole acid (10.0%), epishyobunol acetate (7.2%) [29]
	China (Fruits)	54	NM	NM	<i>N</i> -Carproic acid (25.3%), germacrene A (10.7%), <i>n</i> -dodecanole acid (10.0%), epishyobunol acetate (7.2%), β -caryophyllene (5.44%) [29]
	China (Leaves)	41, 87.5	0.32	MH, 46.5	β -Phellandrene (19.0%), myrcene (17.9%), aromadendrene (17.1%), γ -cadinene (10.1%), (<i>E</i>)- β -ocimene (9.1%) [46]
	Vietnam (Leaves)	34, 90.0	0.21	SH, 54.1	β -Caryophyllene (29.2%), α -humulene (18.0%), (<i>E</i>)- β -caryophyllene (14.6%), humulene epoxide II (5.3%), spathulenol (4.6%) [47]
	China (Fruit)	15, 56.6	1.91	MH	(<i>E</i>)- β -Ocimene (30.5%), β -caryophyllene (5.1%), δ -guaiene (5.0%) [44]
<i>L. melissifolia</i>	USA (Fruit)	35, 86.7	NM	NM	Sabinene (66.2%), (<i>E</i>)- β -ocimene (12.9%), α -phellandrene (4.1%) [48]
<i>L. nacusua</i>	China (Leaves)	22, 64.4	NM	SH, 42.5	β -Caryophyllene (8.7%), hexahydrofarnesyl acetone (6.8%), β -selinene (5.0%), neo-phytadiene (4.5%), palmitic acid (4.4%) [49]
<i>L. neesiana</i>	Nepal (Fruit)	40, 86.0	NM	NM	(<i>Z</i>)-Citral (15.0%), (<i>E</i>)-citral (11.8%), α -copaene (8.7%), citronellal (6.7%) [31]

Table II (continue)

<i>L. obtusiloba</i>	Korea (Leaves)	25, 65.7	0.25	NM	δ -Cadinene (13.8%), limonene (10.2%), β -eudesmol (10.0%), hedycaryol (6.7%), α -pinene (5.7%), bornyl acetate (5.6%) [50]
	Korea (Leaves)	27, 67.8	4.23	NM	β -Caryophyllene (32.1%), α -copaene (31.4%), nerolidol (6.8%), β -farnesene (4.1%) [51]
	Korea (Fruit)	70, 83.7	NM	MH	Camphene (18.4%), α -thujene (13.8%), limonene (12.8%), linalyl acetate (12.5%), dihydromyrcene (11.1%) [52]
	Korea (stems)	58, 87.3	NM	MH, 37.0	Limonene (11.7%), β -phellandrene (7.7%), <i>tert</i> -butyl benzene (5.4%), santorina alcohol (5.0%), 1(10) <i>E</i> ,5 <i>E</i> -germacradien-4-ol (4.0%) [52]
	Korea (roots)	70, 80.8	NM	MH, 27.4	Limonene (6.7%), linalyl acetate (6.2%), camphene (5.1%), β -phellandrene (5.0%), 1-decyne (4.7%) [52]
	Korea (stems)	77, 89.1	NM	MH, 29.5	Linalyl acetate (12.5%), limonene (11.4%), dihydromyrcene (3.9%), β -phellandrene (3.8%), elema-1,3,11(13)-trien-12-ol (3.6%) [52]
	Korea (roots)	78, 98.8	NM	MH, 31.8	Linalyl acetate (13.0%), limonene (12.8%), β -phellandrene (4.6%), cyclododecanone (4.1%) [52]
	Korea (fruits)	70, 93.1	NM	MH, 67.8	Camphene (18.4%), α -thujene (13.8%), limonene (13.4%), β -myrcene (9.2%), α -phellandrene (3.8%) [52]
	Korea (leaves)	78, 87.8	NM	MH, 37.4	Dihydromyrcene (11.1%), germacrene B (7.5%), limonene (5.5%), α -eudesmol (4.3%), 2,2-bis(prop-2-enoxy-methyl)-butan-1-ol (3.87%) [52]
<i>L. pipericarpa</i>	Malaysia (Leaves)	22, 98.3	1.0	SH, 60.0	β -Caryophyllene (32.1%), α -copaene (31.4%), nerolidol (6.1%) [53]
	Malaysia (Wood)	17, 89.9	0.05	MH, 89.9	Limonene (55.4%), linalool (6.6%), geranial (6.7%), neral (5.1%) [53]
<i>L. pulcherrima</i>	India (Leaves)	26, 98.2	NM	OS, 75.3	Furanodienone (49.1%), curzerenone (17.4%), furanosesquiterpenoid (5.2%), furanodiene (3.5%) [54]
	India (Leaves)	35, 97.5	1.12	OS, 89.1	Furanosesquiterpenoid (79.3%), furanodienone (46.6%), curzerenone (17.6%) [54]
	India (Leaves)	28, 83.2	NM	SH, 79.3	Furanodienone (46.6%), germacrene D (26.0%), curzerenone (17.6%), β -caryophyllene (5.4%), β -eudesmol (4.1%) [54]
<i>L. strychnifolia</i>	China (Leaves)	49, 94.9	0.36	OS, 65.9	Sesquithuriferol (35.9%), 14-oxy- α -muurolene (16.5%), 1,8-cineole (5.3%), β -selinene (4.6%) [55]
	China (Leaves)	51, 94.5	0.43	OS, 65.8	Sesquithuriferol (35.9%), 14-oxy- μ -urolene (16.4%), 1,8-cineole (5.3%), β -selinene (4.5%), δ -cadinene (3.8%) [56]
	China (Roots)	58, 91.4	0.31	OS, 39.5	(<i>E</i>)- β -Ocimene (10.2%), 1,8-cineole (8.4%), isoterpinolene (4.7%) [56]
<i>L. umbellata</i>	Japan (Leaves)	27, 89.9	NM	NM	Linalool (42.8%), 1,8-cineole (13.7%), β -myrcene (7.6%), limonene (7.6%) [57]
	Japan (Twigs)	14, 75.2	NM	NM	Linalool (65.7%), geranyl acetate (17.5%), limonene (5.2%) [33]
	Japan (Leaves)	20, 93.2	NM	NM	Linalool (42.8%), 1,8-cineole (13.7%), β -myrcene (7.6%), limonene [33]

NM-not mentioned; MH-monoterpene hydrocarbon; SH-sesquiterpene hydrocarbon; OS-oxygenated sesquiterpene

L. chunii [41]. Besides, nerolidol was reported dominantly in the leaf oil of *L. erythrocarpa* [23,43], whereas spathulenol in the leaf oil of *L. communis* [42] and *L. fragrans* [29].

In addition, limonene was also found as foremost in other *Lindera* species such as *L. pipericarpa* [53], *L. obtusiloba* [50], *L. obtusiloba* [52], and *L. umbellata* [33]. The highest percentage of limonene was reported from the leaf oil of *L. pipericarpa* which gave 55.4% of the total oil [53].

In another study, (*E*)- β -ocimene [29,44,45,56], β -phellandrene [46], (*Z*)-citral [31], camphene [52], and dihydromyrcene [52] were found as major components of monoterpenes in *Lindera* essential oils.

Meanwhile, sabinene and linalool were found as primary components with great percentage in the fruit oil of *L. melissifolia* [48] and twig oil of *L. umbellata* [33], respectively.

According to the above-mentioned findings, the chemical variations between *Lindera* species may be caused by the various stages of development and the unique habitat in which the plant was taken. Furthermore, the chemical and biological variety of aromatic and medicinal plants is influenced by factors such as climatic circumstances, vegetation phase, and genetic changes [58]. Thus, these factors have an impact on the plant's biosynthetic pathways resulting in distinctive chemicals.

5. BIOLOGICAL ACTIVITIES

Many researchers are interested in discovering more about biological actives as it comprises a vast range of natural products with the potential to be used in medicine, agriculture, and industry. Bioactive compounds of *Lindera* essential oils have been reported to exhibit various biological activities as illustrated in Table III [59-68]. Most of the studies were focused on the anti-inflammatory and antibacterial properties of *Lindera* essential oils.

For anti-inflammatory activity, several different assays were used to determine the properties, such as in vitro, animal models, and clinical trials. In vitro assays were used to measure the nitric oxide (NO) production in the cell, in which the leaf and twig oils of *L. obtusiloba* showed a strong inhibition with IC₅₀ values 0.8 and 8.7 μM, respectively [6]. Besides, *L. aggregata*

oil also have significant inhibition of superoxide anion in human neutrophils, hence it is effective to act as anti-inflammatory agent [17]. Eucalyptol and linalool are major components of *Lindera* essential oils, which were reported to exhibit anti-inflammatory properties by lowering the production of inflammatory properties and blocking the activity of enzymes that cause inflammation [67]. For antibacterial activity, the root oil of *L. myrrha* may have a strong activity against *Enterococcus faecium*, *Staphylococcus aureus*, and *Acinetobacter baumannii*, whereas *L. pulcherrima* essential was found to exhibit a strong activity against *Salmonella*, *Pasturella multocida* and *Escherichia coli* [54].

According to a previous study, it is often the unique chemical combination rather than a single component that is responsible for any therapeutic activity. In

Table III – Biological activities of *Lindera* essential oils

Bioactivities	Essential oils	Description
Anti-allergic	<i>L. obtusiloba</i>	The leaf oil have strongly effective in decreasing airway hyper-responsiveness and mucus production in the lung tissue against <i>Streptococcus pneumoniae</i> [59]
		The root oil was reported to inhibit histamine release and pro-inflammatory cytokine production in mast cells with percentage inhibition 66.9% and 68.1%, respectively [60]
		The root oil was reported to have same role as gallic acid, where it can suppressed the release of histamine from mast cells to prevent itching [6]
		The leaf oil have significantly inhibited the expressions of IL-6 and TNF-α in mast cells to reduce allergic symptoms [60]
Anti-arthritic	<i>L. aggregata</i>	The leaf oil shown development of Treg cells through promoting fatty acid oxidation, thus it can controlling immune responses including rheumatoid arthritis with EC ₅₀ value 94% [61]
Antivirus	<i>L. aggregata</i>	The stem oil inhibit the fusion of HIV-1 infected with IC ₅₀ value 5.2 – 31.3 μM [61]
Antibacterial	<i>L. myrrha</i>	The root oil have strong activity against <i>Enterococcus faecium</i> , <i>Staphylococcus aureus</i> , and <i>Acinetobacter baumannii</i> with killing percentage 85.6%, 86.7%, and 92.1%, respectively [54]
	<i>L. pulcherrima</i>	The essential oil revealed a strong activity against <i>Salmonella</i> , <i>Pasturella multocida</i> and <i>Escherichia coli</i> with IC ₅₀ value 19.0, 18.0 and 10.8 μL, respectively [54]
	<i>L. gluca</i>	The fruit oil have strong activity against <i>Shigella flexneri</i> with the inhibition zone diameter 25.4 nm [44]
Anticancer	<i>L. aggregata</i>	The root oil have exhibited human colorectal cancer cells with IC ₅₀ value 6.48 μg/mL [62]
		The root oil exhibit the transforming growth factor (TGF)-β inhibitory activity with IC ₅₀ value 12.9 μg/mL [63]
		The root oil showed strongly inhibited against A549 cell lines and neuroprotective activity in SH-SY5Y cells [63]
		The root oil showed activity in human colon cancer cell line with IC ₅₀ value 9.80 μM [17]
Anti-inflammatory	<i>L. aggregata</i>	The root oil exhibited human neutrophils with IC ₅₀ value 7.45 μM [64]
		The essential oil displayed the significant inhibition of superoxide anion generation in human neutrophils with IC ₅₀ values 8.36 μM [64]
	<i>L. akoensis</i>	The aerial part oil have the ability to decrease the LPS-stimulated production of nitrite in RAW264.7 cell with IC ₅₀ value 38.3 μM [64]
	<i>L. blume</i>	The leaf oil showed strong inhibition in prostaglandins production in an A549 with IC ₅₀ value 0.82 μM [65]
Antitumor	<i>L. obtusiloba</i>	The twig oil inhibited nitric oxide production with IC ₅₀ values 3.6–26.4 μM [6]
	<i>L. gluca</i>	The essential oil showed weak activity against SMMC-7721 cell line with IC ₅₀ value 35.2 μM [66]
	<i>L. strychnifolia</i>	The essential oil showed activity against human esophageal cancer Eca-109 cell line with IC ₅₀ value 24.8 μg/mL [67]
Cytotoxicity	<i>L. glauca</i>	The twig oil showed strong activity against SK-MEL-2 and HCT-15 cell lines with IC ₅₀ values ranging from 9.9 to 12.2 μM [68]
	<i>L. reflexa</i>	The root oil showed the activity against MGC803 and SMMC-7721 cell lines with IC ₅₀ values 2.65 and 4.13 μM, respectively [66]
	<i>L. strychnifolia</i>	The essential oil has strong activity on liver cancer cells line and breast tumor cell line with IC ₅₀ values 2.85 and 3.47 μg/mL, respectively [55]

some cases, that biological activity of the essences from the aromatic plants studied may be attributable both to their major components and to the minor ones in the oils. Hence, the synergistic effects of active chemicals with other components of the essential oil should be taken into consideration [70].

6. CONCLUSION

This current review provides an overview of *Lindera* essential oils in medicinal uses, chemical composition, and biological activities. The *Lindera* essential oils revealed high amounts of β -caryophyllene, limonene, (*E*)- β -ocimene, α -copaene, α -pinene, β -phellandrene, nerolidol, globulol, and 1,8-cineole; which potentially contribute to the bioactivities such as anti-inflammatory, antibacterial and anticancer properties. However, the variation in the amount of its composition within the same species that were reviewed could be varied due to environmental and geographical factors. Hence, more pharmacological investigations should be done to unravel the full therapeutic ability of *Lindera* species. Preclinical analyses and clinical trials for essential oils are also required to evaluate the potential of essential oils from *Lindera* species for drug development. Lastly, it is hoped that within the information provided, it will help researchers in selecting species with an economic potential within a wide range of industries.

REFERENCES

- [1] W.M.N.H.W. Salleh, F. Ahmad, K.H. Yen, Chemical compositions, and biological activities of the essential oils of *Beilschmiedia madang* Blume (Lauraceae). Arch. Pharm. Res. 38, 485-493 (2015).
- [2] W.M.N.H.W. Salleh, F. Ahmad, K.H. Yen, R.M. Zulkifli, S.D. Sarker, Madangones A and B: Two new neolignans from the stem bark of *Beilschmiedia madang* and their bioactivities. Phytochem. Lett. 15, 68-173 (2016).
- [3] W.M.N.H.W. Salleh, F. Ahmad, K.H. Yen, R.M. Zulkifli, J.J. Chen, L. Nahar, J.D. Wansi, S.D. Sarker, Beilschglabrinones A and B: Two new bioactive phenanthrene alkaloids from the stem bark of *Beilschmiedia glabra*. Phytochem. Lett. 16, 192-196 (2016).
- [4] H. Feng, Y. Jiang, H. Cao, Y. Shu, X. Yang, D. Zhu, M. Shao. Chemical characteristics of the sesquiterpenes and diterpenes from Lauraceae family and their multifaceted health benefits: a review. Heliyon 8(12), e12013 (2022).
- [5] W.M.N.H.W. Salleh, F. Ahmad, K.H. Yen, R. Zulkifli, Essential oil compositions of Malaysian lauraceae: a mini review. Pharm. Sci. 22(1), 60-67 (2016).
- [6] M.E. Haque, S. Azam, R. Balakrishnan, M. Akther, I.S. Kim, Therapeutic potential of *Lindera obtusiloba*: focus on antioxidative and pharmacological properties. Plants 9(12), 1765 (2020).
- [7] T. Britannica. Editors of Encyclopaedia Lauraceae. Encyclopedia Britannica (2023).
- [8] M. Nakamura, S. Nanami, S. Okuno, S.K. Hirota, A. Matsuo, Y. Suyama, H. Takumoto, S. Yoshihara, A. Itoh, Genetic diversity and structure of apomictin and sexually reproducing *Lindera* species (Lauraceae) in Japan. Forest 12(2), 227 (2021).
- [9] D. Trivedi, Aromatherapy and Yagya therapy for mental health. Interdis. J. Yagya Res. 3(2), 29-41 (2021).
- [10] F.L.M. Zaki, W.M.N.H.W. Salleh, N.N.M. Noor, S.M. Shaharudin, N.A. Ghani, Characterisation of the essential oil components and their multivariate statistical analysis of the genus *Vitex* and *Plectranthus* (Lamiaceae). Riv. Ital. Sostanze Grasse 99(3), 263-268 (2022).
- [11] A.A.H. Yahaya, W.M.N.H.W. Salleh, *Magnolia* genus - A systematic review on the composition and biological properties of its essential oils. Riv. Ital. Sostanze Grasse 99(3), 249-261 (2022).
- [12] N.H.A. Kadir, W.M.N.H.W. Salleh, A systematic review on essential oils and biological activities of the genus *Syzygium* (Myrtaceae). Riv. Ital. Sostanze Grasse 99(2), 165-178 (2022).
- [13] M.A.M. Azhar, W.M.N.H.W. Salleh, S. Khamis, N.A. Ghani. Variation in essential oil composition of three *Litsea* species from Malaysia. Riv. Ital. Sostanze Grasse 99(1), 57-61 (2022).
- [14] W.M.N.H.W. Salleh, H. Kassim, A. Tawang, Volatile components and biological activities of *Pulicaria* essential oils. A review. Riv. Ital. Sostanze Grasse 98(1), 49-58 (2021).
- [15] N.M. Shakri, W.M.N.H.W. Salleh, N.A.M. Ali, Chemical composition and biological activities of the essential oils of genus *Xylopi* L. (Annonaceae) - a review. Riv. Ital. Sostanze Grasse 97(4), 25-34, (2020).
- [16] D. Moher, A. Liberati, J. Tetzlaff, G.A. Douglas, PRISMA Group. Preferred reporting items for systematic review and meta-analyses: The PRISMA statement. ANN. Intern. Med. 151, 264-269, (2009).
- [17] Y. Lv, Y. Zou, X. Zhang, B. Liu, X. Peng, C. Chu, A review on the chemical constituents and pharmacological efficacies of *Lindera aggregata* (Sims) Kosterm. Front. Nutr. 16(9), 1071276 (2023).
- [18] C.P. Yang, P.H. Shie, G.J. Huang, S.C. Chien, Y.H. Kuo, New anti-inflammatory flavonol glycosides from *Lindera akoensis* Hayata. Molecules 24(3), 563 (2019).
- [19] Q. Zhao, Y. Zhao, K. Wang, Antinociceptive and free radical scavenging activities of alkaloids isolated from *Lindera angustifolia* Chen. J. Ethnopharmacol. 106(3), 408-413 (2006).

- [20] A.Y. Leung, S. Foster, Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics, John Wiley & Sons, New York (1996).
- [21] V. Hajhashemi, A. Ghannadi, B. Sharif, Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of *Lavender angustifolia* mill. J. Enthanopharmacol. 89(1), 67-71 (2003).
- [22] D.K. Patel, Biological importance, therapeutic benefit and analytical aspects of bioactive flavonoid pectolinarin in the nature. Drug Metabol. Lett. 14(2), 117-125 (2021).
- [23] Y.J. Ko, G. Ahn, Y.M. Ham, S.M. Song, E.Y. Ko, S.H. Cho, W.J. Yoon, K.N. Kim, Anti-inflammatory effect and mechanism of action of *Lindera erythrocarpa* essential oil in lipopolysaccharide-stimulated RAW264.7 cells. Excli J. 16, 1103-1113 (2017).
- [24] S. Wang, X. Lan, J. Xiao, J. Yang, Y. Kao, S. Chang, Anti-inflammatory activity of *Lindera erythrocarpa* fruits. Phytother. Res. 22(2), 213-216 (2008).
- [25] C. Du, Y. Li, J. Fan, R. Tan, H. Jiang, Chemical composition, antioxidant and antimicrobial activities of essential oil from the leaves of *Lindera fragrans* Oliv. Rec. Nat. Prod. 15(1), 65-70 (2020).
- [26] Y.H. Fu, Y.D. Hou, Y.Z. Duan, X.Y. Sun, S.Q. Chen, Six undescribed derivatives of stilbene isolated from *Lindera reflexa* Hemsl. and their anti-tumor and anti-inflammatory activities. Fito-terapia 163, 105331 (2022).
- [27] S.C. Joshi, C.S. Mathela, Antioxidant and antibacterial activities of the leaf essential oil and its constituents furanodienone and curzerenone from *Lindera pulcherrima* (Nees.) Benth. ex. Hook.f. Pharmacogn. Res. 4(2), 80-84 (2012).
- [28] W. Zou, L. Gong, F. Zhou, Y. Long, Z. Li, Z. Xiao, B. Ouyang, M. Liu, Anti-inflammatory effect of traditional Chinese medicine preparation Penyanling on pelvic inflammatory disease. J. Ethnopharmacol. 266, 113405 (2021).
- [29] Z. Sun, X. Su, Y. Lin, C. Long, Y. Zhang, T. Zhao, Chemical composition and antioxidant and cholinesterase inhibitory activities of *Lindera glauca* fruit essential oil and molecular docking studies of six selected compounds. Horticulturae 9(2), 289 (2023).
- [30] D. Khakurel, Y. Uprety, G. Ahn, J. Cha, W.Y. Kim, S.H. Lee, S. Rajbhandary, Diversity, distribution, and sustainability of traditional medicinal plants in Kaski district, Western Nepal. Front. Pharmacol. 13, (2022).
- [31] S. Comai, S. Dall'Acqua, A. Grillo, I. Castagliuolo, K. Gurung, G. Innocenti, Essential oil of *Lindera neesiana* fruit: chemical analysis and its potential use in tropical applications. Fito-terapia 81(1), 11-16 (2010).
- [32] K. Candy, M. Akhouni, V. Andriantsoanirina, R. Durand, C. Bruel, A. Izri, Essential Oils as a Potential Treatment Option for Pediculosis. Planta Med. 86(9), 619-630 (2020).
- [33] H. Maeda, M. Yamazaki, Y. Katagata, Kuromoji (*Lindera umbellata*) essential oil inhibits LPS-induced inflammation in RAW 264.7 cells. Biosci. Biotech. Biochem. 77(3), 482-486 (2020).
- [34] H. Lai, Z. Yang, F. Li, W. Pan, C. Xu, L. Zhang, S. Zhang, L. Zhang, M. Huang, Roots extract of *Lindera aggregata* (Sims) Kosterm. Modulates the Th17/Treg balance to attenuate DSS-induced colitis in mice by IL-6/STAT3 signaling pathway. Front. Pharmacol. 12, 615506 (2021).
- [35] M. Shimomura, H. Ushikoshi, A. Hattori, I. Murata, Y. Ohno, T. Aoyama, M. Kawasaki, K. Nishigaki, G. Takemura, T. Fujiwara, H. Fujiwara, S. Minatoguchi, Treatment with *Lindera strychnifolia* reduces blood pressure by decreasing sympathetic nerve activity in spontaneously hypertensive rats. Am. J. Chinese Med. 38(3), 561-568 (2010).
- [36] Y. Cao, B. Xuan, B. Peng, C. Li, X. Chai, P. Tu, The genus *Lindera*: a source of structurally diverse molecules having pharmacological significance. Phytochem. Rev. 15, 869-906 (2015).
- [37] I.H. Lin, The Catalogue of Medicinal Plant Resources in Taiwan. Committee on Chinese Medicine and Pharmacy, Department of Health; Taipei, Taiwan (2003).
- [38] J.H. Kim, J. Lee, S. Kang, H. Moon, K.H. Chung, K.R. Kim, Antiplatelet and antithrombotic effects of the extract of *Lindera obtusiloba* leaves. Biomol. Theraput. 24(6), 659-664 (2016).
- [39] M.H. Oak, J.O. Lee, S.H. Kang, J.D. Sohn, J.H. Kim, J.W. Lim, Y. Na, Y. Oh, S.W. Lee, Method for preventing and treating thrombotic disorders using a pharmaceutical composition comprising an extract of *Lindera obtusiloba*. US Patent. (2014).
- [40] D. Narke, M. Kurade, K. Dholakia, A. Siddique, N. Thakkar, K. Frey, D.S. Pronnoth, Limonene reduces airways hyperreactivity and inflammatory through activation of A_{2A} adenosine receptors. Faseb J. 31(S1), 820 (2017).
- [41] Y. Liu, H. Wang, S. Wei, X. Cai, Characterisation of the essential oil from different aerial parts of *Lindera chunii* Merr. (Lauraceae). Nat. Prod. Res. 27(19), 1804-1807 (2013).
- [42] D. Yang, F. Wang, J. Peng, S. Ren, GC-MS analysis and inhibitory activity of the essential oil extracted from the leaves of *Lindera communis*. J. Chinese Med. Mat. 22(3), 128-131 (1999).
- [43] S.S. Kim, G. Song, T. Oh, K. Kim, E. Yang, J. Kim, N.H. Lee, C. Hyun, Antimicrobial effect of *Lindera erythrocarpa* essential oil against antibiotic-resistant skin pathogens. J. Pure Appl. Microbiol. 3(2), 429-434 (2009).
- [44] F. Chen, X. Miao, Z. Lin, Y. Xiu, L. Shi, Q.

- Zhang, D. Liang, S. Lin, B. He, Disruption of metabolic function and redox homeostasis as antibacterial mechanism of *Lindera glauca* fruit essential oil against *Shigella flexneri*. *Food Contr.* 130, 108282 (2021).
- [45] B. Zhu, X. Hou, J. Niu, P. Li, C. Fang, L. Qiu, D. Ha, Z. Zhang, J. Sun, Y. Li, S. Lin., Volatile constituents from the fruits of *Lindera glauca* (Sieb. Et Zucc.) with different maturities. *J. Essent. Oil Bear. Pl.* 19(4), 926-935 (2016).
- [46] J. Niu, X. Hou, C. Fang, J. An, D. Ha, L. Qiu, Y. Ju, H. Zhao, W. Z. Du, J. Qi, Z. Zhang, G. Liu, S. Lin, Transcriptome analysis of distinct *Lindera glauca* tissues revealed the differences in the unigenes related to terpenoid biosynthesis. *Gene* 559(1), 22-30 (2015).
- [47] D.T.M. Chau, N.T.G. An, L.T. Huong, I.A. Ogunwande, Compositions and antimicrobial activity of essential oils from the leaves of *Beilschmiedia fordii* Dunn. and *Lindera glauca* (Siebold & Zucc.) Blume from Vietnam. *J. Essent. Oil Bear. Pl.* 25(1), 93-102 (2022).
- [48] J. Oh, J.J. Bowling, J.F. Carroll, B. Demirci, K.H. Başer, T.D. Leininger, U.R. Bernier, M.T. Hamann, Natural product studies of U.S. endangered plants: Volatile components of *Lindera melissifolia* (Lauraceae) repel mosquitoes and ticks. *Phytochemistry* 80, 28-36 (2012).
- [49] G. Wei, L. Kong, J. Zhang, C. Ma, X. Wu, X. Li, H. Jiang, Essential oil composition and antibacterial activity of *Lindera nacusua* (D.Don) Merr. *Nat. Prod. Res.* 30(23), 2704-2706 (2016).
- [50] J. Yang, S.Y. Lee, H. Na, S.K. Jang, M.J. Park, Evaluation of anti-asthmatic activity of essential oils from the Lauraceae family in lipopolysaccharide (LPS)-Stimulated NCI-H292 Cells. *J. Korean Wood Sci. Tech.* 50(6), 414-426 (2022).
- [51] I.M. Chung, H. I. Moon, Composition and immunotoxicity activity of essential oils from *Lindera obtusiloba* Blume against *Aedes aegypti* L. *Immunopharmacol. Immunotoxicol.* 33(1), 146-149 (2011).
- [52] S.H. Hwang, S.J. Choi, Y.S. Hwang, S.S. Lim, Comparison analysis of essential oils composition in difference parts from *Lindera obtusiloba* Bl. according to the season by gas chromatography-mass spectrometry (GC-MS). *Korean J. Pharmacogn.* 44(1), 30-40 (2013).
- [53] N.A.M. Ali, I. Jantan, The essential oils of *Lindera pipericarpa*. *J. Trop. For. Sci.* 6(2), 124-130 (1993).
- [54] S.C. Joshi, R.C. Padalia, D.S. Bisht, C.S. Mathela, Terpenoid diversity in the leaf essential oils of himalayan Lauraceae Species. *Chem. Biodivers.* 6(9), 1364-1373 (2009).
- [55] R. Yan, J. Hua, In vitro antitumor activity of *Lindera strychnifolia* root essential oil and its active constituent. *J. Wuhan Univ. (Nat. Sci. Edition)*, 4, 345-348 (2014).
- [56] R. Yan, Y. Yang, Y. Zeng, G. Zou, Cytotoxicity and antibacterial activity of *Lindera strychnifolia* essential oils and extracts. *J. Ethnopharmacol.* 121(3), 451-455 (2009).
- [57] N. Nanashima, M. Kitajima, S. Takamagi, M. Fujioka, T. Tomisawa, Comparison of chemical composition between Kuromoji (*Lindera umbellata*) essential oil and Hydrosol and determination of the deodorizing effect. *Molecules* 25(18), 4195 (2020).
- [58] M. Moghaddam, L. Mehdizadeh, Chemistry of essential oils and factors influencing their constituents. *Soft Chemistry and Food Fermentation, Handbook of Food Bioengineering*, 379-419 (2017).
- [59] B. Lee, J. Ha, H. Shin, S. Jeong, J. Kim, J. Lee, J. Park, H. Kwon, K. Jung, W. Lee, Y. Ryu, J. Jeong, I. Lee, *Lindera obtusiloba* attenuates oxidative stress and airway inflammation in a murine model of ovalbumin-challenged asthma. *Antioxidants* 9(7), 563 (2020).
- [60] H.G. Choi, H.D. Lee, S.H. Kim, M.K. Na, J.A. Kim, S.H. Lee, Phenolic glycosides from *Lindera obtusiloba* and their anti-allergic inflammatory activities. *Nat. Prod. Commun.* 8(2), 181-182 (2013).
- [61] Q. Zhang, Y. Fang, C. Lv, Y. Zhu, Y. Xia, Z. Wei, Y. Dai. Norisoboldine induces the development of Treg cells by promoting fatty acid oxidation-mediated H3K27 acetylation of Foxp3. *Faseb J.* 36(4), 1-16 (2022).
- [62] A. Kwak, J. W. Park, S. Lee, J. Lee, J. Seo, G. Yoon, M. Lee, J. Choi, J. Shim, Isolinderalactone sensitizes oxaliplatin-resistance colorectal cancer cells through JNK/p38 MAPK signaling pathways. *Phytomedicine* 105, 154383 (2020).
- [63] X. Liu, J. Fu, J. Yang, A.C. Huang, R.F. Li, L. P. Bai, L. Liu, Z.H. Jiang, G.Y. Zhu, Linderagrenolides A-N, oxygen-conjugated sesquiterpenoid dimers from the roots of *Lindera aggregata*. *ACS Omega* 6(8), 5898-5909 (2021).
- [64] P.C. Kuo, Y.H. Wu, H.Y. Hung, S.H. Lam, G.H., Ma, L.M. Kuo, T.L. Hwang, H.D. Kuo, T.S. Wu, Anti-inflammatory principles from *Lindera aggregata*. *Bioorg. Med. Chem. Lett.* 30(13), 127224 (2020).
- [65] A.H. Ryen, T. Gols, S. Julia, T. Ammar, J. Per-Johan, B. Andres, U. Ernst, G. Sabine, Bisabolane sesquiterpenes from the leaves of *Lindera benzoin* reduce prostaglandin E2 formation in A549 cells. *Phytochem. Lett.* 38, 6-11 (2020).
- [66] T. Liu, W.Y. Li, X. Liu, Chemical constituents from the roots of *Lindera glauca* and their antitumor activity on four different cancer cell lines. *J. Chinese Med. Mat.* 39(8), 1789-1792 (2016).
- [67] R. Yan, J. Hua, In vitro antitumor activity of *Lindera strychnifolia* root essential oil and its active constituent. *J. Wuhan Univ. (Natural Science Edition)*, 4, 345-348 (2014).

- [68] J.S. Yu, J. Baek, H.B. Park, E. Moon, S.Y. Kim, S.U. Choi, K.H. Kim, A new rearranged eudesmane sesquiterpene and bioactive sesquiterpenes from the twigs of *Lindera glauca* (Sieb. et Zucc.) Blume. Arch. Pharm. Res. 39(12), 1628-1634 (2016).
- [69] A.T. Peana, P.S. D'Aquila, F. Panin, G. Serra, P. Pippia, M.D. Moretti, Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomedicine: Int. J. Phytother. Phytopharmacol. 9(8), 721-726 (2002).
- [70] J. Sharifi-Rad, A. Sureda, G.C. Tenore, M. Daglia, M. Sharifi-Rad, M. Valussi, R. Tundis, M. Sharifi-Rad, M.R. Loizzo, A.O. Ademiluyi, R. Sharifi-Rad, S.A. Ayatollahi, M. Iriti, Biological activities of essential oils: from plant chemoeology to traditional healing systems. Molecules 22(1), 70 (2017).



innovazione e ricerca

Olive oil proficiency tests Chemical-physical parameters and contaminants

154

Since 2003, Innovhub SSI organizes every year an interlaboratory test on olive oil for different commercial categories among various olive oil laboratories. The tests include all the chemical parameters. Since 2016 the main contaminants are also considered. Each participant will have the opportunity to compare his own test results with those obtained by the most accredited Italian and foreign laboratories. The proficiency test has as main purpose, the ability to make corrections from deviation that might occur in the results, compared to the average value obtained by other laboratories. At the end of the laboratory tests, the participants insert the results obtained directly in the web portal on the dedicated page:
<https://proficiencytest.innovhub-ssi.it>
The results will be statistically processed and delivered anonymously to each participant.

For information:
Stefania De Cesarei
Expert Sensorial Analysis
Team Chemistry, Technology and Food Safety
E-mail: pt.ssog@mi.camcom.it
www.innovhub-ssi.it



Nutritional and phytochemical characterisation of *Tunisian Moringa oleifera* Lam. aerial parts

Maha Bergaoui¹ ✉
Mouna Boulares¹
Imen Mahmoudi¹
Youkabd Zarroug²
Nawres Dachraoui¹
Mnasser Hassouna¹

University of Carthage¹, Research Laboratory: "Technological Innovation and Food Security LR22-AGR01" Higher Institute of Food Industries of Tunisia (ESIAT) El Khadhra City, Tunis, Tunisia

² Filed Crops Laboratory National Agronomic Research Institute of Tunisia (INRAT) Ariana, Tunisia

✉ CORRESPONDING AUTHOR:
Maha BERGAOUI
Phone: +21698754188
E-mail: mahabergaoui263@gmail.com

Received: September 11, 2023
Accepted: December 6, 2023

This work aimed to investigate the composition of various aerial parts (leaves, flowers, and seeds) of the Tunisian *Moringa oleifera* Lam as well as their extracted oils.

The *Moringa* seeds showed the highest level of protein ($33.45 \pm 0.07\%$) and fat ($30.32 \pm 0.15\%$). However, flowers and leaves were rich in carbohydrates, and minerals. Furthermore, *Moringa* leaves extract revealed the highest levels of polyphenols (58.77 ± 0.59 mg GAE/g DE) and flavonoids (77.13 ± 0.005 mg QE/g DE), while the highest tannin levels was that of the flower extract (82.90 ± 0.07 mg CE/g DE). Sixteen amino acids were identified in the different *Moringa* aerial parts with a richness of seeds in essential amino acids. This study showed the dominance of unsaturated fatty acids in cold pressed seed oil when compared to that extracted by Soxhlet method. Cold pressed *Moringa* seed oil was rich in beta sitosterol ($50.56 \pm 0.65\%$), stigmasterol ($25.04 \pm 1.06\%$) campesterol ($23.16 \pm 0.81\%$), alpha tocopherol (243.51 ± 0.49 mg/kg), gamma tocopherol (112.6 ± 0.1 mg/kg) and delta tocopherol (11.65 ± 0.2 mg/kg) for the production of specific antioxidants for health promotion in cosmetics, food and pharmacological industries.

Keywords: *Moringa oleifera* Lam, aerial parts, oils fractions, biochemical composition, phytochemical quality.

1. INTRODUCTION

Moringa is considered as the most beneficial tree in the world. It is a native plant of the Himalayan region of Northeastern India. It was cultivated in many other places around the world such as North and South America, Italy, Greece, Africa, and Egypt [1]. *Moringa* is the only genus of the *Moringaceae* family, having 13 species [2]. *Moringa oleifera* Lam (*MO*) is a specie known for its nutritional, agronomic and medical benefits [3], pharmacological, biological, immunomodulatory, antispasmodic, hepatoprotective, anticancer, hypotensive, hypoglycemic, cholesterol lowering effects [4]. In addition, all *MO* parts have been used in traditional foods and dishes for human consumption to prevent malnutrition challenges [3]. Added to this, a high level of proteins, ash, calcium, potassium, sodium, iron, moisture, fat, crude fibre, carbohydrates, β -carotenes, vitamin C, were identified in *Moringa oleifera* [1]. It is a main source of phenolic contents that are mainly found in their leaves [2]. *Moringa oleifera* organs are also known as good sources of secondary metabolites and bioactive compounds, such as terpenoids, flavonoids, tannins, alkaloids, phenolic compounds, anthocyanins and proanthocyanidins with interesting biological activities. While *Moringa* seed oil composition is comparable to olive oil one, that's why it has been used as culinary oil in salads [5]. Recently *Moringa oleifera* has been cultivated in Tunisia [6]. In this context, this study was performed to characterise the nutritional and phytochemical compounds of various aerial parts of *Moringa oleifera* collected from Northern Tunisia and identify their antioxidant activity.

2. MATERIAL AND METHODS

2.1. CHARACTERISATION OF *MORINGA OLEIFERA* AERIAL PARTS

2.1.1. Preparation of plant material

Leaves, flowers, and seeds of *Moringa oleifera* were collected from the North of Tunisia (Morneg, Ben Arous) between August and October 2022. Fresh material of *Moringa oleifera* samples were cleaned and washed under tap water to remove dust then air-dried for one week at room temperature until constant weight and ground to fine powders. All samples were stored in an airtight container at -20°C until further use.

2.2. CHEMICAL CHARACTERISATION OF *MORINGA* AERIAL PARTS

2.2.1. Proximate analysis of *Moringa oleifera*

The Ash, moisture, and fat contents of various powders of *MO* aerial parts were determined according to AOAC (1998) methods. Protein content was determined using the AOAC (1990) procedure using the conversion factor of 6.25 as mentioned by [7]. The carbohydrate was determined according to [8].

2.2.2. Energetic value

The energetic value was calculated according to the Regulation (EC) No. 1169/2011 of the European Parliament and Council as follows:

Energy (kcal/100 g (DW)) = 4 × (g protein + g carbohydrate) + 9 × (g fat).

2.2.3. Determination of amino acids composition

The *MO* samples were hydrolysed using chlorhydric acid for 24h at 105°C. After cooling, sodium hydroxide was added to neutralise the extract. Finally, the filtrated extract was injected to the HPLC-FLD (Agilent Technology, USA). The online amino acid derivatisation reaction was carried out with O-Phthaldialdehyde reagent solution (OPA). The column, used in this work, was column ZORBAX C18 (250×4.60 nm) with 5µm pore size. Due to the wide range of amino acids, it is necessary to perform a gradient to be able to separate them. The analysis was realised in gradient composed of an organic phase A (acetonitrile, methanol, water at 45/45/10 ratio) and an organic phase B (Na₂HPO₄ adjusted with phosphoric acid to pH = 6.5). The flow rate was 1ml/min. The HPLC conditions were adopted as described in the study of [9] with some modifications.

2.2.4. Determination of mineral composition

Mineral elements Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn) were identified by acid mineralisation according to the method described by [10] using an Atomic Absorption Spectrometer. In fact, 2.5 g of raw material was heated in two steps. First, calcination was performed at 500°C to obtain a constant mass.

Second, the resulting ash was burned at 600°C. The obtained ash was dissolved in 10 mL of 40% nitric acid and mixture was heated to obtain wet salts. Subsequently, the solute was dissolved in 15 mL of nitric acid (1 N) and transferred to a 25 mL volumetric flask for analysis.

After mineralisation, potassium (K) and sodium (Na) were determined using the flame photometer. Calcium (Ca) and magnesium (Mg) were determined by atomic absorption. Phosphorus (P) was assayed at 880 nm by the molybdenum blue method.

2.3. PHYTOCHEMICAL CHARACTERISATION

2.3.1. Preparation of *Moringa oleifera* extracts

The *MO* aerial parts seeds, flowers and leaves extracts were prepared according to [11] method. Thirty grams of each sample were mixed with 300 ml of solvent (ethanol Sigma Aldrich) and kept for 24 h à 4°C. The extracts were filtrated with Whatman filter paper No.4 then concentrated at 40°C-50°C using a rotary vacuum evaporator [6].

2.3.2. Determination of total phenolic, flavonoid, and condensed tannins contents

Quantification of total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent according to [12]. Briefly 100 µl of extract were mixed with 500 µL of the Folin-Ciocalteu reagent and 1.5 mL of sodium carbonate (20% w/v). Then, 10 ml of distilled water were added. After keeping the mixture in the dark for 2 h the absorbance was determined at 765 nm using a spectrometer (Jenway 6352 spectrophotometer). The total phenolic content was expressed as mg of gallic acid equivalents per g of dry matter (mg GAE/g DM) according to the standard curve prepared with different concentrations of gallic acid (0.3 M).

Flavonoids content (FVT) was evaluated in each part following the colorimetric method described by [12]. For this, one mL of each extract was mixed with 1 mL of ethanolic solution of aluminium trichloride (AlCl₃; 2% w/v in ethanol). The mixture was incubated for 15 min at room temperature before measuring the absorbance at 430 nm. Total flavonoids were expressed as mg of quercetin equivalents per gram of dry matter (mg QE/g DM), through the calibration curve of quercetin. The absorption of standard quercetin solution was measured under the same conditions.

The determination of condensed tannins (TTC) was carried out using the vanillin method as described by [13]. Two mL of each extract were added to 4 mL of vanillin (1% w/v) in 7 M sulfuric acid [H₂SO₄]. The mixture was maintained at 25°C for 15 min before the assay. The absorbance was measured at 500 nm against a blank. The results were expressed in mg of catechin equivalent per gram of dry matter (mgCE/g DM).

Determination of free radical scavenging of *Moringa oleifera* aerial parts

The free radical scavenging potency of different ethanolic *Moringa* extract was determined by using DPPH according to the method proposed by [14]. A fresh solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was prepared with absolute ethanol at a concentration of 0.2 mM. Briefly, *Moringa* ethanolic extracts with different concentrations was mixed with the DPPH solution in 1:1 ratio. Finally, the mixture was placed in the dark for 30 min and the absorbance was determined at 517 nm. The control samples were prepared using the DPPH solution mixed with ethanol. The results were determined using the standard curve and expressed as IC₅₀ (µg/ml) as the effective concentration of the sample that is required to reduce 50% of the initial DPPH free radicals as mentioned by [15].

3. CHARACTERISATION OF EXTRACTED OILS OF MORINGA OLEIFERA AERIAL PARTS

3.1. OIL EXTRACTION

The extraction of lipid fractions from *MO* aerial parts was carried out using two methods. Seed, leaf, and flower oils were extracted by the soxhlet method using hexane according to [5]. The temperature was set at 50°C for 6 hours than the solvent was removed from oil recovered in the rotary evaporator. The residue oil was kept in opaque vials at -20°C for further investigation.

Moringa seed oil was also obtained by cold press extraction using a Komet DD 85 G vegetable oil screw press (IBG Monforts Oekotec GmbH & Co. KG, Monchengladbach, Germany). The remaining oil was filtrated to remove plant debris and stored under the same conditions until analyses as mentioned by [16].

3.2. DETERMINATION OF FATTY ACIDS COMPOSITION

Fatty acid composition of *Moringa* oils involved their esterification determined using a gas chromatography system (Agilent HP 6890) equipped with flame-ionisation detector (FID) set at 260°C and an Rtx-2330 capillary column (90% biscyanopropyl /10% phenylcyanopropyl polysiloxane, 30 m × 0.32 mm, 0.2 µm film thickness) as explained by [16]. Nitrogen served as the carrier gas with a flow rate of 1.2 mL/min. A total of 1 µL of the sample was injected in splitless mode at a temperature of 240°C. The oven temperature was programmed to increase from 100 to 230°C at a rate of 4°C/min.

Fatty acids' peak identification was conducted referred to the retention times of a mixture of pur standards (CRM47885, Supelco 37 Component FAME Mix).

3.3. CHEMICAL COMPOSITION OF COLD PRESSED MORINGA SEED OIL

The cold pressed seed oil having the best yield of extraction and the more interesting fatty acid com-

position was analysed for its chemical composition. Peroxide value (PI), iodine value, saponification value, specific gravity (using a 10 mL pycnometer at 25°C), and the refractive index at 20°C as described by [16]. Specific absorptivity values K_{232} and K_{270} were determined by using an ultraviolet (UV) spectrophotometer, chlorophylls and carotenoids content were determined respectively as described by [17].

3.4. DETERMINATION OF TOCOPHEROL COMPOSITION

The analytical procedure for determination of tocopherols in cold pressed *Moringa* seed oil involved the use of high-performance liquid chromatography (HPLC) procedure as described by [5] in which 4g oil sample were dissolved in 25 ml of n-Heptane. The detector was set at 295 nm excitation wave lengths whereas the emission wavelength was 330 nm. The injection volume was 20 µl. The rate flow was set at 1 ml/min. n-Heptane and tetrahydrofuran (3.85%) were used as mobile phase. Standard solutions of α, β, γ and δ-tocopherols, formulated using 3–6 µg / mL standard concentrations, were used for quantification purposes.

3.5. DETERMINATION OF STEROL COMPOSITION

The estimation of sterol content involved saponification of 250 mg oil sample using a potassium hydroxide ethanolic solution by boiling under reflux according to [5]. The unsaponifiable matter are derivatized into trimethylsilyl ethers and analysed by capillary DB-5 Agilent column (5% phenyl methyl polysiloxane, 30 m × 0.32 mm internal diameter, 0.25 mm film thickness) gas chromatography (Agilent, HP 6890 series) with split injection and flame ionisation detector according to (COI, 2017) using. Helium was used as a carrier gas at a flow of 2 ml/min. The injector/detector temperatures were held at 280 and 290°C, respectively. The column temperature was set to 240°C and it was next raised to 260°C at a rate of 4°C /min.

4. STATISTICAL ANALYSIS

All the assays were carried out in triplicate. The results were presented as mean values and standard deviation. A Duncan test was used to assess significant differences among plant samples with $\alpha = 0.05$. The analysis was carried out using SPSS V.22.0 IBM SPSS Statistics.

5. RESULTS AND DISCUSSION

5.1. CHEMICAL CHARACTERIZATION OF MORINGA OLEIFERA AERIAL PARTS

5.1.1. Proximate composition

The proximate composition of Tunisian *Moringa oleifera* Lam flowers leaves and seeds is summarised in Table I.

In this study it was shown that carbohydrates were

Table I - Proximate composition of *Moringa oleifera* aerial parts

Parameters	<i>Moringa oleifera</i> aerial parts		
	Leaves	Flowers	Seeds
Moisture (%)	4.43 ± 0.31 ^a	7.02 ± 0.30 ^b	4.12 ± 0.33 ^a
Ash (%)	15,15 ± 0,03 ^c	9,53 ± 0,02 ^b	4,44 ± 0,03 ^a
Crude fat (%)	4,36 ± 0,11 ^b	3,2 ± 0,23 ^a	30,32 ± 0,08 ^c
Crude protein (%)	25,21 ± 0,03 ^b	21,37 ± 0,03 ^a	33,45 ± 0,04 ^c
Carbohydrate (%)	50,85 ± 0,03 ^b	57,4 ± 0,03 ^c	25,62 ± 0,03 ^a
Energetic value (Kcal/100g)	343,48 ± 0,27 ^a	343,88 ± 0,15 ^a	509,16 ± 0,05 ^b

^{a,b,c}: mean values with lowercase letters show a significant difference ($P < 0.05$) between the analysed different samples.

the major compounds in all *Moringa* aerial parts with the major content registered in flower powder (57.4±0.03%). The carbohydrate content was estimated at about 50.85±0.03% and 25.62±0.03%, respectively in leaves and seeds. More seed powder is a very rich source of proteins with about 33.45±0.04% followed by leaf (25.21±0.03%) and flower (21.37±0.03%) powders. This finding was partially in concordance with those 32.9% - 38.3% reported in other studies showing that *Moringa oleifera* seeds are considered as a good source of protein and amino acids [18]. The results obtained on *Moringa oleifera* leaf powder were higher than those (24.84%) found by [19]. The protein content of flowers was lower than that (47.97%) reported by [20].

In this study, the lipid content in leaves, flowers, and seeds of *Moringa oleifera* were respectively about 4.36±0.11%, 3.2±0.23% and 30.32±0.08%. These results agreed with those reported by [21]. However, seed fat content was lower than that (41.7%) reported by [16].

In fact, it was shown that aerial parts were rich in mineral. In fact, leaves showed the highest content (15.15±0.03%) followed by flowers (9.53±0.02%) and seeds (4.44±0.03%). These results were similar to those by [22]

showing similar mineral content varying between 3.9% and 4.4% in *MO* seeds. Concerning the caloric value of leaf, flower, and seed powder of *Moringa oleifera*, it was around 343.48±0.27 Kcal, 343.88±0.15 Kcal and 509.16±0.05 Kcal, respectively. These values were higher than those of some conventional fruits such as mango (73.9 Kcal), pomegranate (80.6 Kcal), kiwi (60.5 Kcal) and papaya (42.2 Kcal) (Anses, 2020). This variation in term of *MO* composition is assigned to different factors, such as the climatic conditions, growing sites, agricultural practices, harvesting period, and genetic characteristics [3].

5.2. AMINO ACIDS COMPOSITION

The results of the essential and non-essential amino acid composition of *Moringa oleifera* aerial parts are shown in Table II.

These results reveal the highest amount of glutamic acid as non-essential amino acid in *Moringa* seed powder with a content of about 2103.24 mg/Kg followed by arginine, threonine, and glycine (1202.01 mg/kg). However, the dominant amino acids in leaf powder were tyrosine 1527.71 mg/kg, leucine 903.14 mg/kg, glutamic acid 782.88 mg/kg. Similarly,

Table II - Amino acids composition (mg/Kg) of *Moringa oleifera* aerial parts

Amino Acids (mg/Kg)	Aerial parts of <i>Moringa Oleifera</i>		
	Seed powder	leaf powder	flower powder
Aspartic Acid	498.96 ± 0.73 ^b	595.87 ± 0.68 ^c	325.4 ± 0.91 ^a
Glutamic Acid	2103.24 ± 0.65 ^c	782.88 ± 0.67 ^b	408.32 ± 0.80 ^a
Serine – Histidine- glutamine	518.69 ± 0.76 ^c	508.13 ± 0.68 ^b	228.6 ± 0.76 ^a
Arginine- Threonine-glycine	1202.01 ± 0.83 ^c	754.36 ± 0.56 ^b	387.1 ± 0.79 ^a
Alanine	816.75 ± 0.67 ^c	516.71 ± 0.56 ^b	352.12 ± 0.87 ^a
Lysine	ND	ND	306.39 ± 0.58
Tyrosine	ND	1527.54 ± 0.87 ^b	1234.5 ± 0.56 ^a
Isoleucine	415.96 ± 0.86 ^c	262.7 ± 0.90 ^b	170.53 ± 0.84 ^a
Phenylalanine	246.72 ± 0.45 ^c	164.66 ± 0.68 ^b	121.22 ± 0.55 ^a
Leucine	1187.98 ± 0.83 ^c	903.14 ± 0.91 ^b	580.72 ± 0.40 ^a
Valine+ Methionine	319.96 ± 0.82 ^b	354.31 ± 0.47 ^c	173.12 ± 0.27 ^a

ND: not detected; ^{a,b,c}: mean values with lowercase letters show a significant difference ($P < 0.05$) between the analysed different samples.

11 amino acids were identified in the flower powder with a high amount of tyrosine of about 1234.5 mg/kg. Lysine was only detected in the flower powder. Moreover, the essential amino acids contents were significantly higher in leaves and seeds when compared to flowers. These findings confirmed that the different parts of *MO* are valuable sources of essential amino acids showing their importance for human or animal nutrition.

5.3. MINERAL COMPOSITION

Data in Table I show that *MO* dry leaves had a high amount of ash, resulting in a high content of P (2565 mg/kg), Mg (4308 mg/kg), Ca (9220 mg/kg), Zn (233 mg/kg), K (16584 mg/kg), Na (1443 mg/kg), Fe (310 mg/kg), and Mn (60.60 mg/kg) as shown in Table III. This result showed that the leaves are a good source of mineral mainly Ca, Mg and K which was in agreement with results published by [23] who found remarkable high amounts for Mg (4036 mg/kg) and K (14988 mg/kg). However, the mineral content in seeds was significantly lower than of leaves. Minerals composition of *MO* flower powder was partially in accordance with that reported by [20]. The high amount of minerals found in this study in the *MO* aerial parts suggested that they can contribute to maintaining the body regulatory functions.

5.4. TOTAL PHENOLIC, FLAVONOIDS AND CONDENSED TANNINS CONTENTS

The phytochemical composition of *Moringa oleifera* was shown in Table IV. In this study, the content of

total phenolic compounds in the *M. oleifera* leaf extract was 58.77 ± 0.59 mg GAE/g DM. This result was slightly lower than that found (62.33 mg GAE/g DM) by [24] and higher than the value (49.29 mg GAE/g DM) reported in *Moringa* leaves extract by [19]. For the total polyphenols content in seeds extract it was about 19.51 ± 0.21 mg GAE/g DM. This content was quite similar to that noted by [25]. This variation was attributed to various factors specific to the plant such as the degree of maturation as well as genetics, climatic and environmental conditions [26]. Indeed, the quantity of extracted polyphenols was directly affected by their location in the plant and by the polarity of solvents [27].

Moreover, the concentration of flavonoids in the leaves of *M. oleifera* was around 77.13 ± 0.005 mg QE/g DM. This finding was significantly important than that (29.90 mg QE/g DM) found by [24]. The obtained flavonoids content in *Moringa* seeds (26.75 ± 0.41 mg QE/g DM) was significantly lower than that (144.07 mg QE/g DM) indicated by [28]. Same results were found by [1] in *MO* extracts showing that total phenol and flavonoid contents were significantly ($P < 0.05$) higher in the leaf extract when compared to seed one. Concerning the condensed tannins content Tunisian *Moringa* seeds had a high content of condensed tannins of around 40 ± 1.76 mg EC/g DM when compared to the value (27.62 mg EC/g DM) [27]. This variability in terms of phytochemical composition of *Moringa oleifera* aerial parts could be attributed to the variation in the environment where the plant was collected, the season and the physiological stage of the plant [29].

Table III - Mineral composition (mg/Kg) of aerial parts of *Moringa oleifera*

Minerals composition (mg/Kg)	Aerial parts of <i>Moringa oleifera</i>		
	Leaves	Flowers	Seeds
K	16584±1.21	-	-
Na	1443±0.52	-	-
Ca	9220±0.69 ^c	4370±0.36 ^b	1950±1.09 ^a
Mg	4308±0.35 ^c	2280±0.43 ^a	2750±0.46 ^b
P	2565±0.17	-	-
Fe	310±0.33 ^c	172.4±0.36 ^b	52.25±0.40 ^a
Mn	60.60±0.51 ^c	30.20±0.36 ^b	11.65±0.52 ^a
Cu	2.65±0.35 ^a	6.75±0.17 ^b	ND
Zn	31.80±0.07 ^c	12.55±0.36 ^a	31.60±0.11 ^b

^{a,b,c}: mean values with lowercase letters show a significant difference ($P < 0.05$) between the different analysed samples.

Table IV - phytochemical composition of *Moringa oleifera*

Extract	PPT (mg GAE/g DM)	FVT (mg QE/g DM)	TC (mg CE/g DM)
Leaves	58.77 ±0.59 ^b	77.13 ±0.005 ^c	41.95 ±0.04 ^a
Flowers	17.82 ±0.04 ^a	22.85 ±0.03 ^b	82.90 ±0.07 ^c
Seeds	19.51 ±0.21 ^a	26.75 ±0.41 ^b	39.33 ±0.55 ^c

PPT: total polyphenols, FT: total flavonoids, TC: condensed tannins, GAE: gallic acid equivalent, CE: catechin equivalent, QE: quercetin equivalent, DM: dry matter, ^{a,b,c}: mean values with lowercase letters show a significant difference ($P < 0.05$) between the different analysed samples.

Table V - Antioxydant activity of *Moringa oleifera* aerial parts

Samples	IC ₅₀ µg/ml
<i>Moringa oleifera</i> leaf extract	39 ±0.03 ^a
<i>Moringa oleifera</i> flower extract	94.42 ±0.04 ^c
<i>Moringa oleifera</i> seed extract	83.59 ±0.59 ^b

^{a,b,c}: mean values with lowercase letters show a significant difference (P<0,05) between the different samples analysed.

ANTIOXYDANT ACTIVITY

For an effective and complete evaluation of the antioxidant potential of *Moringa oleifera* seed, leaf and flower extracts, the results of scavenging free radicals are shown in Table V.

These results obtained showed anti-radical capacity of leaves with a value of IC₅₀ about 39±0.03µg/ml. This result was in disagreement with that of [19] showing a lower leaf anti radical capacity with IC₅₀ value of around 60.07µg/ml. More, the antioxidant activity of *Moringa* leaves was higher than that of the seeds and flowers. In fact, the result indicated that the antioxidant activity of the ethanolic extract of *Moringa* seeds showed a good capacity on the free radical DPPH (IC₅₀ = 83.59±0.59µg/ml) when compared to that (280±0.05 µg/ml) found by [25]. In this study, ethanolic flower extract showed an important anti radical activity (IC₅₀ = 94.42µg/ml). These findings were significantly lower than those obtained by

[1] and [30] and they were attributed to the difference in geographical locations and climatic factors [6]. In this context natural antioxidants are always extremely important for health. They prevent any bad effects of free radicals [14].

6. CHARACTERISATION OF MORINGA OLEIFERA EXTRACTED OILS

6.1. FATTY ACIDS COMPOSITION

The main fatty acids composition in different extracted oils from *M.oleifera* aeriels is shown in Table VI.

In this study 10, 8 and 11 major fatty acids were identified respectively in *MO* leaf, seed and flower extracted oils. It was shown that the leaf oil was mainly composed of 44.68% of linolenic, followed by 18.39% of palmitic and 10.4% of linoleic acids. The result obtained was in accordance with those found by [21] reporting the same fatty acid composition with respective close levels. Concerning flower oil, the fatty acid composition showed the dominance of unsaturated fatty acids (58.63%) with C18:1 the prominent fatty acid (40.31%). Concerning seed oils extracted with cold press and soxhlet method, they showed the same acidic composition with respective high level of monounsaturated fatty acids about (81.54%) and (80.64%). Besides, the predominant poly-unsaturated fatty acids were linoleic acid, linolenic acid, followed by palmitic acid. These results were similar

Table VI - Fatty acids (%) composition of *Moringa oleifera* oil

Fatty acids (%)	Cold press extraction	Soxhlet extraction		
	Seed oil	Seed oil	Leaf oil	Flower oil
Lauric Acid: C _{12:0}	Nd	Nd	1.33±0.06	Nd
Myristic Acid: C _{14:0}	0.18±0.005 ^a	0.15±0.01 ^a	2.47±0.11 ^c	1.07±0.06 ^b
Myristoleic Acid: C _{14:1}	Nd	Nd	Nd	0.54±0.02
Palmitic Acid: C _{16:0}	6.65±0.46 ^a	6.03±0.46 ^a	18.39±0.06 ^c	13.23±0.11 ^b
Palmitoleic Acid: C _{16:1}	1.78±0.46 ^a	2.25±0.06 ^b	2.22±0.03 ^b	1.58±0.11 ^a
Margaric Acid: C _{17:0}	0.08±0.11 ^a	Nd	2.01±0.17 ^b	0.1±0.006 ^a
Margaroleic Acid :C _{17:1}	0.07±0.006 ^a	Nd	Nd	0.52±0.006 ^b
Stearic Acid: C _{18:0}	5.07±0.28 ^b	5.30±0.11 ^b	3.22±0.06 ^a	6.07±0.03 ^c
Oleic Acid: C _{18:1}	81.54±0.11 ^d	80.64±0.17 ^c	7.92±0.11 ^a	40.31±0.06 ^b
Linoleic Acid: C _{18:2}	0.86±0.02 ^a	0.82±0.01 ^a	10.4±0.17 ^c	6.47±0.11 ^b
Linolenic Acid: C _{18:3}	0.32±0.02 ^a	0.45±0.02 ^a	44.68±0.35 ^c	9.21±0.11 ^b
Arachidic Acid: C _{20:0}	2.27±0.06 ^a	2.41±0.03 ^a	7.33±0.11 ^b	20.87±0.17 ^c
Gadoleic Acid :C _{20:1}	1.20±0.13	Nd	Nd	Nd
MUFA	84.59±0.30 ^d	82.89±0.23 ^c	10.14±0.14 ^a	42.95±0.17 ^b
PUFA	1.18±0.04 ^a	1.27±0.03 ^a	55.08±0.52 ^c	15.68±0.23 ^b
ω ₆ /ω ₃	2.68±0.14 ^d	1.82±0.07 ^c	0.23±0.002 ^a	0.70±0.004 ^b
SAFA	14.24±0.80 ^a	13.89±0.62 ^a	33.42±0.36 ^b	41.34±0.38 ^c
Total	100.01±1.14 ^a	98.05±0.89 ^a	99.97±1.08 ^a	99.97±0.80 ^a

ND: not detected; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; Values are means± S three determinations. ^{a,b,c,d}: mean values with lowercase letters show a significant difference (P<0,05) between the different samples analysed.

to those reported by [3] confirming that seed oil was rich in unsaturated fatty acids and that it was similar to olive oil due to its richness mainly in oleic acid [31] which makes it more desirable and more stable when cooking and frying [7]. Furthermore, it should be highlighted that there is no significant difference in terms of fatty acids composition between the extracted seed oil using a different method. This finding agreed with that of [7]. But it should be noted that the cold press process has several advantages over solvent based systems, such as being environment friendly and requiring lesser energy [5]. Furthermore, saturated fatty acids were also detected with minor levels in cold pressed (14.09%) and in soxhlet (9.53%) extracted seed oils. In fact, myristic acid (C14:0) was identified in seed oils extracted using the two methods. However, margaric acid (C17:0) was detected only in cold pressed *Moringa* seed oil with a minor level (0.08%). This result was in perfect agreement with another study [36]. However, these contents did not exceed 15% of the total fatty acid composition which prevent LDL cholesterol, cardiovascular diseases, improve digestion and modulate arterial pressure and blood viscosity [7]. In addition, ω_6 / ω_3 ratio was around 2.68 which must not exceed 4 as recommended by AFSSA in all analysed samples as reported by [32].

6.2. CHARACTERISATION OF COLD PRESSED MORINGA SEED OIL

6.2.1. Tocopherol composition

The relative result to tocopherol composition (Table VII) of cold pressed *Moringa oleifera* seed oil showed the presence of alpha, gamma, and delta tocopherols. Beta tocopherol was not detected in the analysed oil.

Considering these results, the alpha tocopherol was the predominant form of vitamin E in this oil (243.51 mg/kg). This has been reported in most studies. In fact, [33] and [7] found alpha tocopherol contents of *Moringa* seed oil in order of 168.2 and 226.9 mg/kg, respectively. Also, the obtained gamma tocopherol (112.6 mg/kg) and delta tocopherol contents (11.65 mg/kg) were higher than those reported in literature. Additionally, most vegetable oils contain alpha, beta, and gamma tocopherols.

Delta tocopherol exists only in a few vegetable oils such as cotton seed, wheat germ, soybean, castor, and peanut oils. This richness of *Moringa oleifera* oil in tocopherols gives it some protection during storage and processing [34].

6.2.2. Sterol composition

The analysis of the sterol fraction of *Moringa* seed oil revealed the presence of 10 compounds consisting mainly of β -sitosterol, stigmasterol and campesterol representing 90% from total sterols as shown in Table VIII.

These sterols are part of the class of phytosterols

known for their hypocholesterolemic and beneficial effect on human health [7]. It was noticed that *Moringa* seed oil has a remarkable similarity with the soybean oil in terms of major sterol fractions (β sitosterol, stigmasterol, campesterol, avenasterol). Knowing that β -sitosterol is the most representative phytosterol of vegetable oils [35], this study confirmed the observations reported by [16] on the richness of *Moringa* oil in phytosterols. In contrast, the other detected sterol fractions were only present in trace amounts. These findings reported the very interesting nutritional quality of cold pressed *Moringa oleifera* seed oil showing a good climatic, cultivation and storage conditions of seeds.

6.2.3. Physicochemical characterisation of *Moringa* cold pressed seed oil

The physicochemical characterisation of *M.oleifera* cold pressed seed oil is illustrated in Table IX.

Table VII - Tocopherol composition (mg/Kg) of *Moringa* cold pressed seed oil

Tocopherols (mg/Kg)	Cold pressed seed oil
Alpha tocopherol	243.51±0.49
Gamma tocopherol	112.6±0.1
Delta tocopherol	11.65±0.2

Table VIII - Sterols (%) composition of cold pressed seed oil

Sterols (%)	Cold pressed <i>Moringa</i> seed oil
Stigmasterol	25.04±1.06
Campesterol	23.16±0.81
β -sitosterol	50.65±0.65
Chlerosterol	0.395±0.01
Δ 7 avenasterol	0.29±0.01
Δ 5.23 stigmastadienol	0.275±0.01
Δ δ 7stigmastenol	0.215±0.004
Erythrodiol	0.11±0.01
Uvaol	0.05± 0.01

Table IX - Physicochemical characterization of *M.oleifera* cold pressed seed oil

Parameters	Values
Refractive index (20°C)	1.466±0.03
Acid value(mg KOH/ g oil)	0.84±0.03
Saponification value(mg KOH/ g oil)	187.3±2.10
Iodine value (g I ₂ / 100g oil)	64.42 ±0.21
Peroxide value (meq O ₂ /kg oil)	2.45±0.32
Chlorophyll mg/Kg	1.56±0.01
Carotenoid mg/Kg	3.26±0.03
K ₂₃₂	1.22±0.02
K ₂₇₀	0.059±0.01

In this study, the IR value obtained (1.466 ± 0.03) was close to that of olive oil which varies between 1.467 and 1.470. In fact, this result was in agreement with those observed by [16] and [36] reporting RI of about 1.467 and 1.462 respectively for *Moringa oleifera* seed oils. This value was also similar to those recorded on avocado oil (1.465 - 1.474). The analysed specific extinction coefficients K232 (1.22 ± 0.02) and K270 (0.059 ± 0.01) showed an oxidative stability of cold pressed *Moringa* seed oil thanks to its natural antioxidants. The results registered were comparable to those found by [16] who highlighted K232 and K270 values of 1.17 and 0.043 respectively. The low acid value of this oil (0.84 ± 0.03 mg KOH/g oil) showed its good stability. It was also lower than that (1.33 ± 1.15 mg KOH/g of oil) found by [36]. This difference could be explained by the hydrolysis reaction of lipids during the grinding of seeds [7]. The peroxide index (PI) was related to storage conditions and extraction methods. It was noted that PI value of *Moringa* oil was in order of 2.47 ± 0.32 meq O₂/Kg. This result was lower than (10 meq O₂/Kg) required by the Codex Alimentarius, (1992) for most conventional oils. Also, the work carried out by [37] recorded peroxide index value ranging from 3.3 to 4.5 meq O₂/Kg for the oil extracted from pomegranate seeds of different Tunisian varieties. This lower value registered for the PI in *MO* seed oil could be attributed to the richness of this oil in natural antioxidants such as tocopherols and fat-soluble vitamins [7]. The saponification index was related to the length of the fatty acids. The saponification index recorded for the analysed *Moringa* oil was around 188 ± 2.14 mg of KOH/g of oil. This value was close to olive oil ranging between 184 and 196 mg KOH/g oil. This result was like that (185 mg KOH/g oil) found by [36]. The iodine value indicates the level of oleic and linoleic acids present in the oil. Indeed, this index increased with number of long chain fatty acids [7]. In this study the iodine index (64.53 ± 0.25 g/100 g) of *Moringa* seed oil was lower than that (67.42 g/100 g) showed by [16]. The carotenoid and the chlorophyll contents of the cold pressed *Moringa* seed oil was about 3.26 mg/kg and 1.56 mg/kg respectively. These levels were partially in accordance with those reported by [38] showing carotenoid content of about 4 mg/Kg. However, the chlorophylls were not detected. It should be noted that chlorophylls and carotenoids were the major pigments of cold pressed *Moringa* seed oil and they were responsible for the oil colour [16]. Moreover, these pigments were known by their antioxidant activities and their capacity to prevent cardiovascular and eye diseases [17].

7. CONCLUSIONS

In short, the characterisation of Tunisian *Moringa oleifera* aerial parts revealed that seeds are considered as a good source of protein, and fat. Leaves

are rich in ash and mineral. Moreover, *Moringa* leaves and seeds contain good amount in essential amino acids, which confirm their higher nutritional value. The *Moringa* aerial parts showed also good antioxidant activities due to their richness in natural bioactive compounds. On the other hand, the chemical composition of cold pressed seed oil was better than that extracted using the soxhlet method. Oleic acid, beta-sitosterol, and alpha-tocopherol were the dominant compound in *Moringa* cold pressed seed oil which may lead to interesting biological and therapeutic properties when used. This promising result encourages the use of *Moringa* powder and extracted seed oil in cosmetic, pharmaceutical medicinal and food industries.

REFERENCES

- [1] M. Prabakaran, S.H. Kim, A. Sasireka, M. Murugesan Chandrasekaran, M. Chung, Polyphenol composition and antimicrobial activity of various solvent extracts from different plant parts of *Moringa oleifera*, Food Bioscience, (2018).
- [2] E. L. Gómez-Martínez, A. L. San Martín-López, M. J. García-Vásquez, A. García-Rocha, L. V. Mendoza-García, A. San Martín-López, Impacto psicosocial de l'estética dental en alumnos con maloclusiones del Telebachillerato Coxquihui, Veracruz. *Revista Mexicana de Medicina Forense y Ciencias de la Salud*, 4(S1), 54-57, (2020).
- [3] A. Fernandes, A. Bancessi, J. Pinela, M. Inês Dias, A. Liberal, R.C. Calhelha, A. Ćirić, M. Soković, L. CATARINO, I. C.F.R. Ferreira, L. Barros, Nutritional and phytochemical profiles and biological activities of *Moringa oleifera* Lam. edible parts from Guinea-Bissau (West Africa), *Food Chemistry*, (2020).
- [4] A. Sodamade, O.S. Bolaji, O.O. Adeboye, Proximate Analysis, Mineral Contents and Functional Properties of *Moringa oleifera* Leaf Protein Concentrate. *Journal of Applied Chemistry*, 4, 47-51, (2013).
- [5] M. M. Özcan, K. Ghafoor, F. Al Juhaimi, I. A. M. Ahmed, E. E. Babiker, Effect of cold-press and soxhlet extraction on fatty acids, tocopherols and sterol contents of the *Moringa* seed oils. *South African Journal of Botany*, 124, 333-337, (2019).
- [6] N. Bennour, H. Mighri, H. Eljani, T. Zammouri, A. Akrouf, Effect of solvent evaporation method on phenolic compounds and the antioxidant activity of *Moringa oleifera* cultivated in Southern Tunisia, *South African Journal of Botany*, Volume 129, 2020, Pages 181-190, ISSN 0254-6299, (2020).
- [7] S. Louni, Extraction et caractérisation physico-chimique de l'huile de graines de *Moringa Oleifera*. E.N.S.A d'El-Harrach, Algérie. (Mémoire en

vue de l'obtention du diplôme de Magister en Sciences Agronomiques), (2009).

- [8] A. Korus, D. Gumul, M. Krystijan, L. Juszcak, J. Korus, Evaluation of the quality, nutritional value and antioxidant activity of gluten-free biscuits made from corn-acorn flour or corn-hemp flour composites. *European Food Research and Technology*, 243, 1429-1438, (2017)
- [9] X. Huang, S.J. Kao, J. Lin, X. Qin, C. Deng, Development and validation of a HPLC/FLD method combined with online derivatization for the simple and simultaneous determination of trace amino acids and alkyl amines in continental and marine aerosols. *PLoS ONE* 13(11): e0206488, (2018) <https://doi.org/10.1371/journal.pone.0206488>
- [10] Akzhigitova, Zhanna, Aizhamal Baiseitova, Moldyr Dyusebaeva, Yang Ye, and Janar Jenis, "Investigation of Chemical Constituents of *Artemisia Absinthium*". *International Journal of Biology and Chemistry* 11 (1):169-77,(2018).
- [11] J. L. Mau, G. R. Chao, K. T. Wu, Antioxidant properties of methanolic extracts from several ear mushrooms. *Journal of Agricultural and Food Chemistry*, 49 (11), 5461-5467, (2001).
- [12] A. Kumaran, R.J. Karunakaran, In Vitro Antioxidant Activities of Methanol Extracts of five *Phyllanthus* Species from India. *LWT—Food Science and Technology*, 40, 344-352, (2007).
- [13] S. Toda, Antioxidative effects of polyphenols from leaves of *Artemisia princeps* Pamp. on lipid peroxidation in vitro. *J Food Biochem*; 29:305–12,(2005).
- [14] N. Bhalla, N. Ingle, V. Srilakshmi Patri, D. Haranath, Phytochemical analysis of *Moringa Oleifera* leaves extracts by GC-MS and free radical scavenging potency for industrial applications, *Saudi Journal of Biological Sciences*, Volume 28, Issue 12, Pages 6915-6928, (2021).
- [15] I. Mahmoudi, O. Ben Moussa, M. Hassouna, Symbiotic, hypocholesterolemic and antioxidant effects of potential probiotic lactobacilli strains isolated from Tunisian camel milk. *Advances in Microbiology*, 7(4), 328-342, (2017).
- [16] K. Gharsallah, L. Rezig, K. Msaada, A. Chalh, T. Soltani, Chemical composition and profile characterization of *Moringa oleifera* seed oil. *South African Journal of Botany*, 137, 475-482, (2021).
- [17] M. Boulares, A. Bezzezi, M. Arfaoui, A. Boulares, M. Ghrab, O. Ben Moussa, M. Hassouna, S.Boudiche, Improvement of Tunisian 'Chemlali' extra virgin olive oil stability with rosemary and laurel herbs and essential oils, (2021).
- [18] Granella, An approach to recent applications of *Moringa oleifera* in the agricultural and biofuel industries, (2020)
- [19] D.E. Páramo-Calderón, A. Aparicio-Saguilána, A. Aguirre-Cruza, J. Carrillo-Ahumadaa, J.P.Hernández-Uribeb, S. Acevedo-Telloc, J.G. Torruco-Uco, Tortilla added with *Moringa oleifera* flour: Physicochemical, texture properties and antioxidant capacity. *Food Science and Technology*, (2019).
- [20] M. Ndong, S. Wade, N. Dossou, A.T. Guiro, R.D. Gning, Valeur Nutritionnelle du *Moringa Oleifera*, Étude de la Biodisponibilité du fer, Effet de L'enrichissement de Divers Plats Traditionnels Sénégalais Avec la Poudre des Feuilles. *African Journal of Food, Agriculture, Nutrition and Development*, (2007).
- [21] W. C. J. Sagona, P. W. Chirwa, S. M. Sajidu, The miracle mix of *Moringa*: Status of *Moringa* research and development in Malawi. *South African Journal of Botany*, 129, 138-145, (2020).
- [22] S. Boukandoul, S. Casal, F. Zaidi, *Moringa Oleifera* seed oil: Production, uses and health benefits. In: Hong, N.Kh.D. (Ed.), *Seed oil: Production, uses and benefits*. NovaScience Publishers, New York, 2018. 1–27 , (2019).
- [23] [F. H. El-Massry, M. E. MOSSA, S. M. YOUSSEF, *Moringa oleifera* plant. *Egyptian Journal of Agricultural Research*, 91(4), 1597-1909,(2013).
- [24] T. Prabsattroo, J. Wattanathorn, S. Iamsaard, *Moringa oleifera* extract enhances sexual performance in stressed rats. *J. Zhejiang Univ. Sci. B* 16, 179–190, (2015).
- [25] H. Barakat, G.A. Ghazal, Propriétés physico-chimiques de *Moringa Oleifera* Graines et leur huile comestible cultivées dans différentes régions d'Égypte. *Sciences de l'alimentation et de la nutrition*, 7, 472-484, (2016).
- [26] S. Mohamed, A. Youssef, M. Issa, H. Abdel Salam, A. El-Ansary, Validated HPLC Method for Quantitative Analysis of Gallic Acid and Rutin in Leaves of *Moringa Oleifera* Grown in Egypt. *Egyptian Journal of Chemistry*, 64(3), 1583-1591,(2021).
- [27] S. Djermoune, N. Henoune, Composition chimique et teneur en composés phénoliques des graines de *Moringa oleifera*. Mémoire de Fin de Cycle En vue de l'obtention du diplôme de Master of science. Département des Sciences Alimentaires, Université A. MIRA-Bejaia, Algérie 89 p, (2015).
- [28] W. R. Compaoré, P. A. Nikièma, H. I. N. Bassolé, A. Savadogo, J. Mouecoucou, Chemical composition and antioxidative properties of seeds of *Moringa oleifera* and pulps of *Parkia biglobosa* and *Adansonia digitata* commonly used in food fortification in Burkina Faso. *Current Research Journal of Biological Sciences*, 3(1), 64-72, (2011).
- [29] M. Saadoudi, L. Hambaba, M. Abdeddaim, A. Lekbir, A. Bacha, S. Boudraa, S. Zidani, nutritional composition, physical properties and sensory evaluation of biscuit produced from jujubes (fruits of *Zizyphus lotus* L.) *Annals. Food Science and Technology*, (2017).

- [30] S. Vats, T. Gupta, Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of *Moringa oleifera* Lam. from Rajasthan, India. *Physiol Mol Biol Plants* 23, 239–248, (2017).
- [31] R.S. G. Singh, P. S. Negi, C. Radha, Phenolic composition, antioxidant and antimicrobial activities of free and bound phenolic extracts of *Moringa Oleifera* seed flour. *Journal Of functional Foods*. 5: 1883 –1891, (2013).
- [32] M. Boulares, M. Mankai, S. El Adab, O. Ben Moussa, R. Teber, M. Hassouna, Anti-Listerial Activity of Oregano and Cinnamon Essential Oils in Vacuum Packed Ground Ovine Meat during Refrigerated Storage. *Journal of Agricultural Science and Technology*, 25(1), 61-73, (2023).
- [33] M. A. Salama, M. A. Owon, M. F. Osman, A. I. Esmail, B. Matthäus, Characterization of Egyptian *Moringa oleifera* lipids (whole seeds and kernels). *Journal of Food and Dairy Sciences*, 9(12), 379-384, (2018).
- [34] M. E. Cuvelier, M. N. Maillard, “Stabilité des huiles alimentaires au cours de leur stockage”, *Oléagineux. Corps Gras Lipides*, 19(2), 125132, (2012).
- [35] M. V. Aïssi, S. Mohamed, F.P. Tchobo, Etude comparative de la qualité des huiles végétales alimentaires raffinées en usage au Bénin. *Bulletin d’Informations de la Société Ouest Africaine de Chimie N°06 ; 25 – 3*, (2009).
- [36] A. Leone, A. Spada, A. Battezzati, A. Schiraldi, J. Aristil, S. Bertoli, *Moringa Oleifera* Seeds and Oil: Characteristics and Uses for Human Health: An overview. *Int. J. Mol. Sci*, 17, 2141. édition Maurizio Battino université de milan, Italie, (2016).
- [37] M. Khemakhem, Y. Zarroug, K. Jabou, S. Selmi, N. Bouzouita, Physicochemical characterization of oil, antioxidant potential, and phenolic profile of seeds isolated from Tunisian pomegranate (*Punica granatum* L.) cultivars. *Journal of Food Science* Vol. 0, Iss. 0, (2021).
- [38] N. Foidl, H. P. S. Makkar, K. Becker, The potential of *Moringa oleifera* for agricultural and industrial uses. In: *What development potential for Moringa products*. October 20th - November 2nd 2001. Dar Es Salaam, (2001).

Effects of different postharvest storage conditions of black cumin seeds on the oxidative stability of cold pressed oil

Derya Arslan*
Ayşenur Acar

Department of Food Engineering
Faculty of Engineering
Necmettin Erbakan University
Koycegiz Campus
Konya, Turkiye

The high peroxide values encountered in black cumin seed oil, even when obtained from newly harvested seeds can be closely related to the storage conditions of the seeds. The oxidative stability of black cumin seed oil from unroasted and mildly roasted (65°C) seeds was evaluated under three different storage conditions; refrigerated at 4°C, shaded and exposed to direct sunlight both at room temperature. Seeds were stored in airtight jars or tied cloth bags as two different storage materials under these conditions. Storage in jars under shaded storage and in cloth bags at refrigeration condition resulted in lower free fatty acidity in the oils. Shaded storage showed better results in terms of PV, TPC and DPPH (4 months). Refrigerated conditions in cloth bags provided higher values of total phenolics and DPPH radical scavenging activity for the unroasted seeds. Mild roasting produced oils with lower free fatty acidity, higher amounts of total phenolics and stronger DPPH radical scavenging activity, however reduced the oxidative stability. A positive correlation was detected between the peroxide values of the oil and the L* values of the oil, and a negative correlation with the induction time. Free fatty acidity and induction time of the oil obtained from minimally heat-treated seeds showed a strong negative correlation.

Keywords: Black cumin seed oil; Cold pressing; Oxidative stability; Peroxide value; Storage conditions

165

1. INTRODUCTION

For many years, black cumin seeds (*Nigella sativa* L.) have been used for medicinal and culinary purposes [1]. Black cumin seeds (BCS) contain a high amount of oil and have an important place in the formulation of cosmetic and dietary supplement products, as well as health and nutrition due to their phytochemical content (phenolic and bioactive compounds) [2].

Black cumin seed oil (BCSO) is usually extracted by cold pressing, which is an inexpensive, safer and simpler process than solvent extraction [3]. Since it is unrefined, cold-pressed BCSO contains high amounts of natural antioxidants and thymoquinone, which is a main bioactive component of BCSO [1,2]. Due to the increasing demand of consumers for safe and native food products, cold-pressing has become an alternative to conventional extraction procedures [1].

Lipid oxidation, which has adverse effects on human health and food quality, is one of the major causes for the loss of quality characteristics such as colour, odour, taste and nutritional value [1]. For this reason, some measures should be taken to reduce oxidation and increase the oxidative stability of lipid products [4]. Autoxidation and light-sensitive oxidation are responsible for the oxidation of edible oils during processing and storage. Due to the crude oils obtained after extraction not being processed, phenolic compounds with high stability against autoxidation and polar lipids are protected [5].

✉ CORRESPONDING AUTHOR:

Derya Arslan
NEÜ Köyceğiz Yerleşkesi, 42080 Meram
Konya, Turkiye
Email: dears@erbakan.edu.tr
Tel: +905355640994

ORCID ID
[https://orcid.org/
0000-0002-6655-9312](https://orcid.org/0000-0002-6655-9312)

Received: April 14, 2023
Accepted: February 9, 2024

Considering the results of many studies, it was understood that BCSO showed peroxide values varying between a wide range [6-10]. Notably some literature data are high in terms of peroxide value [11, 12]. We believe that these high peroxide values are due to storage conditions of BCS. Accordingly, the effects of factors such as storage temperature, storage media and packages (light exposure and air) and duration, which is thought to cause an increase in peroxide value of BCSO, were investigated.

Thus, in this study, BCSs were stored in cloth bags and jars under different conditions (refrigerator temperature, shaded at room temperature and directly sun exposure). The impact of these storage materials and conditions on oxidative stability and some quality characteristics of the corresponding oils was examined. On the other hand, effect of roasting was also evaluated with this regard, since roasting is a process with important contributions in oil extraction.

2. MATERIALS AND METHODS

2.1. BLACK CUMIN SEEDS

BCSs were obtained in a week after harvest in December 2019 from Konya, Turkiye. BCS (24 kg for each replicate) were brought to the laboratory having 3 replicates for each treatment. Equal batches (1 kg) of BCS were packed in two types of storage materials, sealed airtight jars and tied cloth bags, which were stored at three different conditions as 4°C in a laboratory refrigerator, shaded and exposed to direct

sunlight, both at room temperature (Fig. 1). Samples were analysed for oxidative stability on months 4 and 8 of storage time.

A mild roasting step was included in the experimental design for some batches of the stored seeds. For this purpose, water with a ratio of 10% of seed weight was homogeneously sprayed onto the ground seeds. Samples were incubated for 3 h, at 60°C and mixed at regular intervals.

2.2. OIL EXTRACTION FROM SEEDS BY COLD-PRESSING

BCSO was extracted using a manual screw press extractor (Karaerler NF 500, Turkiye, 1.5 kW power, 50 kg seed.h⁻¹ capacity, single head, 15 hz rotation speed). The temperature remained below 50°C during the extraction. The oils were filled in dark coloured glass bottles and kept at +4°C.

2.3. DETERMINATION OF FREE FATTY ACIDITY (FFA) AND PEROXIDE VALUES (PV) OF BCSO

FFA and PV were measured according to practices Ca 5a-40 and Cd 8b-90 recommended by AOCS, respectively [13]. PV was given as milliequivalent active oxygen per kg of oil (meq O₂/kg oil) while FFA was expressed as a percentage of oleic acid (% oleic acid).

2.4. OXIDATION STABILITY OF BCSO (RANCIMAT TEST)

Oxidative stability stated as the induction time (h) was evaluated with an accelerated controlled test

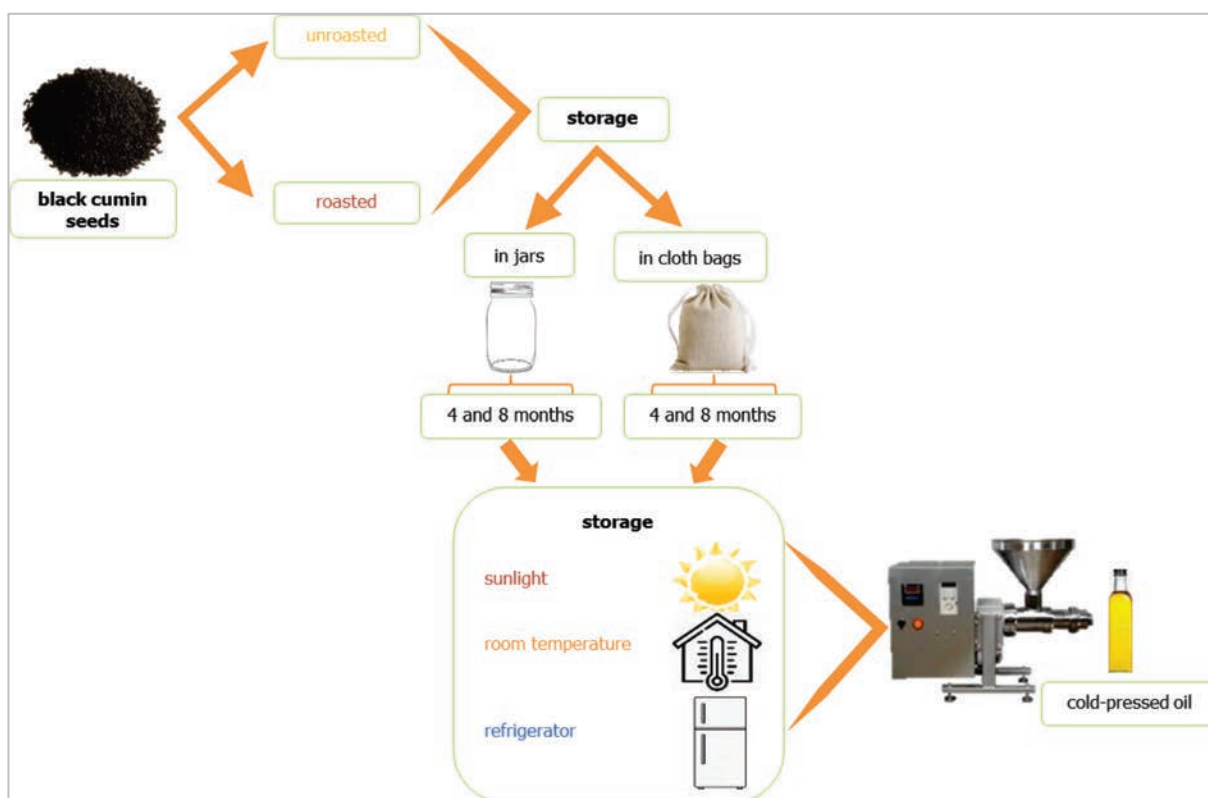


Figure 1 - Flow chart of the process of obtaining oil from BCSs stored at different conditions

on Rancimat device (Metrohm Co., Herisau, Switzerland). By passing an air flow of 20 L/h from inside 3 g oil heated at 120°C, volatile degradation products were kept in distilled water and the conductivity of the water increased [13].

2.5. COLOR MEASUREMENT

The colour of the oils was determined using the Minolta CR400 (Minolta Co., Osaka, Japan) instrument according to the Hunter colour system. Measurements were performed in triplicate [14].

2.6. TOTAL PHENOLIC COMPOUNDS (TPC)

n-hexane (5 mL) and methanol water (10 mL, 80:20, v/v) were added to 10 g BCSO and vortexed for 2 min. The mixture was centrifuged at 3000 rpm for 5 min and the extract was separated from the lipid phase through a separatory funnel. Extraction was performed by adding 3 mL of n-hexane and 5 mL of methanol water to the oily residue and this procedure was repeated. Purified phenolics were concentrated in a vacuum evaporator (Heidolph, Hei-Vap Core, Germany) at 40°C. The TPC of oil samples was determined by spectrophotometric method described previously by Rigane et al. [15].

2.7. 2,2-DIPHENYL-1-PICRYLHYDRAZYL RADICAL SCAVENGING ACTIVITY (DPPH-RSA) ANALYSIS

The antioxidant activity was evaluated by measuring the radical scavenging effect of BCSO phenolic extracts towards the DPPH radical [16]. 2 mL of DPPH and 0.9 mL of HCl buffer (pH 7.4) solutions were added to 0.1 mL of the BCSO extracts. The tubes were allowed to stand at room temperature for 30 min. The change in absorbance was determined at 517 nm in a spectrophotometer (Biochrom LibraS22, UK). DPPH-RSA was calculated as percent inhibition (%)

with the following formula:

$$\text{Inhibition \%} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right] \times 100.$$

2.8. STATISTICAL METHODS

The experimental procedure was conducted with three replications. Analysis of variance (ANOVA) was performed using a factorial experiment in a completely randomised design. Significant differences at 0.05 level were determined using Duncan's multiple range post hoc experiment. Pearson correlation analysis was conducted separately for the oil samples from roasted and unroasted seeds (SPSS 16.0 statistical software (Chicago, IL, USA)).

3. RESULTS AND DISCUSSION

Regarding the unroasted samples, at the end of 8 months of storage, free fatty acids were higher in sun exposed samples for both in jar and cloth bag storage. FFA was lower in cloth bags under refrigerated conditions. Lower moisture levels retained in cloth bag might have contributed to suppressing the FFA formation. The acidity was lower in the jar for 4 months of storage; however, it was impossible to reach this conclusion for 8 months of storage. In other words, a significant increase was detected in the samples stored in the jar at the 8th month compared to the 4th month. It can be said that roasting provides a slight decrease in acidity in general, which was most likely due to moisture loss during roasting regarding the stored seeds, but in the case of unroasted samples FFA of cold-pressed fresh seed oil was lower than the results of unroasted samples (Table I, Table II). According to Mazaheri et al. [17], the FFA of BCSO was comparatively high owing to the lipase activity, which was significantly decreased by pretreatments such as microwaving and roasting. For sto-

Table I - Effects of different storages condition on some analytical parameters of oil from unroasted BCSs (mean ± SD, n=3)

	Storage conditions	Storage time (month)	Free fatty acids (% oleic acid)	Peroxide value (meq O ₂ /kg oil)	Induction time (h)
	fresh seeds	0	8.72±0.07 ^{ht}	13.76±0.11 ^f	7.07±0.05 ^a
Cloth bag	sunlight	4	11.71±0.36 ^d	18.05±0.52 ^e	4.07±0.28 ^b
		8	12.15±0.08 ^c	29.34±0.20 ^a	3.16±0.26 ^d
	shaded	4	11.71±0.16 ^d	13.93±0.48 ^g	3.88±0.83 ^{bc}
		8	11.96±0.04 ^{cd}	25.26±0.82 ^c	3.33±0.11 ^{cd}
	refrigerator	4	10.35±0.10 ^f	19.27±0.69 ^e	3.96±0.45 ^{bc}
		8	10.57±0.09 ^{ef}	29.42±0.16 ^a	0.97±0.10 ^f
Jar	sunlight	4	11.66±0.15 ^d	25.25±0.54 ^c	4.35±0.16 ^b
		8	14.76±0.44 ^a	29.59±0.34 ^a	2.22±0.27 ^e
	shaded	4	9.15±0.07 ^g	22.80±1.80 ^d	3.85±0.30 ^{bc}
		8	10.82±0.22 ^e	26.77±0.25 ^b	2.04±0.32 ^e
	refrigerator	4	10.49±0.08 ^{ef}	15.43±0.51 ^f	3.42±0.26 ^{cd}
		8	12.63±0.39 ^b	29.39±0.67 ^a	1.73±0.20 ^e

† Small case letters within a column show significant differences between values belong to oils of samples stored at different conditions (P≤0.05).

rage in jars, the refrigerator was superior to the shaded storage with FFA values at levels as low as those of oil from the fresh sample. The negative effect of sun exposed storage became evident, especially after 8 months of storage. The hydrolysis and translation of triglycerides to free fatty acids during extraction and storage occur by hydrolytic reactions caused by the lipolytic activity of enzymes [3, 6].

The PVs of oils ranged between 15.43-29.59 meq O₂/kg (Table I). Considering the recommended PV of 10 meq O₂/kg [18] in edible oils, the high PV of BCSO even immediately after extraction is attributed to the lipoxygenase enzyme activity [17, 19]. On the other hand, Ramadan and Mörsel [20] attributed the high PV of black cumin oil to its relatively high free fatty acidity. High free fatty acidity increases the solubility of oxygen in oil due to the emulsion medium it creates. Indeed, also in this study, the free fatty acidity of the samples was considerably high with values varying between 8.85 and 13.14%.

For both roasted and unroasted samples, 4 months of shaded storage in a cloth bag had a significant suppressive effect on the increase of peroxide value even ensured that PV of unroasted seeds remained at almost the same value as the fresh sample. However, this effect was not observed in samples stored in jars. Regarding storage in jars, refrigeration provided lower peroxide formation compared to the sun exposed and shaded storage. These evaluations are relevant for the analysis performed on the 4th month, as the difference between PVs got closer each other at the 8th month. Roasting had no positive effect in terms of PV. In fact, in case of storage in cloth bags, unroasted samples showed lower PVs compared to the roasted samples (4th month values). Consistent with this, when the induction time is taken into ac-

count, it is obvious that the time was shorter for the roasted samples compared to the unroasted ones. A 4-month storage in jars under refrigerated conditions resulted in the lowest PV values. At the end of 8 months of storage, it was understood that shaded storage showed the best values in terms of oxidative stability. The induction time decreased gradually with increasing storage time (from 4 to 8 months) in oils from unroasted and roasted BCS. The lowest induction time was found in the oils pressed from seeds (unroasted and roasted) stored in the refrigerator in cloth bags for 8 months. As expected, oxidative stability decreased with extended storage time in all samples. Even the longest induction time detected in oils from stored samples was half the induction time of oil from fresh seeds.

At the end of the 8-month storage period, BCSOs stored in both jars and cloth bags at refrigerator had higher PVs than that of oils of shaded storage and oxidised rapidly during Rancimat analysis. However, the study of Aidos et al. [21] showed that the lipid hydroperoxides formed 3.7 times faster at 0°C than at 20°C. Thus, the authors recommend 0°C as the best temperature to keep the fish oil.

It cannot be said that there was a strong relationship between peroxide values and induction times. The formation of high amounts of hydroperoxides does not always mean that high amounts of secondary oxidation products will be formed [22].

The oxidative stability of oil, which is a safety and quality parameter, has an important place for its use in food products and potential commercial applications. OSI, which closely depends on the content of antioxidants in oils, basically evaluates the formation of oxidation products (primary or secondary) [23]. Peroxides that increase during storage, after that are

Table II - Effects of different storages condition on some analytical parameters of BCSO from roasted seeds (mean ± SD, n=3)

Packaging material	Storage conditions	Storage time (month)	Free fatty acids (% oleic acid)	Peroxide value (meq O ₂ /kg oil)	Induction time (h)
	fresh seeds	0	8.72±0.07 [†]	13.76±0.11 ^h	7.07±0.05 ^a
Cloth bag	sunlight	4	10.75±0.08 ^e	26.76±0.71 ^{bc}	3.37±0.10 ^b
		8	11.62±0.18 ^{cd}	28.15±0.37 ^{ab}	1.71±0.22 ^{de}
	shaded	4	10.74±0.11 ^e	16.05±0.88 ^g	2.92±0.10 ^c
		8	11.90±0.03 ^c	25.09±1.50 ^d	2.06±0.45 ^d
	refrigerator	4	11.39±0.08 ^d	20.09±0.18 ^f	3.55±0.20 ^b
		8	13.14±0.08 ^a	28.19±0.26 ^{ab}	0.91±0.06 ^f
Jar	sunlight	4	9.04±0.07 ^f	25.19±2.20 ^{cd}	2.85±0.36 ^c
		8	12.41±0.30 ^b	29.29±0.49 ^a	1.49±0.09 ^e
	shaded	4	8.45±0.09 ^g	22.39±0.76 ^e	3.39±0.34 ^b
		8	11.85±0.28 ^c	23.02±0.20 ^e	2.00±0.14 ^d
	refrigerator	4	8.49±0.21 ^g	16.18±0.05 ^g	3.15±0.27 ^{bc}
		8	11.65±0.30 ^{cd}	26.22±0.17 ^{cd}	1.57±0.26 ^{de}

[†] Small case letters within a column show significant differences between values belong to oils of samples stored at different conditions (P<0.05)

transformed into secondary oxidation products (such as alcohol, aldehyde, ketone). During the oxidation of fat and oil, sudden oxidation, i.e., the propagation period, occurs after the induction period once the antioxidants in food have been consumed during the induction period [24]. When the results of induction time and phenolic content are examined, it can be said that there is a relationship among these data. Phenolic compounds are able to delay lipid oxidation as they are effective antioxidants [25].

The TPC of the oils increased with the roasting process (Table III, Table IV). Roasting is known to increa-

se the phenolic content and antioxidant activity of the oil [26]. TPCs of oils of unroasted samples were in the range of 2730.83 and 4751.01 mg GAE/kg oil. After 4 months of storage, lower levels of TPC were detected in the samples stored in the refrigerator than those exposed to the sun and shade. However, contrary to this situation, phenolic values were found to be higher in samples stored under refrigerated conditions after 8 months compared to 4 months (in all storage materials and conditions). The increase in TPC with storage can be explained by the breakdown of complex phenolic compounds in oils into simple phenols

Table III - Effects of storage on total phenolics and DPPH radical scavenging activity of oils from unroasted BCSs (mean \pm SD, n=3)

Packaging material	Storage conditions	Storage time (month)	Total phenolics (mg GAE/kg oil)	DPPH-RSA (% inhibition)
Cloth bag	fresh seeds	0	4410.41 \pm 88.60 ^{ab†}	69.81 \pm 1.61 ^a
		4	3088.38 \pm 42.01 ^e	50.07 \pm 1.33 ^d
	sunlight	8	2808.31 \pm 16.12 ^g	38.67 \pm 0.59 ^h
		4	3156.98 \pm 20.88 ^d	50.44 \pm 1.00 ^d
	shaded	8	2965.70 \pm 41.75 ^f	38.81 \pm 1.09 ^h
		4	2785.71 \pm 54.57 ^{gh}	40.04 \pm 1.71 ^{gh}
Jar	refrigerator	8	3648.51 \pm 55.55 ^c	47.33 \pm 0.68 ^e
		4	4751.01 \pm 43.94 ^a	66.93 \pm 0.46 ^b
	sunlight	8	2730.83 \pm 17.01 ^h	38.41 \pm 0.76 ^h
		4	4142.45 \pm 56.54 ^b	57.33 \pm 0.78 ^c
	shaded	8	3591.20 \pm 51.15 ^c	43.63 \pm 1.48 ^f
		4	2838.98 \pm 24.21 ^g	41.15 \pm 1.01 ^g
refrigerator	8	2807.34 \pm 12.43 ^g	36.33 \pm 1.46 ⁱ	

† Small case letters within a column show significant differences between values belong to oils of samples stored at different conditions ($P \leq 0.05$).

Table IV - Effects of storage on total phenolics and DPPH radical scavenging activity (DPPH-RSA) of oils from roasted BCSs (mean \pm SD, n=3)

Packaging material	Storage conditions	Storage (month)	Total phenolics (mg GAE/kg oil)	DPPH-RSA (% inhibition)
Cloth bag	fresh seeds	0	4410.41 \pm 88.60 ^{ab†}	69.81 \pm 1.61 ^a
		4	3403.95 \pm 20.16 ^g	52.78 \pm 1.17 ^c
	sunlight	8	3111.78 \pm 53.34 ⁱ	39.48 \pm 1.11 ^{fg}
		4	4215.90 \pm 40.61 ^d	59.96 \pm 1.19 ^b
	shaded	8	3180.39 \pm 32.03 ^h	39.52 \pm 1.48 ^{fg}
		4	3071.43 \pm 25.28 ⁱ	41.48 \pm 1.07 ^f
Jar	refrigerator	8	4365.21 \pm 49.88 ^c	48.70 \pm 1.58 ^{de}
		4	4992.33 \pm 53.34 ^a	75.63 \pm 1.49 ^a
	sunlight	8	3043.18 \pm 42.79 ^g	38.93 \pm 1.54 ^g
		4	4653.35 \pm 46.09 ^b	60.81 \pm 1.57 ^b
	shaded	8	3524.21 \pm 24.21 ^f	39.89 \pm 0.56 ^{fg}
		4	3184.42 \pm 32.96 ^h	50.70 \pm 1.51 ^{cd}
refrigerator	8	3685.63 \pm 32.52 ^e	47.70 \pm 1.52 ^e	

† Small case letters within a column show significant differences between values belong to oils of samples stored at different conditions ($P \leq 0.05$).

[7]. For both the roasted and unroasted samples, the highest TPC values (4142-4992 mg GAE/kg oil) were determined in the oils of seeds stored under sun and shade for 4 months in the jar. Additionally, high values were determined for the roasted samples in cloth bags after 4 months in the shade and 8 months in the refrigerator (4213 and 4365 mg GAE/kg oil, respectively).

All these high values are close to the value detected in fresh seeds on average. BCSOs from the seeds exposed to sun contained the lowest levels of total phenolics. Hydrolytic activities and oxidation are probably caused by temperature, oxygen and enzymes during storage that leads to a decrease in phenolic concentration. TPC, which is a significant quality parameter

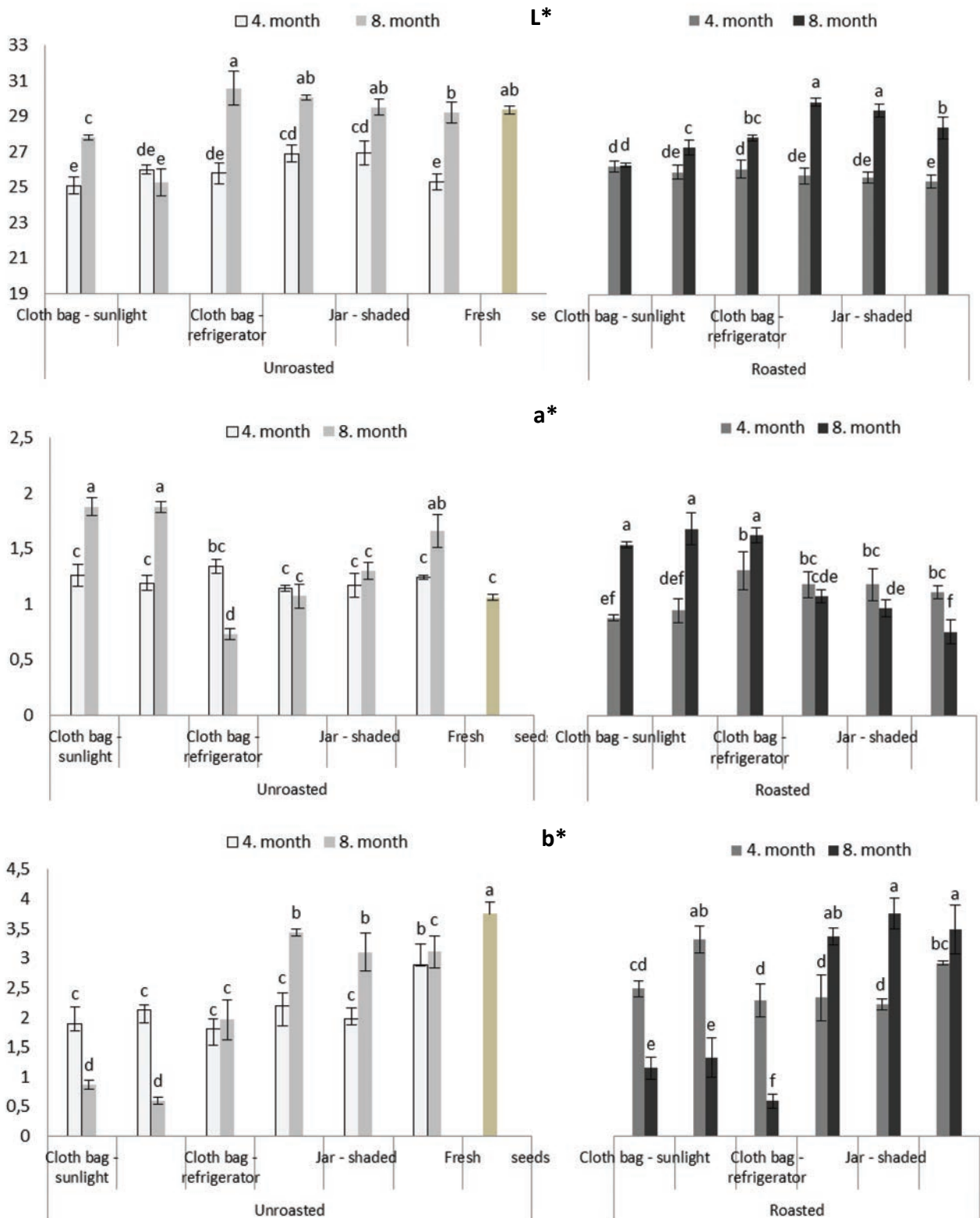


Figure 2 - Effects of the storage conditions on color indices of BCSO from unroasted and roasted seeds (mean ± SD, n=3).

especially as an antioxidant in oil, has nutritional and organoleptic properties [7]. It has been indicated that BCSO is a rich source of polyphenols, which possess antihydrolytic effect and antioxidant activity [27]. Total phenolic component content of BCSO was found 2-3.5 times higher in the present study compared to that reported by Lutterodt et al. [1] (1.02-1.40 mg /g). This difference may be due to the variety of seeds, growing conditions, and methods and conditions of obtaining both the oil and the phenolic extract.

In a study in which various cooking oils were exposed to heat and light, it was reported that the change in peroxide values differed according to the oil [28]. While this conversion was reported to be fast in olive oil and rapeseed oil it was slower in sunflower oil and safflower oil as the conversion began when the hydroperoxide concentration reached a certain amount [22]. Likewise, the rate of hydroperoxides formation was different for walnut, linseed and rapeseed oils in the study of Guillén and Ruiz [29]. The difference between the conversion of primary oxidation products to secondary oxidation products from oil to another oil was attributed to the degradation rate of acyl groups which is closely related to the formation and degradation of primary and secondary oxidation compounds and the nature and evolution of these products. In addition to the degree of unsaturation of these oils, they stated that different behaviours were observed in their oxidation due to the difference in the minor components they contain. For instance, it has been previously shown that tocopherols are more effective in delaying the formation of hydroperoxides than carbonyls. It was stated that total polar compounds and phenolic compounds, rather prevent the formation of off-flavour, that means delay in secondary oxidation [30]. BCSO is rich in linoleic and oleic acids and contains high amounts of unsaponifiable elements. Tovar et al. [31] did not detect a direct correlation between phenolic compounds and oxidative stability in some oils examined in their study. It was suggested that stability in oils was not related to total phenolics but to some major phenolic compounds. Similarly, Lutterodt et al. [1] samples with the highest phenolic content showed the lowest oxidative stability. Therefore, they stated that phenolic components partially affect oil stability.

The DPPH inhibition rate results were in line with the concentrations of total phenolics. As shown in Table III, oils from stored seeds exhibited lower DPPH-RSA than the oil of fresh seeds. Roasting of the seeds resulted in higher DPPH-RSA in the pressed oils. In this respect, higher activity was obtained with storage in a jar than with storage in a cloth bag, except for samples stored in the refrigerator for 8 months. For both unroasted and roasted BCSOs, the highest DPPH-RSA was observed in oils from seeds stored in cloth bags at room temperature and in jars exposed to sunlight for 4 months. Storage for 4 months produced higher DPPH-RSA than 8 months of storage

(except for storage in the cloth bag under refrigerated conditions). Roasting revealed higher DPPH-RSA in BCSOs mostly overall storage conditions, so that it was also mentioned above that roasting resulted in higher TPC in the oils. Liang et al. [32] reported that the RSA increased in BCSs when the time of heating was prolonged. Lutterodt et al. [1] reported that DPPH-RSA may vary depending on the seed variety and growing conditions of BCSs and was significantly affected by seed storage and processing practice.

At the end of 8 months of storage, L^* values increased compared to 4 months of storage. This means there was some lightening of the colour. Roasting did not show any significant effect on the L^* values (Fig. 2). The L^* values of the seeds stored for 4 months under all packaging and ambient conditions were lower than those of the oil obtained from the fresh sample. The highest L^* values were determined at samples stored in jars for 8 months exposed to sunlight which were similar to the L^* values of fresh seed oil (the free acidity of these samples was also higher than the others). Although this phenomenon was the same for roasted and unroasted seeds, slightly higher L^* values were obtained for the unroasted samples.

Regarding the roasted samples, there was an increase in the a^* values (more redness) of the samples stored in cloth bags, and decrease (less redness) in those stored in jars (regarding all storage conditions) after 8 months compared to 4 months. a^* values of the unroasted seed oils were closer to that of fresh seed oil, however the oils of unroasted seeds stored in a cloth bag under sun or shade showed higher a^* values compared to fresh seed oil. Likewise, 8 months storage in the cloth bag resulted in higher a^* values in the oils of roasted seeds. Lower b^* values were measured in stored samples compared to fresh seed oil. Storage in cloth bags resulted in lower b^* values for both roasted and unroasted seeds, although roasted seed oils had even lower values. In all the three conditions, the oils of roasted seeds stored in jars had b^* values equivalent to the fresh seed oil. Both roasted and unroasted samples showed lower b^* (more yellowness) values at the 8th month compared to the 4th month when stored in cloth bags, and b^* values increased as the storage progressed when the seeds were stored in jars. The oils from seeds stored in jars had significantly higher b^* values than those from seeds stored in cloth bags, indicating a decrease in red colour. Roasting had a clear impact on the a^* and b^* values of BCSOs stored in cloth bags, at room temperature and under sunlight. A similar effect was also seen in the study of Suri et al. [33].

A positive relationship was detected between the peroxide value and the L^* value of the oil, especially for unroasted samples (Table V). Consistent with this, a significant negative correlation was observed between the L^* value and the induction time. The results showed that the oxidation products released during oil storage affected the darkening of the colour of the

Table V - Pearson correlation coefficients between the analyzed parameters of oils from unroasted and roasted BCSs

		L*	Free fatty acids	Peroxide value	Induction time	Total phenolics
unroasted	L*	1				
	Free fatty acids	-	1			
	Peroxide value	0.736**	0.259	1		
	Induction time	-0.784**	-0.220	-0.658**	1	
	Total phenolics	-	-0.429**	-	-	1
	DPPH-RSA	-	-0.373*	-	0.465**	0.911**
roasted	L*	1				
	Free fatty acids	-	1			
	Peroxide value	0.530**	0.548**	1		
	Induction time	-0.765**	-0.702**	-0.547**	1	
	Total phenolics	-	-0.386*	-	-	1
	DPPH-RSA	-	-0.676**	-	0.378*	0.851**

* and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively

oil. Data obtained especially in roasted seed oil showed positive correlations between free fatty acids and peroxide number and strong negative correlations between induction time. The increased formation of oxidation products due to the mild roasting process may have caused a significant correlation; in contrast no significant correlations were seen at this level for oil from unroasted seeds. There was also dependency of the increase in free fatty acidity and the decrease in DPPH-RSA which can probably be attributed to the fact that free fatty acids have emulsifying properties, making the extraction of antioxidant compounds with hydrophilic and lipophilic characters difficult. The strong positive correlations between DPPH-RSA and the induction time along with DPPH-RSA and total phenolics content were expected results.

CONCLUSIONS

The quality and functional properties of the oil were negatively affected when storage exceeded 4 months. Storage in a cloth bag under shaded conditions resulted in higher oxidative stability, confirmed by lower a peroxide value and prolonged induction time. Under shade, storage in jars is recommended for lower free fatty acidity in the oil. Additionally, if the seeds are stored unroasted under refrigerator conditions, the cloth bag has an advantage over the jar due to lower free fatty acid increase during storage and higher oxidative stability. However, in general, both total phenolic content and DPPH radical scavenging activity were lower in oils obtained from seeds stored in cloth bags. Minimal roasting of the seeds increased the peroxide value of the oil from the seeds stored in cloth bags and shortened the induction time. Sun exposure during storage showed the most notable negative effects on free fatty acidity and peroxide value. Correlation analysis revealed a positive relationship between increased oxidation in the oil and darkening of the oil color. The free fatty acidity of the oil and the induction time exhibited strong negative correlation

due to the increased formation of oxidation products with mild roasting.

Conflict of interest

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

- [1] H. Lutterodt, M. Luther, M. Slavin, J.J. Yin, J. Parry, J.M. Gao, L.L.Yu, Fatty acid profile, thymoquinone content, oxidative stability, and antioxidant properties of cold-pressed black cumin seed oils. *LWT - Food Science and Technology*, 43, 1409–1413 (2010).
- [2] A. Hajib, H. Harhar, S. Gharby, I. Nounah, B. Matthäus, D. Guillaume, Z. Charrouf, Is geographic origin a good marker for cumin seed oil (*Cuminum cyminum* L.), *Riv. Ital. Sostanze Grasse*, 95(3), 155-159 (2018).
- [3] H. Bakhshabadi, H.O. Mirzaei, A. Ghodsvali, S.M. Jafari, A.M. Ziaifar, V. Farzaneh, The effect of microwave pretreatment on some physico-chemical properties and bioactivity of black cumin seeds' oil. *Industrial Crops and Products*, 97, 1–9 (2017).
- [4] F. Shahidi, Y. Zhong, Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews*, 39, 4067–4079 (2010).
- [5] M.T. Satue, S.W. Huang, E.N. Frankel, Effect of natural antioxidants in virgin olive oil on oxidative stability of refined, bleached, and deodorized olive oil. *Journal of the American Oil Chemists' Society*, 72(10), 1131-1137 (1995).
- [6] M. Soleimanifar, R. Niazmand, S.M. Jafari, Evaluation of oxidative stability, fatty acid profile, and antioxidant properties of black cumin seed oil and extract. *Journal of Food Measurement and Characterization*, 13, 383–389 (2019).

- [7] Y. Mazaheri, M. Torbati, S. Azadmard-Damirchi, G.P. Savage, Oil extraction from blends of sunflower and black cumin seeds by cold press and evaluation of its physicochemical properties. *Journal of Food Processing and Preservation*, 43(10), pp. e14154 (2019b).
- [8] M. Kiralan, G. Çalik, S. Kiralan, A., Özaydin, G. Özkan, M.F. Ramadan, Stability and volatile oxidation compounds of grape seed, flax seed and black cumin seed cold-pressed oils as affected by thermal oxidation. *Grasas y Aceites*, 70(1), e295 (2019).
- [9] T. Dedebas, L. Ekici, O. Sagdic, Chemical characteristics and storage stabilities of different cold-pressed seed oils. *Journal of Food Processing and Preservation*, 45(2), e15107 (2021).
- [10] Ž. Tarasevičienė, V. Laukagalis, A. Paulauskienė, A. Baltušnikienė, E. Meškinytė, Quality changes of cold-pressed black cumin (*Nigella sativa* L.), safflower (*Carthamus tinctorius* L.), and milk thistle (*Silybum marianum* L.) seed oils during storage. *Plants*, 12(6), 1351 (2023).
- [11] M. Kiralan, G. Özkan, A. Bayrak, M.F. Ramadan, Physicochemical properties and stability of black cumin (*Nigella sativa*) seed oil as affected by different extraction methods. *Industrial Crops and Products*, 57, 52-58 (2014).
- [12] M.A. Mamun, N. Absar, Major nutritional compositions of black cumin seeds – cultivated in Bangladesh and the physicochemical characteristics of its oil. *International Food Research Journal*, 25(6), 2634-2639 (2018).
- [13] AOCS, 1998. AOCS Official Methods and Practices of the AOCS (fifth ed.), AOCS Press, Champaign, USA (1998).
- [14] S.M. Lee, K.T. Lee, S.H. Lee, J.K. Song, Origin of human colour preference for food. *Journal of Food Engineering*, 119(3), 508-515 (2013).
- [15] G. Rigane, A. Yahyaoui, A. Acar, S. Mnif, R.B. Salem, D. Arslan, Change in some quality parameters and oxidative stability of olive oils with regard to ultrasound pretreatment, depitting and water addition. *Biotechnology Reports*, 26, e00442 (2020).
- [16] K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos, D.H. Bryne, Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19, 669-675 (2006).
- [17] Y. Mazaheri, M. Torbati, S. Azadmard-Damirchi, G.P. Savage, Effect of roasting and microwave pre-treatments of *Nigella sativa* L. seeds on lipase activity and the quality of the oil. *Food Chemistry*, 274, 480-486 (2019a).
- [18] FAO/WHO, (2009). Report of the 21st session of the Codex Alimentarius Committee on fats and oils. Kola Kinabalu, Malaysia, 16-20 February (2009).
- [19] M. Kiralan, M. Ulaş, A. Özaydin, N. Özdemir, G. Özkan, A. Bayrak, M.F. Ramadan, Blends of cold pressed black cumin oil and sunflower oil with improved stability: A study based on changes in the levels of volatiles, tocopherols and thymoquinone during accelerated oxidation conditions. *Journal of Food Biochemistry*, 41, e12272 (2017).
- [20] M.F. Ramadan, J.T. Mörsel, Oxidative stability of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.) and niger (*Guizotia abyssinica* Cass.) crude seed oils upon stripping. *European Journal of Lipid Science and Technology*, 106(1), 35-43 (2004).
- [21] I. Aidos, S. Lourenclo, A. Van der Padt, J.B. Luten, R.M. Boom, Stability of crude herring oil produced from fresh byproducts: influence of temperature during storage. *Journal of Food Science*, 67(9), 3314-3320 (2002).
- [22] M.D. Guillen, N. Cabo, Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. *Food Chemistry*, 77, 503-510 (2002).
- [23] E. Pereira, M.C. Ferreira, K.A. Sampaio, R. Grimaldi, A.J. de Almeida Meirelles, G.J. Maximo, Physical properties of amazon fats and oils and their blends. *Food Chemistry*, 278, 208-215 (2019).
- [24] N. Gotoh, S. Wada, The importance of peroxide value in assessing food quality and food safety. *Journal of the American Oil Chemists' Society*, 83, 473-474 (2006).
- [25] A. Siger, M. Nogala-Kalucka, E. Lampart-Szczapa, The content and antioxidant activity of phenolic compounds in cold-pressed plant oils. *Journal of Food Lipids*, 15(2), 137-149 (2008).
- [26] N. Chandrasekara, F. Shahidi, Effect of roasting on phenolic content and antioxidant activities of whole cashew nuts, kernels, and testa. *Journal of Agricultural and Food Chemistry*, 59, 5006-5014 (2011).
- [27] A. Asdadi, H. Harhar, S. Gharby, Z. Bouzoubaâ, A. Yadini, R. Moutaj, M.E. Hadek, Chemical composition and antifungal activity of *Nigella sativa* L. oil seed cultivated in Morocco. *International Journal of Pharmaceutical Science Invention*, 3, 9-15 (2014).
- [28] A. Kaleem, S. Aziz, M. Iqtedar, Investigating changes and effect of peroxide values in cooking oils subject to light and heat. *Fuuast Journal of Biology*, 5(2), 191-196 (2015).
- [29] M.D. Guillén, A. Ruiz, Monitoring the oxidation of unsaturated oils and formation of oxygenated aldehydes by proton NMR. *European Journal of Lipid Science and Technology*, 107(1), 36-47 (2005).
- [30] R. Farhoosh, S. Pazhouhanmehr, Relative contribution of compositional parameters to the primary and secondary oxidation of canola

- oil. *Food Chemistry*, 114(3), 1002-1006 (2009).
- [31] M.J. Tovar, J. Motilva, M.P. Romero, Changes in the phenolic composition of virgin olive oil from young trees (*Olea europaea* L. cv. Arbequina) grown under linear irrigation strategies. *Journal of Agricultural and Food Chemistry*, 49, 5502-5508 (2001).
- [32] L.C. Liang, W. Zzaman, T.A. Yang, A.M. Easa, Effect of conventional and superheated steam roasting on the total phenolic content, total flavonoid content and DPPH radical scavenging activities of black cumin seeds. *Pertanika Journal of Tropical Agricultural Science*, 41(2), 663-676 (2018).
- [33] K. Suri, B. Singh, A. Kaura, M.P. Yadav, N. Singh, Impact of infrared and dry air roasting on the oxidative stability, fatty acid composition, Maillard reaction products and other chemical properties of black cumin (*Nigella sativa* L.) seed oil. *Food Chemistry*, 295, 537-547 (2019).



innovazione e ricerca

Virgin Olive Oil Organoleptic Assessment



174

Reg. UE 2022/2104 and 2022/2105 establish the chemical-physical parameters and methods for quality control of olive oil.

The organoleptic assessment (Panel test) contributes to the definition of the quality of the oil, the Regulation classifies virgin olive oil in the categories:

- EXTRA VIRGIN OLIVE OIL
- VIRGIN OLIVE OIL
- LAMPANTE OLIVE OIL

according to the intensity of the defects and of the fruitiness perceived, as determined by a group of tasters selected, trained and monitored as a panel, using statistical techniques for data processing. It also provides information on the organo-leptic characteristics for optional labeling.

The organoleptic assessment is qualified by a level of reliability comparable to that of the analytical tests.

Our Panel is recognized by the IOC (International Olive Council), by the Italian Ministry of Agricultural, Food and Forests as a tasting committee in charge of the official control of the characteristics of virgin olive oils and designation of origin (D.O.) oils. The organoleptic assessment is accredited by ACCREDIA (Italian Accreditation Body).

The Panel serves industry, production consortia, certification bodies and large-scale distribution.

For further information: Stefania De Cesarei ✉ stefania.decesarei@mi.camcom.it
Expert Sensorial Analysis and Head of Panel Test

General considerations on pesticide residues in olive oils

Tiziana Generali

Istituto Superiore di Sanità
Dipartimento Ambiente e Salute
Reparto Esposizione a contaminante in Aria, Suolo e da Stili di Vita (ECASS)
Sezione Antiparassitari

✉ CORRESPONDING AUTHOR:

Tiziana Generali
Tel: +390649902603
Email: tiziana.general@iss.it

RISG
Anniversario
100

Presentato al centenario RISG

**Sostanze grasse:
ricerca, innovazione
e scenari futuri**

15 novembre 2023

The olive tree is one of the most important and ancient crops in the Mediterranean area where, according to the latest data from the International Olive Council, 92% of the world's olive oil is produced. The olive tree can be attacked by a large variety of parasites, resulting in a reduction in the quality and quantity of olives and olive oil produced.

Most of the plant protection products used on olive trees are insecticides, acaricides and fungicides and also herbicides considering that olives can also be harvested with the beating technique from tents placed on the ground. The traces of plant protection products that can be found in olives are named "residues".

The Maximum Residue Level (MRL) is defined as the highest level of a pesticide residue that is legally tolerated in or on a food or feed when plant protection products are applied according to Good Agricultural Practice (GAP).

The MRL values of pesticide residues in olives (as for all crops) are defined in Regulation (EC) 396/2005 and subsequent updates for all possible food-pesticide combinations and can be consulted in a European Commission database.

To calculate MRLs in olive oil, process factors must be applied to the MRL values on olives. In the European Union coordinated multi-annual control programmes, each Member State is required to report the process factors used to analyze olive oil samples. The SANTE 10704/2021 document represents an information document from the European Commission for the application of process factors which are however set by the National Authorities. Currently in Italy the process factor for olive oil is equal to 5.

1. INTRODUCTION

Pesticides or plant protection products (PPP) are compounds belonging to different chemical classes, used in agriculture, livestock farming, public hygiene, domestic life and industrial products to control many harmful or unwanted organisms, plant or animal. They are divided according to their field of action into different categories as example: insecticides/acaricides, herbicides, fungicides, phyto regulators.

Depending on their chemical structure and associated chemical-physical properties and the type of use and method of application, not all of the applied dose is degraded and small quantities of original pesticides can persist in foods, animals and the environment as explained in Figure 1.

The olive tree is one of the most important and ancient crops in the Mediterranean area where, according to the latest data from the International Olive Council, 92% of the world's olive oil is produced

Olive oil is one of the main components of the Mediterranean diet and also an export product with a high economic impact.

The olive tree can be attacked by numerous parasites, also as a result of climate changes and consequent increase in average temperatures leading in a reduction in the quality and quantity of olives and olive oil produced. Most of the PPP used on olive trees are insecticides, acaricides and fungicides and also herbicides considering that olives can also be harvested with the beating technique from tents placed on the ground.

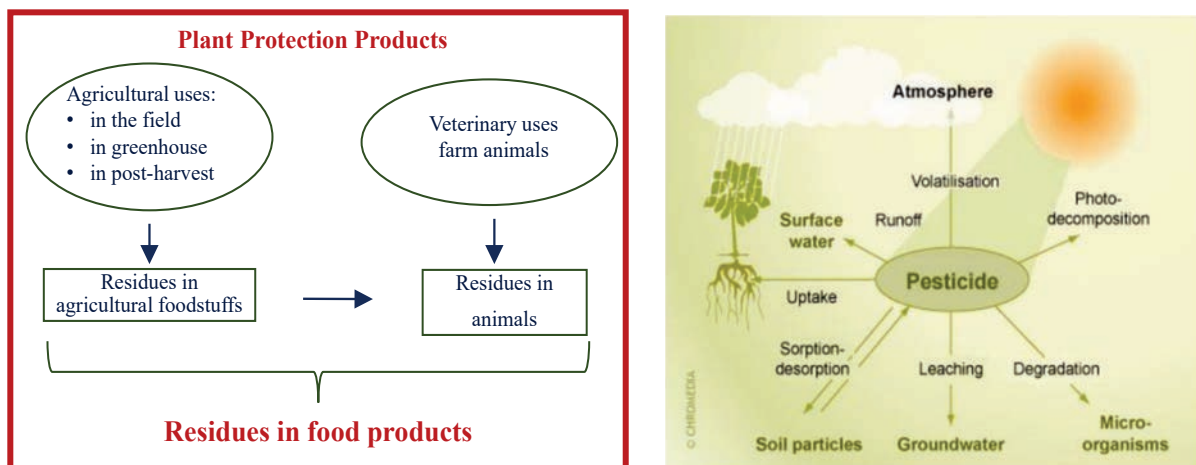


Figure 1 – Scheme of possible presence of pesticide residues in food products and in the environment

The official control on residues of phytosanitary products in olives, olive oil and in all foods falls within the controls provided for by European Commission (EC) Regulation 2017/625 [1] and within the scope of EC Regulation 396/2005 [2] and represents one of the most relevant health priorities in the field of food safety, with the aim of guaranteeing a high level of consumer protection.

Regulation (EC) 396/2005 [2] indicates the procedure for setting the Maximum Residue Level (MRL) for each pesticide in or on food products and feed of plant and animal origin.

MRL means the maximum allowable quantity of active ingredient residue present in agricultural commodities, after treatments with PPP which does not pose risks to the health of the consumer and it is expressed in mg/kg.

The structure of Regulation (EC) 396/2005 [2] is described in Table I.

Table I – Structure of Regulation (EC) 396/2005

Regulation (EC) 396/2005	
50 articles governing procedures required for fixing or deleting MRL and their provisions for official controls at National and Community level	
ANNEX I	list of products to which the regulation is applied, last update Regulation (EU) 2018/62
ANNEX II	list of MRLs for each active substance/product combination included in annex I, continuous updating
ANNEX III	list of provisional MRL
ANNEX IV	list of active substances for which it is not necessary to fix MRL
ANNEX V	list of MRLs not present in Annexes II and III or for active substances not listed in Annex IV
ANNEX VI	list of processing factors not yet published
ANNEX VII	list the combinations of active substance/product subject to derogation as regards post-harvest treatment with a fumigant

The setting of MRL is a complex process harmonized by EFSA which takes into account good agricultural practices (GAP), conditions of use, doses, number of treatments, safety intervals which are determined by residual field studies. These residue levels are assessed both during the authorization phase of active substances, during the authorization phase of PPP and in the event of a non-compliant product being found on the market. This assessment is carried out by comparing consumer exposure estimates with toxicological parameters such as acute reference doses (ARfD) (for acute risk assessment) and acceptable daily doses (ADI) (for chronic risk assessment).

The MRL are not considered as toxicological limits, so exceeding them does not pose a danger to humans, but rather constitute the maximum quantity of residue that could be present on a product of plant origin when GAP are respected during use of PPP.

The established limits MRL are uniformly applied throughout Europe and are continuously updated. All crop/pesticide combinations can be consulted using a specific database on the EC website.

The official control on residues of PPP in foods falls within the controls provided for by EC Regulation 2017/625 [1] and within the scope of EC Regulation 396/2005 [2] mentioned above.

The EC issues coordinated three-year control programs to ensure compliance with maximum levels of pesticide residues and to assess consumer exposure to pesticide residues in and on food products of plant and animal origin.

These regulations establish the pesticide/food product combinations that each member state must monitor as well as the minimum number of samples to be analyzed, considering processed food products such as olive oil among the samples to be analyzed. The scheme of the last control program is Regulation (EU) 2023/731 [3] is shown in Figure 2 where it is highlighted that in the year 2024 one of food products subjected to official control is olive oil. Member States are required to communicate the processing factors (Pf) used in the case of analysis of olive oil as all processed products considered.

L 95/28 EN Official Journal of the European Union 4.4.2023

COMMISSION IMPLEMENTING REGULATION (EU) 2023/731
of 3 April 2023

concerning a coordinated multiannual control programme of the Union for 2024, 2025 and 2026 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin and repealing Implementing Regulation (EU) 2022/741

ANNEX I
PART A
Products ⁽¹⁾ of plant origin ⁽²⁾ to be sampled in 2024, 2025 and 2026

2024 (b)	2025 (c)	2026 (a)
(0151010) Table grapes ⁽³⁾	(0130010) Apples ⁽³⁾	(0110020) Oranges ⁽³⁾
(0163020) Bananas ⁽³⁾	(0152000) Strawberries ⁽³⁾	(0130020) Pears ⁽³⁾
(0110010) Grapefruits ⁽³⁾	(0140030) Peaches, including nectarines and similar hybrids ⁽³⁾	(0162010) Kiwi fruits ⁽³⁾
(0231030) Aubergines ⁽³⁾	Wine (red or white) made from (0151020) Wine grapes (where no specific processing factors for wine are available, Member States shall report the wine processing factors used).	(0241020) Cauliflowers ⁽³⁾
(0241010) Broccoli ⁽³⁾	(0251020) Lettuces ⁽³⁾	(0220020) Onions ⁽³⁾
(0233010) Melons ⁽³⁾	(0242020) Head cabbages ⁽³⁾	(0213020) Carrots ⁽³⁾
(0280010) Cultivated fungi ⁽³⁾	(0231010) Tomatoes ⁽³⁾	(0211000) Potatoes ⁽³⁾
(0231020) Sweet peppers/bell peppers ⁽³⁾	(0252010) Spinaches ⁽³⁾	(0300010) Beans (dried) ⁽³⁾
(0500070) Wheat grain ⁽³⁾	(0500050) Oat grain ^{(3), (4)}	(0500070) Rye grain ⁽³⁾
Virgin olive oil from (0402010) Olives for oil production (where no specific oil processing factor is available, Member States shall report the processing factors used).	(0500010) Barley grain ^{(3), (4)}	(0500060) Brown rice (husked rice), defined as rice after the removal of the hull from paddy rice ⁽³⁾

⁽¹⁾ Only processed products shall be analysed. In case of products sampled in frozen state, a processing factor shall be reported, if applicable.
⁽²⁾ If no sufficient samples of rye, wheat, oat or barley grains are available, also rye, wheat, oat or barley whole grain flour can be analysed and a processing factor shall be reported.
⁽³⁾ If no sufficient samples of oat grains are available, the part of the required sample number for oat grains that could not be taken, can be added to the sample number for barley grains, resulting in a reduced sample number for oat grains and a proportionately increased sample number for barley grains.
⁽⁴⁾ If no sufficient samples of barley grains are available, the part of the required sample number for barley grains that could not be taken, can be added to the sample number for oat grains, resulting in a reduced sample number for barley grains and a proportionately increased sample number for oat grains.
⁽⁵⁾ Where appropriate, also polished rice grain can be analysed. It shall be reported whether polished or husked rice was analysed. If polished rice was analysed, a processing factor shall be reported.

Figure 2 – Scheme of Regulation (EU) 2023/731

2. PROCESSING FACTORS (Pf)

The Regulation (EC) 396/2005 [2] established MRL values for primary food matrices only. In order to establish the maximum acceptable residue in and on the relevant processed products, the article 20 of this Regulation states: "For processed food and feed products and/or composites for which MRLs have not been fixed, those laid down for the relevant product referred to in Annex I shall apply, taking into account changes in the residue content resulting from the processing and/or to mixing. Specific concentration or dilution factors for certain processing operations and/or mixtures or for certain processed and/or composite products may be included in the list set out in annex VI"

As mentioned above the Pf list (Annex VI) have not yet been defined by the EC.

The EC published the SANTE 10704/2021 document [4] entitled: *Information note on Article 20 of Regulation (EC) No 396/2005 as regards processing factors, processed and composite food and feed*.

The purpose of this document is not to establish factors of harmonized processing at EU level, but provide one tool for Member States on how to implement the provisions of Article 20 of Reg. 396/2005 in a harmonized way, so that the processing factors established by a Member State may be mutually accepted by the other Member States. In the Figure 3 is shown the definition of processing factor as presented in the SANTE document.

$$Pf = \frac{\text{Residue in the processed fraction (mg/kg)}}{\text{Residue in the raw commodity (mg/kg)}}$$

Pf is > 1: Residues concentrated in the processed product.

Pf is < 1: Residues declined in the processed product (due to dilution, removal or degradation).

Pf = 1: Processing did not result in a change of residue concentrations.

Figure 3 – Definition of processing factor according to the SANTE 10704/2021 document

SANTE document defined two different kinds of Pf: substance-specific processing factors and generic processing factors for certain standard processing operations (e.g., drying by removing of water). Generic processing factors should only be used when substance specific factors are not available Available sources of specific processing factors are listed below:

- *EFSA publications and EFSA (EU) database* The EFSA (EU) database includes processing factors from EFSA publications until June 2016. Work is currently ongoing to implement additional processing information from more recent EFSA publications
- *National databases in the European Union*. The German Federal Institute for Risk Assessment (BfR), the Dutch National Institute for Public Health and the Environment (RIVM), the Spanish Agency for Food Safety and Nutrition (AESAN)
- *Joint Meeting on Pesticide Residues (JMPR) reports*. The residue definitions for enforcement derived by JMPR should match with the EU residue definitions for enforcement
- *Other sources* (e.g., Souci-Fachmann-Kraut database (SFK) for food composition, the database from the European Spice Association (ESA), the dataset of the Association of Organic Processors etc.)

When multiple Pf are available it should be taken the most appropriate for the specific situation considering that there is no hierarchy between the national databases of the Member States.

If several Pf are available, it would be recommended to use the median processing factors from the EFSA process (EU) or national databases.

When specific substance processing factors are not available it is possible used generic processing factors as in the case of olive oil explained in the SANTE document and showed in the Figure 4.

Example: the yield factor between olive oil (processed commodity) and olives (unprocessed product) is considered to be 20%. If no other processing factors are available, this factor could be considered, but only if information on the physico-chemical properties of the residues, in particular their fat/water solubility (via log Kow, also known as logP), is also taken into account.

Assuming that residues fully concentrate into the processed commodity of olive oil, the processing factor is derived according to the following equation:

$$Pf = \frac{1}{\text{yield factor}} = \frac{\text{mass olives}}{\text{mass olive oil}} = \frac{100}{20} = 5$$

The derived MRL of the olive oil (processed commodity) can be calculated by multiplication of the MRL of olives (unprocessed product) by the processing factor of 5.

Figure 4 – Generic processing factor of olive oil according to the SANTE 10704/2021 document

According to the SANTE document the Pf are applied:

- approved and non-approved active substances in the EU
- refer to the residue definition for enforcement laid down in Regulation (EC) No 396/2005
- MRLs established at a specific LOQ or at the default level of 0.01 mg/kg

The SANTE/10704/2021 [4] document has been conceived as an information note of the Commission Services and it does not represent the official position of the Commission. It does not intend to produce legally binding effects. However, it remains ultimately the Member States responsibility to decide, after analysis of available information, on whether to use or not to use processing factors and, if used, to decide on an appropriate factor as a basis for taking enforcement action

Regarding the Pf for olive oil, many countries member of European Community or however many olive oil producers of Mediterranean area, used a Pf of 5 as in Italy where this Pf is established by an Official Circular of Health Minister dated 17th November 2015.

3. OFFICIAL CONTROLS

As mentioned previously the official control on residues of PPP in foods falls within the controls provided for by EC Regulation 2017/625 [1] and within the scope of EC Regulation 396/2005 [2].

The Ministry of Health coordinates and defines official control programs on food products in Italy, also including annual plans regarding residues of PPP in foods. Part of the controls carried out at national level are an integral part of the coordinated official control programs envisaged by the European Union.

The controls are carried out on both internally produced and imported foods to ascertain the respect of the MRL of pesticides in foods on the national territory.

Furthermore, to improve the uniformity and reliability of European controls in the field of pesticide residues in food, the EC establishes, pursuant to Article 93 of EC Regulation 2017/625 [1], four European Reference Laboratories (EURL) that deal with residues of pesticides in various sectors:

- Cereals and feed
- Food products of animal origin and foods with a high fat content
- Fruits and vegetables, including foods with high water and acid content
- Single residues methods

The main task of these Reference Laboratories is to ensure the quality and comparability of the data provided by the various European countries on pesticide residues in food.

Member state also designate, according to Article 100 of the EC Regulation 2017/625 [1], one or more National Reference Laboratories (NRL) for each EURL.

In Italy, four NRL have been designated for residues of PPP in foods: three of these are at the Istituto Superiore di Sanità:

- National Reference Laboratory for Fruit and Vegetables
- National Reference Laboratory for Food of Animal Origin and foods with a high fat content
- National Reference Laboratory Single Residues Methods

one laboratory at the Istituto Zooprofilattico Sperimentale (IZS) del Piemonte e Valle D'Aosta:

- National Reference Laboratory for Cereals and Feeding Stuff

Official control are carried out by the official control laboratories (A.R.P.A., AASSLL and IZS) identified by the Regional Health Departments. These laboratories must be accredited as required by EC Regulation 2017/625 [1], according to the UNI ISO/17025 standard [5], with analysis methods validation according to a European guide document SANTE/11312/2021 [6]: *Analytica Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed*, updated every two years. They must also participate in the Proficiency Test (PT) organized by the EURL and by NRL as required by Regulation 396/2005 [2].

The Istituto Superiore di Sanità in its role of NRL annually organize a PT for the determination of pesticide residues in olive oil in which European NRL, European official control and private laboratories participate.

Results of the Italian Control Plans for pesticide residues in food are summarized in a final report published by the Ministry of Health and the last one published concerns the monitoring data for the year 2020 [7].

Considering the olive oil, 214 samples were analysed. The percentage of samples without detectable residues was found to be 93.5% and no samples showed residues exceeding the legal limit. The data regarding the olive oil matrix certainly shows a situation of absence of risk for the consumer.

Overall, as in the past few years, the results of official Italian controls continue to be in line with those found in other EU countries and indicate a high level of consumer protection.

4. REFERENCES

- 1] Europe. Regulation (EU) 2017/625 of the European Parliament and the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products. *Official Journal of the European Union* L95/1, 7 April 2017.
- 2] Europe. Regulation (EC) NO 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. *Official Journal of the European Union* L70/1, 16 March 2005.
- 3] Commission Implementing Regulation (EU) 2023/731 of 3 April 2023 concerning a coordinated multiannual control programme of the Union for 2024, 2025 and 2026 to ensure compliance with maximum residues levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin and repealing Implementing Regulation (EU) 2022/741. *Official Journal of the European Union* L95/28, 4 April 2023.
- 4] European Commission – Directorate-General for Health and Food Safety. Note on Article 20 of Regulation (EC) No 396/2005 as regards processing factors, processed and composite food and feed. Brussels: European Commission (SANTE/10704/2021).
- 5] ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories. Geneva: International Organization for Standardization; 2005. Upgraded 2018.
- 6] SANTE/11312/2021) document "Analytica Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed" Supersedes Document No SANTE/2019/12682. Implementing by 01/01/2022.
- 7] Ministero della Salute, Direzione Generale per l'Igiene e la Sicurezza degli Alimenti e la Nutrizione. Relazione sul controllo ufficiale sui residui di prodotti fitosanitari negli alimenti - Dati anno 2020. (https://www.salute.gov.it/imgs/C_17_pubblicazioni_3284_allegato.pdf)

FCM: Overall Regulatory Framework

Fabiana Vanni, Giorgio Padula, Veruscka Mannoni,
Silvia Giamberardini, Marco De Felice, Cinzia Gesumundo

Istituto Superiore di Sanità
DAMSA Dipartimento di Ambiente e Salute
(Department of Environment and Health)
Reperto ECASS (Esposizione a Contaminanti in Aria Suolo e da Stili di vita)

✉ CORRESPONDING AUTHOR:

Fabiana Vanni
Email: fabiana.vanni@iss.it
Viale Regina Elena, 299 – 00161 Roma

RISG
Anniversario
100

Presentato al centenario RISG

**Sostanze grasse:
ricerca, innovazione
e scenari futuri**

15 novembre 2023

Food Contact Materials (FCM), present at all stages of the food chain (production, processing, storage, consumption), are fully within the scope of Food Safety, as illustrated by the European Union in the White Paper (Brussels 2000). They shall be subject to the Reg. (EC) 178/2002, to the general rules, as well as to the official control procedures established by the Reg. (EU) 2017/625; Member States are required to verify that FCM meets food safety requirements by carrying out appropriate checks at borders and on national territory.

More specifically, the "framework rule" of FCM is the Reg (EC) no. 1935/2004 which establishes general principles of safety and inertia (art. 3), also anticipating a harmonized EU regulatory framework and allowing the Member States to maintain or adopt specific measures where specific EU laws do not exist (art. 6).

Currently the materials covered by EU regulations are plastic [Reg. (EU) 10/2011 as amended and supplemented], polyamide/melamine FCM [Reg. (EU) 284/2011] and materials "Active & Intelligent" [Reg. (EC) 450/2009]. For ceramics and regenerated cellulose, specific directives have been issued.

At the national level, the DM 21/03/1973 as amended and supplemented shall legislate on paper and board, rubber, glass, stainless steel and the non-harmonized part of plastic FCM. Specific provisions are also laid down for aluminium FCM (DM No. 76 of 18/04/2007), tinfoil (DM No. 18/02/1984 and DM No. 405 of 13/07/1995) and tin-free steel FCM (DM No. 243 of 01/06/1988).

In addition to the abovementioned Reg. (EC) No 1935/2004, the general regulatory framework also provides national provisions valid for all FCM, regardless of the possible presence of specific provisions for certain materials; they are Presidential Decree No. 777 of 23/08/1982 and Legislative Decree No. 108 of 25/01/1992.

Furthermore, specific provisions regarding recycled plastic FCM are laid down in Reg. (EU) 2022/1616 that repealed the Reg. (EC) 282/2008.

Finally, all FCM shall respond to Reg (EC) 2023/2006 on good manufacturing practices.

1. FCM: GENERAL SAFETY PRINCIPLES

Food Contact Materials (FCM) are ready-to-use materials and objects that could be already in contact with food or would come into contact with food during their lifecycle.

They are present in all stages of the food chain, from production (agricultural harvesting, automatic milking) and processing (contact with equipment, work benches) up to the stages of packaging and storage (canned, sliced, frozen foods). Finally, cooking stages (industrial cooking or cooking pots) and final consumption (cutlery, dishes) are included.

According to the chemical properties of food they are expected to come into contact, FCM need to be projected properly. Infact they could come into contact with acid or alkaline solutions or they may have to withstand thermal shock. To ensure food safety, proper technological suitability and appropriate packaging technologies need to be foreseen.

FCM can be made by a wide type of materials of natural and synthetic origin as well and they are synthetically illustrated in Table I.

Table I – Materials constituting the FCM

Classes of Materials	Specific Materials
Synthetic polymers	Plastic polymers Rubbers Non-woven
Cellulosic materials	Papers Cardboard Wood Cork Regenerated cellulose
“Silicea” based materials	Ceramics Glass Crystal
Metallic materials	Coated and uncoated metals and alloys Tinplate Tin-free steel

They range from synthetic polymers (plastic, rubber, non-woven) to different cellulosic materials (paper, cardboard, wood ecc.). Even “silicea” based materials are also widely used (ceramic, glass and crystal) as well as metallic materials (coated and uncoated metals and alloys, tinplate and tin-free steel). It is important to highlight that the final product could be made by different materials used alone or in combination. All materials have different characteristics but nothing is absolutely inert or insoluble, therefore all materials can be a potential source of food contamination if placed in contact with it. The contaminants might migrate from materials to food and the level of contamination depends on many combined factors. They are the nature of the migrant substance and the nature of the materials, but also the nature of food as well as the contact conditions (time, temperature, surface). From a general point of view, the risk depends on three factors:

- the interaction between material and food, that is the migration from FCM to the food; it is evaluated by experimental tests or calculation through migration models
- the toxicological properties of the migrant substance (hazard) known from toxicological studies or literature data
- the consumer’s exposure known through the amount of food consumed and type of consumer (child, adult etc.)

As regards the risk related to the use of the FCM, the normative framework applicable is very complex; the rules apply, as a matter of priority, to producers, importers and distributors, but also end-users are required to comply with the provisions.

2. GENERAL REGULATORY FRAMEWORK

The FCM, present at all stages of the food chain, are fully within the scope of Food Safety, as illustrated by the European Union in the White Paper – Brussels 2000 [1]. The adopted measures laid out the actions needed to modernise European legislation on food, proposing a comprehensive and integrated strategy applicable to the whole food chain “from farm to fork”. The provisions regarding “official controls” are also part of this strategy and, as part of a complete and correct management of issues related to food safety, the strategy also contemplates the need to provide correct information to end consumers.

The regulatory structure relating to food contact materials and articles provides for general provisions (summarized in Table II and Table III).

Table II – General regulatory framework

European legislation	National legislation
Regulation (EC) N. 178/2002 Regulation (EU) N. 2017/625 Commission Communication (2022/C 467/02)	Legislative Decree No. 27 of 02/02/2021

Table III – FCM general provisions

European legislation	National legislation
Regulation (EC) No. 1935/2004 Regulation (EC) 2023/2006	Presidential Decree No. 777 of 08/23/1982 Legislative Decree No. 108 of 01/25/1992 Legislative Decree No. 29 of 10/02/2017

Moreover, provisions are set for certain materials (summarized in Table IV).

Table IV – FCM specific provisions

European legislation	National legislation
Regulation (EU) No. 10/2011 Regulation (EU) No. 284/2011 Regulation (EU) No. 2022/1616 Regulation (EC) No. 1895/2005 Regulation (EC) No. 450/2009 Regulation (EU) No. 2018/213 Directive 84/500/EEC Directive 2005/31/EC Directive 2007/42/EC Directive 93/11/EEC	Ministerial Decree 21/03/1973 as amended Ministerial Decree 04/04/1985 Ministerial Decree 01/02/2007 Ministerial Decree No. 76 of 18/04/2007 Ministerial Decree 18/02/1984 Ministerial Decree No. 405 del 13/07/1995 Ministerial Decree No. 243 del 01/06/1988

First of all, FCM fall within the scope of Regulation (EC) No 178/2002 [2], as already indicated in the recitals where it is written that *“In order to take a sufficiently comprehensive and integrated approach to food safety, there should be a broad definition of food law covering a wide range of provisions with a direct or indirect effect on the safety of food and feed, including provisions on materials and articles in contact with food, animal feed and other agricultural inputs at the level of primary production”*.

Article 6 specifies the concept of Risk Analysis, linked to food safety, and defined as a process consisting of three interconnected components: risk assessment, risk management and risk communication. A key role in the risk assessment phase in all fields related to food safety, including FCM, is played by the European Food Safety Authority (EFSA), established by the Regulation. Indeed, as written in article 22, the Authority, as a primary function, *“...shall provide scientific advice and scientific and technical support for the Community's legislation and policies in all fields which have a direct or indirect impact on food and feed safety”*. In addition, in article 28, in the Description of the Scientific Committee and the Scientific Panel, the *Panel on food additives, flavourings, processing aids and materials in contact with food* is expressly indicated.

FCM fall within the scope of Regulation (EU) 2017/625 [3] on official controls and other official activities. As indicated in Article 1, *“This Regulation shall apply to the official controls performed for the verification of compliance with the rules, whether established at Union level or by the Member States, to apply Union legislation, in the areas of food and food safety [...omissis...] and the manufacture and use of materials and articles intended to come into contact with food”*. The Regulation, in establishing common standards for official controls, also ensures that the legislation is correctly applied and enforced, while specifying the full applicability of both European standards, where existing, and national standards.

In order to assist national authorities in the application of Regulation (EU) 2017/625, the Commission Communication (2022/C 467/02) [4] has been issued. Moreover, the Legislative Decree No. 27 of 02/02/2021 [5] has been issued to adapt national legislation to the provisions of the regulation.

2.1 GENERAL PROVISION FOR FCM

General provisions for FCM are established at EU and national level. As regards the EU regulatory structure, the FCM framework legislation is the Regulation (EC) No. 1935/2004 [6]. The purpose of the Regulation is *“to ensure the effective functioning of the internal market for the FCM...securing a high level of protection of human health and the interests of consumers”*.

However the core of the regulation is represented by the article 3 *“Materials and articles, including active and intelligent materials and articles, shall be manufactured in compliance with good*

manufacturing practice so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could: (a) endanger human health; or (b) bring about an unacceptable change in the composition of the food; or (c) bring about a deterioration in the organoleptic characteristics thereof”.

All FCM shall comply with the requirements of Article 3, which includes the concept of risk assessment.

The regulatory tools useful for risk management can be, for example, lists of substances authorized, purity standards, specific conditions of use, specific and overall limits on migration etc.

In the absence of specific measures, the Regulation shall not prevent Member States from maintaining or adopting national provisions.

Article 15 contains important indications regarding labelling, it shall be made clear that the object can be intended for food contact and any restrictions on use shall be indicated; while article 17 specifies that the traceability of materials and articles shall be ensured at all stages in order to facilitate control.

It is important to underline what indicated in article 16 about Declaration of Compliance (DoC), a written declaration stating that the products comply with the rules applicable to them. Moreover, a supporting documentation (SD) to demonstrate such compliance shall be made available to the competent authorities on demand.

The DoC and the SD are always present, both in European laws and in our national legislation; although there are differences between EU and national legislation, the fundamental principles and consequences arising from the application of the DoC and SD rules are common.

The DoC constitutes a key point both in the assumption of responsibility of FCM producers and in the correct transfer of information between companies in the supply chain, whereas SD is a mean of demonstrating the compliance of a FCM to the competent authority, in case of inspections or controls.

For the drafting of the DoC there are legislative indications, while for the SD there is only a generic strategy of shared approach. It should have an organized collection, containing the specification of composition and procurement, the certification/declaration of compliance issued by the supplier, when applicable, the test reports on starting material, raw materials, semiprocessed and/or finished articles, or any other documents which enables the business operator to demonstrate to the Competent Authorities that what their company produces comply with the rules on FCM.

The European regulatory framework also provides that the FCM comply with Regulation (EC) 2023/2006 [7]. It means that all companies that produce, market and import FCM shall operate according to Good Manufacturing Practice (GMP) and shall prepare a Quality Assurance System and a Quality Control System.

Since 2009, the Istituto Superiore di Sanità, in collaboration with the Industrial Associations of the FCM sector, has launched the CAST Project (Contatto Alimentare Sicurezza e Tecnologia) and several Guidelines for the application of the Regulation (EC) 2023/2006 to the supply chain of FCM have been developed and updated over time [8, 9, 10, 11]. Specific documents have also been drawn up to provide information relating to the SD to be made available on demand to the competent authorities [12]. It is important to underline that the guidelines do not have a binding value, but they are a valid tool for a harmonized and shared approach with stakeholders to the abovementioned issues.

The general regulatory framework also provides national provisions valid for all FCM, regardless of the possible presence of specific provisions for certain materials. They are Presidential Decree No. 777 of 23/08/1982 [13] and Legislative Decree No. 108 of 25/01/1992 (constituting an amendment to Presidential Decree no. 777). They contain general provisions, including the mandatory presence of the DoC and the necessary indications on labelling. In these decrees, penalties are also indicated even if the specific Legislative Decree No. 29 of 10/02/2017 [14] is currently in force.

2.2 SPECIFIC PROVISION FOR FCM

Plastic is a material widely used in FCM; it is regulated at European level by Regulation (EU) No. 10/2011 [15], by now in its 17th amendment. It applies to FCM of homogeneous single- and multi-layer plastics (i.e. materials made entirely of plastic, although of different types) held together by adhesives or other means, to plastics whether or not molded or coated, to plastic layers or coatings forming seals

of lids and closures. Instead heterogeneous multi-layer multi-material plastics (FCM consisting of plastics coupled to materials other than plastic) are regulated by national legislation, as falling within the scope of Ministerial Decree 21/03/1973 [16] as amended. In the case of multi-material multi-layer plastics, only the layer that comes into direct contact with food shall comply with the regulatory requirements, provided that it acts as a barrier preventing the migration of material constituents, not directly in contact with the food, to the food itself. For this purpose, the legislation provides for specific migration tests. More generally, European and national regulatory instruments consist primarily of positive lists of monomers and additives, overall and specific migration limits. Other useful instruments are restrictions on use and compliance testing (migration test) as specified in the legislation (food simulants, conditions of contact such as time and temperature). As indicated above, the DoC must be present along all the stages of the supply chain and the SD must be available upon request of the competent authorities. Annex IV of the Regulation (EU) No. 10/2011 highlights nine points with detailed descriptions of the information that must be present in the DoC.

Special conditions apply to polyamide and melamine plastic kitchenware from the People's Republic of China and Hong Kong according to Regulation (EU) No. 284/2011 [17]; these FCM shall be accompanied by a further declaration and test report of the analytical determinations of primary aromatic amines and formaldehyde.

For recycled plastic materials intended to come into contact with food, the Regulation (EU) 2022/1616 [18] is in force; it provides for suitable recycling technologies and processes. The Regulation also states the authorization procedures for recycling technologies and the official controls to be carried out on installations.

Among the European regulations, some are foreseen in more specific areas such as Regulation (EC) No. 1895/2005 for the use of certain epoxy derivatives in FCMs [19]; Regulation (EC) No. 450/2009 on active and intelligent material in FCM [20].

Ceramics is also a material regulated at European level. Directive 84/500/EEC [21], subsequently amended by Directive 2005/31/EC [22] are in force; in Italy they were implemented with the Ministerial Decree 04/04/1985 [23] and the Ministerial Decree 01/02/2007 [24] respectively. Ceramic FCM require maximum levels of cadmium and lead to be met, moreover the presence of DoC up to the retail stage is needed.

Further European directives are foreseen for regenerated cellulose films, Directive 2007/42/EC [25] and for the release of the N-nitrosamines and N-nitrosatable substances from elastomer or rubber, Directive 93/11/EEC [26]; both are implemented in Italy with specific updates of the Ministerial Decree 21/03/1973.

As required by Regulation (EC) No. 1935/2004, for materials not subject to specific European provisions, national regulations are applicable, where existing. Several FCM fall within the scope of the abovementioned Ministerial Decree 21/03/1973 as amended and they are stainless steel, glass, paper and cardboard, rubber, regenerated cellulose and non-harmonised parts related to plastics (heterogeneous multi-material multi-layer).

As regards stainless steel FCM, the Ministerial Decree 21/03/1973 provides positive lists with the types of stainless steels can be used; they are identified using acronyms provided by international standards or, alternatively, they are identified with chemical cast analysis. These lists may be updated periodically; the last update is Ministerial Decree No. 208 of 25/11/2022 [27]. Overall migration and specific migration limits for chromium, nickel and manganese are also foreseen.

For glass FCM, there are no positive lists, but three categories of glass are indicated with their respective limitations and tolerances of use. For all categories, there are global migration limits; while specific migration limits are provided for lead in crystals.

For paper and board FCM, the Ministerial Decree 21/03/1973 shall include positive lists for fibrous materials, fillers, auxiliary substances, optical whiteners and processing aids. Paper and board FCM shall meet specific composition and purity requirements. All the updates of the Ministerial Decree 21/03/1973 on paper and board flow into the Ministerial Decree no. 217 of 25/09/2007 [28].

Positive lists are also provided for rubber FCM; the compliance shall be verified by migration tests; the Ministerial Decree 21/03/1973 also contains indications for dyes in rubber FCM.

Aluminum FCM are subject to the provisions of Ministerial Decree No. 76 of 18/04/2007 [29]. They shall meet specific purity and composition requirements. No migration tests are foreseen.

Finally, specific provisions are provided for tinplate FCM (Ministerial Decree 18/02/1984 [30], updated by the Ministerial Decree No. 405 del 13/07/1995 [31]) and for tin-free steel (Ministerial Decree No. 243 del 01/06/1988 [32]). In both cases, global migration test and specific migration test of some metals shall be carried out.

On the sidelines of the current legislation, several Notes from the Ministry of Health are also issued. To facilitate their use, DGISAN Note 32249 of 11/10/2011 concerning the DoC of FCM and Note 20072 of 20/05/2014 on objects in metal alloys and objects coated with porcelain enamel are cited.

It is important to underline that the general and specific regulatory framework applicable to FCM, complex and detailed, pursues the aim of the consumer health and it is currently being carefully considered in order to favour updates with primary objectives of safety and sustainability.

3. REFERENCES

- [1] White Paper on Food Safety, COM (99) 719 final, 12 January 2000
- [2] Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, *Official Journal of the European Communities* L31/1-24, February 2, 2002
- [3] Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation), *Official Journal of the European Union* L95/1-142, April 7, 2017
- [4] Commission Notice on the implementation of Regulation (EU) 2017/625 of the European Parliament and of the Council (Official Controls Regulation), *Official Journal of the European Union* C467/2-32, December 8, 2022
- [5] Decreto Legislativo 2 febbraio 2021, n.27 Disposizioni per l'adeguamento della normativa nazionale alle disposizioni del Regolamento (UE) 2017/625 ai sensi dell'articolo 12, lettere a), b), c), d) ed e) della legge 4 ottobre 2019, n.117, *Gazzetta Ufficiale Serie Generale* n.60, 11 marzo 2021
- [6] Regulation (EC) No. 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC, *Official Journal of the European Union* L338/4-17, November 13, 2004
- [7] Commission Regulation (EC) No 2023/2006 of 22 December 2006 on good manufacturing practice for materials and articles intended to come into contact with food, *Official Journal of the European Union* L384/75-78, December 29, 2006
- [8] M.R. Milana, M. Denaro, R. Feliciani, A. Maggio, A. Maini, G. Padula, CAST Project, Guidelines for the application of the Regulation (EC) 2023/2006 to the supply chain of materials and articles intended to come into contact with food, *Rapporti ISTISAN* 11/37
- [9] M.R. Milana, M. Denaro, R. Feliciani, C. Gesumundo, A. Maggio, V. Mannoni, O. Panico, G. Padula, Progetto CAST (Contatto Alimentare Sicurezza e Tecnologia), Linee guida per il riscontro documentale sull'applicazione del Regolamento (CE) n. 2023/2006, *Rapporti ISTISAN* 13/14
- [10] M.R. Milana, M. Denaro, R. Feliciani, C. Gesumundo, A. Maggio, V. Mannoni, O. Panico, G. Padula, Progetto CAST (Contatto Alimentare Sicurezza e Tecnologia), Linee guida per il riscontro documentale sull'applicazione del Regolamento (CE) 2023/2006. Vernici, adesivi e inchiostri da stampa, *Rapporti ISTISAN* 16/43

- [11] C. Gesumundo, M.R. Milana, V. Mannoni, S. Giamberardini, F. Vanni, M. De Felice, M. Denaro, R. Feliciani, M. Massara, G. Padula, Progetto CAST (Contatto Alimentare Sicurezza e Tecnologia), Linee guida per l'applicazione del Regolamento (CE) 2023/2006 alla filiera dei materiali e oggetti destinati al contatto con gli alimenti. Edizione 2023, Rapporti ISTISAN 23/4 Rev.
- [12] M.R. Milana, M. Denaro, R. Feliciani, C. Gesumundo, A. Maggio, V. Mannoni, O. Panico, G. Padula, Progetto CAST (Contatto Alimentare Sicurezza e Tecnologia), Linee guida sulla documentazione di supporto per la dichiarazione di conformità alla legislazione sui materiali e oggetti a contatto con alimenti, Rapporti ISTISAN 18/24
- [13] Decreto del Presidente della Repubblica 23 agosto 1982, n.777, Attuazione della direttiva (CEE) n. 76/893 relativa ai materiali e agli oggetti destinati a venire a contatto con i prodotti alimentari, *Gazzetta Ufficiale Serie Generale* n.298, 28 ottobre 1982
- [14] Decreto Legislativo 10 febbraio 2017, n. 29, Disciplina sanzionatoria per la violazione di disposizioni di cui ai regolamenti (CE) n. 1935/2004, n. 1895/2005, n. 2023/2006, n. 282/2008, n. 450/2009 e n. 10/2011, in materia di materiali e oggetti destinati a venire a contatto con prodotti alimentari e alimenti, *Gazzetta Ufficiale Serie Generale* n.65, 18 marzo 2017
- [15] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, *Official Journal of the European Union* L12/1-89, January 15, 2011
- [16] Decreto Ministeriale 21 marzo 1973, Disciplina igienica degli imballaggi, recipienti, utensili, destinati a venire in contatto con le sostanze alimentari o con sostanze d'uso personale, *Supplemento Ordinario alla Gazzetta Ufficiale* n.104, 20 aprile 1973
- [17] Commission Regulation (EU) No 284/2011 of 14 January 2011 laying down specific conditions and detailed procedures for the import of polyamide and melamine plastic kitchenware originating in or consigned from the People's Republic of China and Hong Kong Special Administrative Region, China, *Official Journal of the European Union* L77/25-29, March 23, 2011
- [18] Commission Regulation (EU) 2022/1616 of 15 September 2022 on recycled plastic materials and articles intended to come into contact with foods, and repealing Regulation (EC) No 282/2008, *Official Journal of the European Union* L243/3-46, September 20, 2022
- [19] Commission Regulation (EC) No 1895/2005 of 18 November 2005 on the restriction of use of certain epoxy derivatives in materials and articles intended to come into contact with food, *Official Journal of the European Union* L302/28-32, November 19, 2005
- [20] Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food, *Official Journal of the European Union* L135/3-11, May 30, 2009
- [21] Council Directive of 15 October 1984 on the approximation of the laws of the Member States relating to ceramic articles intended to come into contact with foodstuffs (84/500/EEC), *Official Journal of the European Communities* L277/12-16, October 20, 1984
- [22] Commission Directive 2005/31/EC of 29 April 2005 amending Council Directive 84/500/EEC as regards a declaration of compliance and performance criteria of the analytical method for ceramic articles intended to come into contact with foodstuffs, *Official Journal of the European Union* L110/36-39, April 30, 2005
- [23] Decreto Ministeriale 4 aprile 1985, Disciplina degli oggetti di ceramica destinati ad entrare in contatto con i prodotti alimentari, *Gazzetta Ufficiale Serie Generale* n.98, 26 aprile 1985
- [24] Decreto 1 febbraio 2007. Recepimento della direttiva n. 2005/31/CE della Commissione del 29 aprile 2005, che modifica la direttiva n. 84/500/ CEE del Consiglio, per quanto riguarda una dichiarazione di conformità e i criteri di efficienza dei metodi di analisi per gli oggetti di ceramica, destinati ad entrare in contatto con i prodotti alimentari, *Gazzetta Ufficiale Serie Generale* n.66, 20 marzo 2007
- [25] Commission Directive. 2007/42/EC of 29 June 2007 relating to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs, *Official Journal of the European Union* L172/71-82, June 30, 2007
- [26] Commission Directive 93/11/EEC of 15 March 1993 concerning the release , of the N-

nitrosamines and N-nitrosatable substances from elastomer or rubber teats and soothers, *Official Journal of the European Communities* L93/37-38, April 17, 1993

- [27] Decreto 25 novembre 2022, n. 208. Regolamento recante l'aggiornamento al decreto del Ministro della sanità 21 marzo 1973, recante: «Disciplina igienica degli imballaggi, recipienti, utensili, destinati a venire a contatto con le sostanze alimentari o con sostanze d'uso personale», limitatamente agli acciai inossidabili, *Gazzetta Ufficiale* Serie Generale n.15, 19 gennaio 2023
- [28] Decreto 25 settembre 2007, n. 217. Regolamento recante aggiornamento del decreto ministeriale 21 marzo 1973, concernente la disciplina igienica degli imballaggi, recipienti, utensili destinati a venire a contatto con le sostanze alimentari o con sostanze d'uso personale, *Gazzetta Ufficiale* Serie Generale n.270, 20 novembre 2007
- [29] Decreto 18 aprile 2007, n. 76. Regolamento recante la disciplina igienica dei materiali e degli oggetti di alluminio e di leghe di alluminio destinati a venire a contatto con gli alimenti, *Gazzetta Ufficiale* Serie Generale n.141, 20 giugno 2007
- [30] Decreto 18 febbraio 1984, Disciplina dei contenitori in banda stagnata saldati con lega stagno-piombo ed altri mezzi, *Gazzetta Ufficiale* Serie Generale n.76, 16 marzo 1984
- [31] Decreto 13 luglio 1995, n.405 Regolamento recante aggiornamento del decreto ministeriale 18 febbraio 1984 concernente la disciplina dei contenitori in banda stagnata saldati con lega stagno-piombo ed altri mezzi, *Gazzetta Ufficiale* Serie Generale n.228, 29 settembre 1995
- [32] Decreto 1 giugno 1988, n.243 Disciplina degli oggetti in banda cromata verniciata destinati a venire in contatto con gli alimenti, *Gazzetta Ufficiale* Serie Generale n.153, 1 luglio 1988

Pubblicazioni del passato commentate alla luce delle conoscenze odierne

N. 9 - SETTEMBRE 1956 - Anno XXXIII

RIVISTA MENSILE

Spediz. in abbon. postale Gruppo - III

OLII - GRASSI - COLORI MINERALI E SAPONI E VERNICI

RIVISTA UFFICIALE DELLA STAZIONE SPERIMENTALE PER LE INDUSTRIE DEGLI OLII E DEI GRASSI DELLA COMMIS. TECNICA GOVERNAT. PER GLI OLII MINERALI, GRASSI E SAPONI, COLORI E VERNICI E DELLA DIVISIONE MATERIE GRASSE DELL'UNIONE DI CHIMICA PURA ED APPLICATA - SEZ. ITALIANA

Direz. e Amm.: Olli Minerali - Grassi e Saponi - Colori e Vernici - Milano (36) - Via G. Colombo 79 (Città degli Studi) - Tel. 292.169 - 292.850
Abbonamento annuo: Italia L. 4000 - Estero L. 6000 - Numero separato L. 400

Riproduzione vietata

GIOVANNI D'ARRIGO

Centro Sperimentale Regionale per l'Industria degli Olii, dei Grassi e dei Saponi - Catania

Ricerche spettrofotometriche nell'ultravioletto su olii di semi e sulle loro miscele con olio di oliva^()*

Olii Minerali, Grassi e Saponi, Colori e Vernici 33 (9), 299-304 (1956)

Una nota di

Paolo Bondioli e Lanfranco Conte

SISSG – Società Italiana per lo Studio delle Sostanze Grasse – Milano

Accademia dei Georgofili – Firenze

✉ paolo.bondioli1956@gmail.com

Quando abbiamo iniziato le ricerche bibliografiche con l'obiettivo di rivedere con le conoscenze di oggi articoli di un passato non recente, ci siamo imbattuti in questo lavoro del 1956, che gettava le basi per l'analisi spettrofotometrica degli oli, tecnica analitica ancor oggi in uso e di grande rilevanza diagnostica. Le prime ricerche spettrofotometriche nel campo degli oli risalgono agli anni '30 del secolo scorso (1-3) e si stavano sviluppando in Italia negli anni '50.

L'introduzione passa in rassegna i gruppi cromofori delle sostanze grasse che hanno interesse nella regione dello spettro Ultravioletto (200 – 400 nm). Riportiamo di seguito l'estratto del lavoro, che rappresenta un utile compendio della situazione.

E' noto che i gruppi cromofori presenti nei grassi sono il carbossile —COOR ed il doppio legame —CH=CH— i quali, da soli, assorbono a lunghezze d'onda molto basse e precisamente intorno ai 200 m μ .

Nel caso però in cui compaiono dei doppi legami coniugati, lo spettro ultravioletto presenta delle bande di assorbimento a lunghezze d'onda superiori ai 220 m μ . Tali bande sono caratteristiche per ogni sistema di doppi legami coniugati (sistemi dienici, trienici, tetraenici ecc.) per cui ad ognuno di questi compete una banda, o sistema di bande, in posizioni corrispondenti a lunghezze d'onda determinate.

Gli assorbimenti UV nella regione compresa tra 220 e 300 nm sono attribuibili alla presenza di sistemi dienici e trienici coniugati. Già allora si sapeva che questi sistemi coniugati sono presenti a livelli molto bassi in sostanze grasse vergini o comunque non processate e che l'assorbimento UV aumenta con la raffinazione. Ricordiamo inoltre che, al tempo di questa pubblicazione, la gascromatografia era soltanto agli albori e non risultano applicazioni analitiche sulle sostanze grasse. Da qui l'idea di sottoporre a isomerizzazione alcalina campioni di olio per provocare la coniugazione di acidi grassi con sistemi dienici e trienici in forma nativa (e quindi a ponte metilenico interrotto) per indurre assorbimento nella regione UV. Scopo dell'isomerizzazione era quindi di realizzare una stima indiretta della presenza di acidi di e triinsaturi nel campione, nell'ottica di differenziare l'olio di oliva da altri oli vegetali.

Il fatto che alcuni olii, pur contenendo degli acidi insaturi, non posseggono sistemi di doppi legami coniugati, sembrò limitare notevolmente, in un primo tempo, le possibilità d'impiego della spettrofotometria ultravioletta nel campo dei grassi, fino a quando non si riuscì, mediante trattamento con alcali, a trasformare tali prodotti insaturi non coniugati nei loro isomeri coniugati.

Il primo a notare tale possibilità fu il Moore (4).

Metodi quantitativi per l'analisi dei grassi, basati su tale trattamento con alcali, furono proposti per la prima volta da Mitchell, Kraybill e Zscheile (5) e successivamente modificati e perfezionati da altri (6, 7, 8) per cui oggi tale procedimento di «isomerizzazione alcalina» viene largamente impiegato nell'analisi di una grande varietà di olii e grassi.

L'Autore passa poi ad esaminare l'impatto della presenza di prodotti di ossidazione nel comportamento dei campioni all'analisi UV e che possono quindi interferire con la determinazione dei dieni e trieni coniugati allo stato nativo.

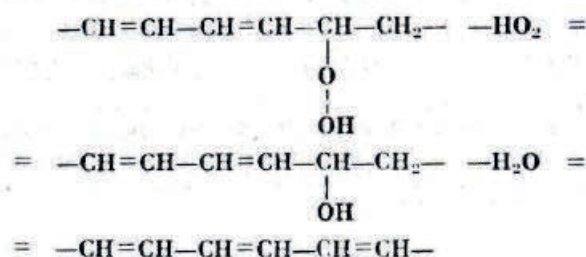
Per poter più agevolmente interpretare le curve di assorbimento nell'ultravioletto degli olii, riportate nella parte sperimentale, è necessario accennare, sia pur brevemente, al fenomeno della ossidazione spontanea degli olii con riferimento alle alterazioni che essa apporta negli spettri di questi.

Secondo recenti teorie, la ossidazione dei sistemi insaturi non-coniugati porterebbe, in seguito a spostamento di doppi legami, alla formazione di idroperossidi coniugati per cui, negli olii contenenti tali sistemi insaturi, si nota durante i primi stadi di ossidazione, un aumento nella intensità di assorbimento intorno a 230 m μ , dovuto a coniugazione diecnica (9, 10).

La ossidazione dei composti poliinsaturi coniugati è notevolmente diversa dalla precedente e porta delle alterazioni, negli spettri ultravioletti di tali composti, che si manifestano con una diminuzione dell'assorbimento nella regione compresa fra 260 e 280 m μ ed un aumento intorno ai 230 m μ (11, 12). Tale comportamento si spiegherebbe ammettendo la formazione di perossidi dienici, a spese dei sistemi trienici, accompagnata anche da polimerizzazione.

Notevoli alterazioni negli spettri di assorbimento degli oli, si manifestano anche in seguito ai processi di raffinazione. Ciò fu messo in evidenza da Mitchell e Kraybill (13), i quali trovarono che la deodorazione ed in particolare la decolorazione con terre fanno salire l'assorbimento intorno ai 270 m μ . Tale incre-

mento nella regione trienica potrebbe spiegarsi, alla luce delle moderne teorie sulla ossidazione, ammettendo una demolizione degli idroperossidi dienici con perdita di un atomo di ossigeno e formazione del corrispondente composto ossidrilato dal quale, per eliminazione di una molecola di acqua, si otterrebbe un triene.



Tale eliminazione di acqua sarebbe favorita dalle terre impiegate nella decolorazione.

Nella parte sperimentale vengono descritte le procedure di laboratorio utilizzate per la preparazione e l'analisi dei campioni. Interessante e curioso allo stesso tempo è l'impiego di etanolo 96° come solvente, mentre in tutti gli articoli successivi e anche attualmente si utilizzano idrocarburi (esano, isotano, cicloesano, etc.) dotati di maggiore potere solvente nei confronti delle sostanze grasse.

Il lavoro prosegue quindi con la pubblicazione di spettri UV di oli di semi di lino, cotone, papavero, girasole, arachide, soia, vinaccioli, sesamo, colza e ravizzone. Questi spettri sono messi in comparazione con quello di un olio di oliva di pressione di buona qualità. L'Autore osserva che, con l'eccezione dei primi tre campioni, per tutti gli altri gli assorbimenti nell'intorno di 230 e 270 nm sono molto spiccati. Da qui la preparazione di miscele di olio di oliva genuino (Nota 1) con il 20 % di oli di girasole, arachide, soia, vinaccioli, sesamo, colza e ravizzone. Gli spettri riportati dimostrano in maniera evidente la presenza degli oli estranei.

In tutto questo ragionamento esiste però un errore di base: gli assorbimenti UV degli oli estranei sono dovuti alla presenza di sistemi dienici e trienici coniugati che si sono generati dalla raffinazione degli oli vegetali, che in base alla legge Salari diventerà obbligatoria nel 1968. È a tutti oggi noto che oli di semi ottenuti per spremitura a bassa temperatura e non raffinati non presentano assorbimenti UV tali da renderli individuabili quando in miscela con olio di oliva genuino.

Anche l'Autore ne è consapevole, come dimostra nel paragrafo che segue. Dobbiamo considerare che all'epoca di pubblicazione di questo lavoro era ancora possibile la vendita di olio sfuso e frequenti e possibili erano le aggiunte di oli estranei (oli di semi raffinati) all'olio di oliva nella catena distributiva, fino alla vendita al dettaglio. Al tempo vigeva anche l'obbligo di aggiungere agli oli raffinati una piccola percentuale di olio di sesamo, che consentiva al personale ispettivo di rivelare in loco la commistione con una semplice reazione cromatica, realizzata a carico del sesamolo.

Nota 1: la denominazione dell'olio di oliva vergine è quella del periodo cui si fa riferimento. Solo nel 1960 con Legge 13 Novembre 1960, n. 1407 l'olio di oliva commestibile viene classificato in 4 diverse categorie (extra vergine, sopraffino, fino, corrente) in funzione dell'acidità libera. La classificazione attuale invece trae origine dal Regolamento Europeo n. 2568/91, ora abolito e sostituito da dal Regolamento UE 2022/2104.

E' però da considerare il fatto che generalmente gli oli di semi non vengono impiegati allo stato genuino per scopi commestibili, ma vengono prima sottoposti ai comuni procedimenti di raffinazione (deacidificazione, deodorazione, decolorazione) allo scopo di attenuare le sgradevoli proprietà organolettiche che molti di essi posseggono, sì da alterare il meno possibile l'aspetto dell'olio d'oliva cui possono venire miscelati.

E' risaputo però, come abbiamo fatto rilevare nella prima parte di questa nota, che la raffinazione apporta delle profonde alterazioni negli spettri di assorbimento ultravioletti degli oli in genere, alterazioni che si manifestano con una diminuzione dell'assorbimento intorno ai 230 m μ (regione dienica) ed un aumento nella regione trienica con un massimo principale a 270 m μ e due secondari, ad esso associati, a 260 e a 280 m μ . Era quindi prevedibile che tali alterazioni negli spettri si riscontrassero anche per le miscele degli oli raffinati con l'olio genuino d'oliva, per cui ci siamo proposti di indagare fino a che punto fosse possibile svelarne la presenza. Pertanto abbiamo dapprima rilevato gli spettri di alcuni oli di semi raffinati e poi quelli delle miscele, a diversi tenori, di questi con olio d'oliva di pressione e le curve ottenute sono riprodotte qui di seguito.

La vendita di olio sfuso al dettaglio è stata poi vietata con Legge 27 gennaio 1968, n. 35, art. 7 "Norme per il controllo della pubblicità e del commercio dell'olio di oliva e dell'olio di semi."

L'articolo si conclude con la pubblicazione degli spettri UV ottenuti da oli di oliva raffinati, secondo la terminologia dell'epoca identificati come Rettificato A e Rettificato B (Nota 2) ed è in questa occasione che, riferendosi agli assorbimenti nelle regioni di 230 e 270 nm, si intravede probabilmente per la prima volta la possibilità di distinguere tra oli di oliva vergini, raffinati e loro miscele.

Nota 2: la terminologia utilizzata (Rettificato A e B) si riferisce agli oli raffinati di oliva e di sansa rispettivamente, che non potevano essere messi in commercio tal quali, ma previa aggiunta di olio di oliva vergine. Le miscele assumevano la definizione di Oli di Oliva e Olio di Sansa e di Oliva, tuttora vigente (Legge 13 Novembre 1960, n. 1407 art. 3)

Possiamo quindi affermare che questo lavoro, sicuramente insieme ad altri pubblicati nello stesso periodo, è l'antenato dell'attuale metodo COI/T.20/Doc. No 19/Rev. 5: 2019 "Spectrophotometric investigation in the ultraviolet", per il quale è prescritto l'impiego di isotano o di cicloesano. Interessante è notare che il massimo di assorbimento per i sistemi trienici è stabilito a 268 o 270 nm in funzione del solvente impiegato, come conseguenza dell'effetto batocromo dovuto alla diversa polarità dei solventi.

Una versione meno recente della metodica in oggetto, pubblicata come metodo NGD C40-1976, così come la norma COI ed il Reg (CEE) 2568/91 prevedevano un passaggio su allumina per campioni che non rispettavano i parametri UV degli oli vergini, con lo scopo di rimuovere i prodotti di ossidazione, alcuni dei quali hanno assorbimenti nell'intorno dei 270 nm. A seguito del passaggio su allumina si ripeteva la lettura UV, i risultati della quale a questo punto erano attribuibili solo alla presenza di sistemi di doppi legami coniugati. Il passaggio su colonna di allumina venne eliminato per problemi di affidabilità del metodo di purificazione così come veniva realizzato, che prevedeva un passaggio su colonna difficilmente riconducibile ad una pratica cromatografica, in quanto veniva percolato un rilevante volume di soluzione al 10%, il che comportava un recupero di poco superiore al 30-35%, recupero che peraltro non dava origine ad un campione rappresentativo di quello di partenza, in quanto la composizione acidica post purificazione differiva sensibilmente da quella del campione di partenza. La Commissione Tecnica Italiana realizzò esperienze di purificazione utilizzando silice anziché allumina e gestendo l'eluizione secondo le regole della cromatografia (eluizione in banda ristretta), avendo come risultato un recupero quantitativo dell'olio e nessuna alterazione della composizione degli acidi grassi.

Un'altra considerazione che portò alla eliminazione della purificazione fu una semplificazione; se per attribuire un olio ottenuto dalla lavorazione delle olive alla categoria "extra vergine" devono essere rispettati determinati limiti di estinzione specifica a 232 e 268/270 nm, poco importa per quale motivazione ciò non avvenga, se per ossidazione, raffinazione o miscelazione con oli estranei raffinati.

Probabilmente si trattava di una necessità di discriminare tra un olio ossidato che quindi poteva nascere come extra vergine ed essere andato incontro ad ossidazione, configurando così, in qualche modo, una non volontà di dolo nel perpetrare una frode, da un olio miscelato ad un raffinato, di oliva o di seme, nel qual caso invece il dolo era evidente.

Si riporta qui la bibliografia originale pubblicata a corredo dell'articolo, a favore di chi volesse ulteriormente approfondire l'argomento.

BIBLIOGRAFIA

- 1) A. CHEVALLIER, J. GUILLOT, P. CHABRE: *Bull. Soc. Chim. Biol.*, 15, 358-68 (1933).
- 2) J. GUILLOT: *Ann. Fals. Fraudes*, 28, 69-75 (1935).
- 3) A. DINGWALL, J. C. THOMSON: *J. Am. Chem. Soc.*, 56, 899 (1934).
- 4) T. MOORE: *Biochem. J.*, 31, 138 (1937).
- 5) J. H. MITCHELL, H. R. KRAYBILL, F. P. ZSCHEILE: *Ind. Eng. Chem. Anal. Ed.*, 15, 1 (1943).
- 6) M. L. SWAIN, B. A. BRICE: *J. Am. Oil Chem. Soc.*, 26, 272 (1949).
- 7) T. P. HILDITCH, C. B. PATEL, J. P. RILEY: *The Analyst*, 76, 81 (1951).
- 8) E. G. HAMMOND, W. O. LUNDBERG: *J. Am. Oil Chem. Soc.*, 30, 433 (1953).
- 9) R. T. HOLMAN, W. O. LUNDBERG, W. M. LAUER, G. O. BURR: *J. Am. Chem. Soc.*, 67, 1285 (1945).
- 10) E. H. FARMER, D. A. SUTTON: *J. Chem. Soc.*, 122 (1943).
- 11) R. W. BRAUER, L. T. STEADMAN: *J. Am. Chem. Soc.*, 66, 563 (1944).
- 12) R. R. ALLEN, F. A. KUMNEROW: *J. Am. Oil Chem. Soc.*, 28, 101 (1951).
- 13) J. H. MITCHELL, H. R. KRAYBILL: *J. Am. Chem. Soc.*, 64, 988 (1942).
- 14) R. RAMB: *Fette und Seifen*, 47, 575 (1940).
- 15) J. P. WOLFF: *Rev. Franc. Corps Gras*, 3, 17 (1956).
- 16) A. UZZAN: *Rev. Franc. Corps Gras*, 3, 9 (1956).

Una copia dell'articolo originale di D'Arrigo può essere richiesta inviando una email a: risg@mi.camcom.it

La storia per quanto ci riguarda non finisce qui: sulla Rivista abbiamo reperito altri articoli successivi a questo, che illustrano in maniera dettagliata le basi molecolari e il significato diagnostico delle metodiche analitiche spettrofotometriche.

Una nota di prossima pubblicazione si occuperà di una di questi articoli ormai dimenticati, ma che costituiscono una solida base teorica per il chimico delle sostanze grasse dei nostri giorni.

Riteniamo estremamente opportuno fare una azione di recupero di queste basi teoriche, pur nella convinzione che queste costituiscano patrimonio comune dei moderni ricercatori.

Tuttavia, in considerazione della impressionante evoluzione delle apparecchiature analitiche che ha fatto seguito alla nascita ed allo sviluppo dei sistemi informatici, appare quanto mai opportuno ricordare e rivedere cosa si trova alla base delle moderne tecniche analitiche. In altre parole pensiamo sia necessario andare a scavare al di sotto di questi apparecchi per ricordare o a riscoprire la teoria chimica sulla quale queste metodologie sono fondate.

Per concludere, ci ha molto incuriosito l'affiliazione dell'Autore Giovanni D'Arrigo, il Centro Sperimentale Regionale per l'Industria degli Olii, dei Grassi e dei Saponi di Catania.

Da una ricerca sul web è emerso un filmato del 1960 prodotto dall'Istituto Luce, che annuncia la fondazione del Centro, reperibile all'indirizzo:

<https://patrimonio.archivioluca.com/luce-web/detail/IL5000036034/2/nasce-grazie-alla-regione-sicilia-centro-sperimentale-l-industria-degli-olii-grassi-e-saponi-garantire-assistenza-tecnica-3.html?startPage=0>

Nel filmato si menziona la fondazione nel 1960; al contrario l'articolo a firma D'Arrigo con la citata affiliazione è del 1956.

Un documento reperito negli archivi online dell'università di Catania

<http://archivia.unict.it/bitstream/10761/328/4/4.%20LA%20SICILIA%20E%20IL%20PIANO%20MARSHALL.pdf>

menziona il Centro Sperimentale per l'Industria dei Grassi e dei Saponi, istituito con Decreto Presidenziale n. 72/A del 2 Maggio 1951.

In atti Parlamentari della Camera dei Deputati, VII legislatura il Centro risulta essere soppresso con Legge 8 marzo 1971, n. 5 e i 7 dipendenti messi in carico all'ESA (Ente di Sviluppo Agricolo).

http://legislature.camera.it/dati/leg07/lavori/stampati/pdf/015_118001_F002.pdf

Riguardo la figura dell'Autore, Giovanni D'Arrigo, è stato reperito sulla Gazzetta Ufficiale della Regione Siciliana il Decreto 29 Marzo 1968 n. 292 che attribuisce a D'Arrigo la funzione temporanea della Direzione del Centro, in sostituzione del Prof. Guglielmo Stagno D'Alcontres, dimissionario.

<https://www2.regione.sicilia.it/beniculturali/dirbenicult/soprintendenze/vincoli/Paesaggistici/CARTELLA%20DECRETI%20E%20VERBALI%20VINCOLI%20PROVINCIE%20DELLA%20SICILIA/SIRACUSA/6%20-%20-%20Isola%20di%20Ortigia.pdf>



ANNUNCI DI RICERCA PARTNER per progetti di ricerca e trasferimento tecnologico

Enterprise Europe Network (EEN)

195

Anno 2024
(aggiornato al 30 settembre 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 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 patented 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 RDRRO20230526011

A Romanian research institute is looking for partners for Eurostars calls

The Romanian research institute is looking for international partners (SMEs, SME + research orga-

nization) 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 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 established 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

Progetto BOFR20240223025

A French company offers a subcontract to produce white-label natural liquid food supplements for foreign partners

A French company offers manufacturing and packaging products without heat treatment, preservatives or additives in glass ampoules (5-15mL), bottles (20mL-1L) or sterile bags (10-1000L). The SME offers subcontracting agreement to companies in sectors of nutraceuticals, sport nutrition, health nutrition, nutricosmetics, animal nutrition or pet food.

23 Apr 2025

Progetto TODE20241004015

A German research institute specialized in nutrition food technology and bioeconomy offers opportunities for product and process development

The german research institute is specialized in the development, analytics and production in the fields nutrition, food science and biotechnology. It wants to support and help companies, that have a need in for product development or process optimization, but do not have the necessary technical, analytical or human resources. In addition to participation in

research projects, collaboration is also sought in the form of commercial agreements with technical support.

Dead-line for EOIs:16 Oct 2025

Progetto TRES20240612015

Spanish agricultural company is looking for new techniques for agricultural, ecological, self-made products through a technical cooperation or financial agreement

This agricultural company is located in Valencia (Spain). It has more than ten years of experience in the agriculture sector. They grow fresh and seasonal fruits and vegetables based on modern, advanced and sustainable agriculture. One of the advantages offered by the project is to strengthen the farmer-consumer relationship, so that the acquisition of products is made directly. They are searching for new crop-growing techniques to expand through a technical cooperation or a financial agreement.

Dead-line for EOIs:12 Jun 2025

Progetto BOUA20240529003

Production of dairy products and purchase of equipment in the dairy industry

The company produces a wide range of products, including also export-oriented (butter 82% fat, casein, hard cheeses). The company plans to conclude both short-term and one-time agreements for the supply of goods of its own production.

Dead-line for EOIs:03 Jun 2025

Progetto BOIT20240411011

An Italian organic olive oil producer is looking for distributors in specific European countries

An Italian organic farm offers its organic extra virgin olive oil under distribution services agreements in specific European countries.

The company carries out all the production process phases, from the harvest to the packaging, processing local varieties of olives grown at their own plantings. It is a JAS (Japanese Agricultural Standard) and COR (Canada Organic) certified firm.

Dead-line for EOIs:11 Apr 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



RECENSIONI DI LIBRI

LA CONSERVAZIONE DEI CEREALI

A CURA DI:
GIANNI BACCARINI, ANDREA VILLANI



II Edizione - 2024
€ 29,00 - Edagricole di New Business Media srl
ISBN: 978-88-506-5651-6
Pagine 256 - formato 17 x 24 cm
e-mail: libri.edagricole@newbusinessmedia.it
www.edagricole.it

Una nuova edizione completamente rivisitata e con nuovi approfondimenti

La conservazione, quale anello di congiunzione intermedio e multidisciplinare fra la produzione e la trasformazione delle materie prime, è oggi chiamata a concorrere all'assicurazione dei due pilastri fondamentali del sistema alimentare - quantità e qualità - riassunti nei concetti della Food safety e della Food security, con la seconda destinata ad assumere una sempre maggiore attualità, in un mondo in cui le risorse produttive si avviano a diventare limitate e devono diventare sostenibili in termini ambientali ed economici.

Questo libro, grazie all'apporto dei migliori esperti del settore e attraverso l'approfondimento delle numerose problematiche connesse allo stoccaggio dei cereali e alle filiere mercantili, vuole invitare a una nuova e moderna pratica conservativa, capace di ridurre l'impatto ambientale tramite un uso corretto delle risorse e dei mezzi di difesa, in una visione d'insieme che abbracci sostenibilità, rispetto del lavoro e del territorio.

INDICE:

Strutture e tecniche di conservazione - Il ruolo della conservazione nella valorizzazione dei prodotti di filiera - Conservazione dei cereali, destinazioni d'uso e casi specifici (riso e sementi) - Conservazione e gestione contaminanti, residui, allergeni - Infestanti e tecniche di gestione - Caratterizzazione merceologica dei cereali e dei semi oleosi: le difettosità dei cereali e dei semi oleosi - Norme e schemi di certificazione applicabili alla conservazione.

CURATORI:

Gianni Baccarini, esperto del settore cerealicolo, è consulente in ambito qualità, sostenibilità delle produzioni e sicurezza agroalimentare. Partecipa a diversi gruppi di lavoro istituzionali, è docente di Corsi di formazione e autore di diverse pubblicazioni di settore.

Andrea Villani, agronomo, Accademico corrispondente dell'Accademia Nazionale di Agricoltura e Accademico Ordinario dell'Accademia di Agricoltura di Torino, ha maturato la propria esperienza professionale nell'ambito della Borsa Merci di Bologna e di altre istituzioni di settore.

AUTORI:

Gianni Baccarini | Andrea Villani | Eugenio Giambastiani | Andrea Padovani | Amedeo Reyneri | Fabrizio Piva | Massimo Gregori | Andrea Demonitis | Lorenzo Petrini | Marcello Gatti | Carlo Brera | Paolo Guerra | Gianluca Avoni | Davide Busani.

VINO SOSTENIBILE

Gli standard nazionali e internazionali

A CURA DI:

ETTORE CAPRI, ELISA FRASNETTI, LUCREZIA LAMASTRA



I Edizione - 2024

€ 27,50 - Edagricole di New Business Media srl

ISBN: 978-88-506-5629-5

Pagine 216 - formato 17 x 24 cm

E-mail: libri.edagricole@newbusinessmedia.it -
www.edagricole.it

Il testo si propone come una guida pratica alla sostenibilità del vino attraverso la storia dei sistemi di certificazione - nazionali e internazionali - che mirano a supportare le comunità, ridurre gli impatti ambientali e migliorare la competitività delle aziende, includendo anche aspetti di formazione e incoraggiando l'uso delle tecnologie per il miglioramento continuo.

Per le aziende, l'importanza dei sistemi di certificazione - in un momento storico in cui si fa sempre più necessaria la riflessione su cambiamenti climatici, biodiversità e risorse - risiede anche nella loro natura di strumento di autovalutazione, all'interno di un mercato sempre più orientato alle certificazioni di sostenibilità, capaci di generare impatto in termini di reputazione, innovazione, differenziazione e creazione di reti imprenditoriali.

Il libro è pensato sia per coloro che hanno già intrapreso percorsi di sostenibilità, sia per coloro che, ancora agli inizi, vogliono comprendere meglio i vantaggi culturali, sociali e tecnici connessi alla certificazione dei processi produttivi ed organizzativi della propria azienda.

INDICE:

Gli impegni nazionali e internazionali - La vitivinicoltura sostenibile - I programmi nazionali - Lo standard unico nazionale - Gli elementi imprescindibili di una gestione sostenibile - La misura della sostenibilità - La procedura di certificazione per le aziende - La formazione - Dichiarare il proprio impegno sostenibile.

AUTORI:

Ettore Capri è professore ordinario in Chimica agraria e direttore del centro di ricerca OPERA, l'osservatorio per lo sviluppo sostenibile in agricoltura (Università Cattolica del Sacro Cuore). Dal 2009 idea e sviluppa standard di sostenibilità per il settore agro-alimentare e la ristorazione. In questo contesto ricopre ruoli istituzionali presso le agenzie europee e presso i ministeri italiani.

Elisa Frasnetti, specialista di sostenibilità sociale per una catena della GDO, ha acquisito competenze nel campo della sostenibilità vitivinicola durante la sua esperienza come assegnista di ricerca presso l'Università Cattolica del Sacro Cuore. Qui si è dedicata alla sostenibilità nel settore agroalimentare e ristorativo, concentrando la sua atten-

zione sull'analisi degli impatti ambientali e sull'uso innovativo delle tecnologie per favorire lo sviluppo sostenibile.

Lucrezia Lamastra è professore associato in Chimica agraria presso l'Università Cattolica del Sacro Cuore. È stata responsabile scientifico del programma VIVA - La sostenibilità della vitivinicoltura in Italia, promosso dal Ministero dell'Ambiente e della Sicurezza Energetica, ed è coordinatore del comitato Scientifico del programma siciliano SOSTain.

..... CONGRESSI

DAIRY EXPO TECH 2024

5-6 December 2024 | Piacenza, Italy

The event is 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/>

Hands-on Annual Vegetable Oil Deep Frying Course with Live Demonstrations

9-11 December 2024 | College Station, Texas, USA
Program:

- December 09, 2024

Registration and course Briefing

- December 10, 2024

8:10 AM Basic Chemistry of Oils and Fats – Dr. Alam

9:30 AM Oxidative Stability and Shelf Life of Frying Oils and Fats – Dr. N. Senanayake

10:35 AM Hi-Oleic Oil: Availability, Price, Frying Data and Comparison with other Frying Oils” – B. Stobaugh

11:35 AM "Analytical Methods for Deep Frying Assessment”- J. Ulahanan

1:35 PM Formation of 3-MCPD and GE in Frying Oils and their negative health effects – J. Bello

2:35 PM Practical Demonstration: Live Frying of French fries with various selected frying oils and sensory evaluation of the fried products along with group discussion.

- December 11, 2024

8:10 AM Refining of Frying Oil and why it is Important to Remove Contaminants – J. Bello

9:10 AM "Industrial Frying System and the Criteria for Selecting Industrial Fryers” – C. Ryes

10:15 AM "Frying Oils: Chemistry, Filtration, and Adsorbents” – B. Cooke

11:15 AM "Use of Specialty Oils in Deep Frying” – Dr. Alam

12:15 PM Graduation Lunch-Diplomas will be handed over.

1:30 PM "Acrylamide in Frying Foods- A Concern: Dr. Alam

2:15 PM Use of Palm/Red Palm Oil in Deep Frying – TBA

3:15 PM Practical Demonstration: Frying Battered Chicken in Various frying oils and sensory evaluation of the fried product and oil evaluation

Event page: <https://fatsandoilsrnd.com/annual-courses/>

Convegno SISSG

11-13 December 2024 | Parma, Italy

The Biennial 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 Nutraceuticals
- 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/congresso-sissg-2024-parma/>

Fuels of the Future 2025

20-21 January 2025 | Berlin, Germany

The 22nd International Conference on Renewable Mobility "Fuels of the Future 2025" will take place on 20 & 21 January 2025 as an in-person event in Berlin. As customary, the conference will be held bilingually (German-English). It is anticipated that there will be in excess of 60 speakers and more than 700 national and international participants from various industries.

Participants from the following areas are expected to attend the conference: biofuels, renewable fuels, green hydrogen, e-fuels, agriculture/agricultural engineering, recycling/waste industry, oil industry, trade, automotive industry, e-mobility, shipping, aviation, chemical industry, academia, consulting firms, municipalities, heavy haulage, freight transport, energy industry, certification companies, politicians, government/public servants, embassies and consulates, trade associations, press.

See the event page: <https://www.fuels-of-the-future.com/en>

Clean Fuels Conference 2025

20-23 January 2025 | San Diego, California, USA

The Clean Fuels Conference connects key players of the biodiesel, renewable diesel and sustainable aviation fuel industry for one can't-miss event. Clean fuels for land, sea and sky come together for a week of expert sessions, exhibits and showcases. Attendees include clean fuels producers and marketers, distributors, feedstock providers, fleet managers, Original Equipment Manufacturers (OEMs), ESG officers and members of the media.

Program overview:

- January 20

4:00 PM - PRE-CONFERENCE: Clean Fuels 101

4:00 PM - PRE-CONFERENCE: Carbon Programs 101

- January 21

8:30 AM - California Dreamin': Clean Fuels' Time to Shine

10:45 AM - Carbon Confessions: From CIs to Scope Emissions

10:45 AM - Changing the Narrative on Food and Fuel

1:15 PM - Acceleration of Change: A New Era for Clean Fuels

3:30 PM - Getting Federal Policy into Gear

- January 22

8:30 AM - All Aboard: Rail Contemplates Life After Diesel

10:45 AM - The Need for Speed: States Taking the Wheel

10:45 AM - New Frontiers: Expanding Markets for Low Carbon Liquid Fuels

1:00 PM - Fast-Forward: ASTM and Biodiesel Look to 2030 and Beyond

1:45 PM - TBA

2:45 PM - Pedal to the Metal: Original Equipment Manufacturers and the Latest in Diesel Technology

4:30 PM - Clean Fuels Happy Hour

• January 23

8:00 AM - Feedstock Revolution: Unleashing the Power for Clean Fuels (Closing breakfast session)

For more info, check the event page: <https://www.cleanfuelsconference.org/>

International Rendering Symposium, part of the International Production & Processing Expo

30 January 2025 | Atlanta, Georgia, USA

Rendering is an integral and often invisible aspect of animal agriculture's economic and environmental sustainability. This program will discuss rendering's contributions, impact, and the future of the industry.

Date: Thursday, Jan. 30, 11:00 a.m. - 6:00 p.m.

Registration Fee: \$250

See the event page:

<https://www.ippexpo.org/education-programs/>

Palm & Lauric Oils Price Outlook Conference & Exhibition (POC2025)

24-26 February 2025 | Kuala Lumpur, Malaysia

Event page: <https://www.pocmalaysia.com/>

Fats & Oils International Conference - Exhibition (FOIC)

6-8 March 2025 | JW Marriott Sahar, Mumbai, India

The Oil Technologists Association of India (OTAI) – Western Zone has organized technological conferences and seminars for decades, consistently aiding research scholars, technology providers, and industry professionals enhancing their skills.

To better serve the Fats & Oils Industry, OTAI – WZ launched the "FATS & OILS INTERNATIONAL CONFERENCE – EXHIBITION (FOIC)" in 2019, with events planned every two years. FOIC 2019 was a success, although FOIC 2021 was canceled due to the COVID-19 pandemic. OTAI – WZ returned with FOIC 2023, which exceeded expectations.

FOIC always selects themes relevant to the industry's global context, making it a significant and beneficial event for participants worldwide.

The upcoming FOIC 2025, scheduled for 6th – 8th March 2025 at JW Marriott, Sahar, Mumbai, will focus on "Technological Innovations, Efficiency & Value Addition Following Principles of Green Chemistry."

This theme emphasizes value addition in Fats & Oils processing, from seed to oil, protein, and by-products. Given the industry's thin margins, it's essential to maximize value from all products, whether for human or animal consumption, oleochemicals, or biodiesel feedstock. Energy efficiency is also crucial, as it improves process econom-

ics and supports environmental sustainability.

Emerging biotech techniques, such as enzyme usage, are set to revolutionize oil processing and byproduct production.

Food safety is another key focus, particularly minimizing undesirable products during processing and the role of packaging materials.

The event will also highlight the latest analytical instruments for quality control and the importance of process automation through PLC/DCS systems for precise control.

FOIC 2025 will be an invaluable event, providing a platform for global industry leaders to discuss and interact through presentations and deliberations.

More info: <https://foic.org.in/invitation.php#>

NIOP Annual Convention

16-18 March 2025 | Omni Rancho, Las Palmas Resort & Spa, California, USA

The NIOP Annual Convention is the hub for industry-wide networking, offering a unique platform to connect with fellow professionals, exchange insights, and build valuable relationships. Attendees can look forward to engaging discussions with industry thought leaders, gaining invaluable perspectives on the latest trends and innovations. Beyond the enriching experience of knowledge sharing, the Annual Convention also offers a vibrant social atmosphere, providing opportunities to unwind, relax, and simply enjoy the camaraderie of like-minded individuals.

The 2025 NIOP Annual Convention promises to be an exceptional event, setting the standard for industry-wide networking, featuring enlightening discussions with leading industry figures, and providing a captivating environment for socializing and relaxation. With the picturesque backdrop of the Omni Rancho Las Palmas Resort and Spa in the heart of Palm Springs, California, attendees will not only bask in the wealth of professional opportunities but also immerse themselves in the breathtaking beauty of the California landscape. It's a combination of business and leisure that ensures an unforgettable experience for all who attend.

See the event page: <https://niop.org/annual-convention-2024-live/>

2025 International Biomass Conference & Expo

18-20 March 2025 | Cobb Galleria Centre, Atlanta, Georgia, USA

The 18th annual International Biomass Conference & Expo will take place March 18-20, 2025, at the Cobb Galleria Centre in Atlanta, GA. This dynamic event unites industry professionals from all sectors of the world's interconnected biomass utilization industries—biobased power, thermal energy, fuels and chemicals.

Organized by BBI International and produced by

Biomass Magazine, this event brings current and future producers of bioenergy and biobased products together with waste generators, energy crop growers, municipal leaders, utility executives, technology providers, equipment manufacturers, project developers, investors and policy makers. It's a true one-stop shop – the world's premier educational and networking junction for all biomass industries.

International Biomass Conference & Expo is where future and existing producers of biobased power, fuels and thermal energy products go to network with waste generators and other industry suppliers and technology providers. It's where project developers converse with utility executives; where researchers and technology developers rub elbows with venture capitalists; and where Fortune 500 executives and influential policy makers sit side-by-side with American farmers and foresters. International Biomass Conference & Expo is the largest, fastest-growing event of its kind. In 2024, this event is expected to draw nearly 900 attendees. In 2024, the event drew more than 900+ attendees. This growth is fueled by a world-class Expo and an acclaimed program.

Once again, the 2025 International Biomass Conference & Expo program will include 30-plus panels and more than 100 speakers, including 90 technical presentations on topics ranging from anaerobic digestion and gasification to pyrolysis and combined heat and power, all within the structured framework of four customized tracks:

Track 1: Pellets & Densified Biomass
Track 2: Biomass Power & Thermal
Track 3: Biogas & Renewable Natural Gas (RNG)
Track 4: Advanced Biofuels & Sustainable Aviation Fuel (SAF)

Inflation Reduction Act

International Biomass Conference & Expo will help biomass industry stakeholders identify and evaluate technical and economic solutions that fit their operation. It's time to tap into the revenue generating potential of sustainable biomass resources.

Program:

- Tuesday, March 18
- 1:00 - 2:30 PM: Concurrent Tracks
- Track 1: Pellets & Densified Biomass
- Track 2: Biomass Power & Thermal
- Track 3: Biogas & Renewable Natural Gas (RNG)
- Track 4: Advanced Biofuels & Sustainable Aviation Fuel (SAF)
- 2:30 - 2:45 PM: Refreshment Break
- 2:45 - 4:15 PM: Concurrent Tracks
- Track 1: Pellets & Densified Biomass
- Track 2: Biomass Power & Thermal
- Track 3: Biogas & Renewable Natural Gas (RNG)
- Track 4: Advanced Biofuels & Sustainable Aviation Fuel (SAF)
- 4:15 - 4:30 PM: Networking Break
- 4:30 - 6:00 PM: Grand Opening & Welcome Re-

ception

- Wednesday, March 19
- 8:00 - 9:00 AM: Breakfast
- 9:00 - 11:00 AM: General Session
 - Welcome & Awards
 - Keynote Speaker
 - Roundtable Discussion: Biomass Industry Associations
- 11:00 - 6:00 PM: Expo Open
- 11:00 - 1:00 PM: Dedicated Expo Time
- 11:30 - 1:00 PM: Lunch in the Expo
- 1:00 - 2:30 PM: Concurrent Tracks
- Track 1: Pellets & Densified Biomass
- Track 2: Biomass Power & Thermal
- Track 3: Biogas & Renewable Natural Gas (RNG)
- Track 4: Advanced Biofuels & Sustainable Aviation Fuel (SAF)
- 2:30 - 3:00 PM: Refreshment Break in the Expo
- 3:00 - 4:30 PM: Concurrent Tracks
- Track 1: Pellets & Densified Biomass
- Track 2: Biomass Power & Thermal
- Track 3: Biogas & Renewable Natural Gas (RNG)
- Track 4: Advanced Biofuels & Sustainable Aviation Fuel (SAF)
- 4:30 - 6:00 PM: Networking Reception in the Expo

- Thursday, March 20
 - 7:30 - 8:30 AM: Breakfast
 - 7:30 - 1:30 PM: Expo Open
 - 8:30 - 10:00 AM: Concurrent Tracks
 - Track 1: Pellets & Densified Biomass
 - Track 2: Biomass Power & Thermal
 - Track 3: Biogas & Renewable Natural Gas (RNG)
 - Track 4: Advanced Biofuels & Sustainable Aviation Fuel (SAF)
 - 10:00 - 10:30 AM: Refreshment Break in the Expo
 - 10:30 AM – Noon: Concurrent Tracks
 - Track 1: Pellets & Densified Biomass
 - Track 2: Biomass Power & Thermal
 - Track 3: Biogas & Renewable Natural Gas (RNG)
 - Track 4: Advanced Biofuels & Sustainable Aviation Fuel (SAF)
 - Noon - 1:00 PM: Lunch in the Expo
 - 1:00 - 2:30 PM: Concurrent Tracks
 - Track 1: Pellets & Densified Biomass
 - Track 2: Biomass Power & Thermal
 - Track 3: Biogas & Renewable Natural Gas (RNG)
 - Track 4: Advanced Biofuels & Sustainable Aviation Fuel (SAF)
 - 2:30 PM: Sessions Conclude
- See the event page:
<https://ibce.bbiconferences.com/ema/DisplayPage.aspx?pagelId=Home>

AOCS Annual Meeting & Expo

27-30 April 2025 | Portland, Oregon, USA

OCS is a community of scientists, technicians, nutritionists, researchers and other industry professionals advancing the science and technology of edible oils, fats, proteins, surfactants and related materials. AOCS is a leading international society

with more than 2,000 members around the world. The AOCS Annual Meeting & Expo is a premier international science and business forum on fats, oils, surfactants, proteins and related materials. The extensive technical program in 10 interest areas features more than 600 oral and poster presentations, plus additional break out sessions around important trending topics:

- Analytical
- Biotechnology

- Edible Applications
- Health and Nutrition
- Industrial Oil Products
- Lipids and Oxidation
- Processing
- Protein and Co-Products
- Surfactants and Detergents

Call for paper is open!

More info: <https://annualmeeting.aocs.org/program>



INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

DETERMINAZIONE DEGLI AMMINOACIDI

L'analisi della composizione in amminoacidi è una tecnica ampiamente utilizzata in vari settori industriali al fine di valutare la composizione chimica e la presenza di eventuali adulterazioni del campione sottoposto a controllo.

Innovhub SSI effettua l'analisi su un'ampia tipologia di campioni: alimenti, mangimi, sostanze proteiche vegetali, bevande, prodotti caseari, prodotti per la detergenza (relativamente al contenuto in enzimi).

Gli amminoacidi analizzati includono sia i 20 standard che quelli fisiologici (fino a 40 composti diversi), presenti nel campione in forma libera o dopo idrolisi delle proteine. L'analisi è effettuata mediante un analizzatore automatico che impiega la cromatografia a scambio cationico e la derivatizzazione post-colonna con ninidrina per la separazione e la quantificazione.

I nostri laboratori offrono servizi di consulenza, analisi e ricerca applicata conto terzi.

Analisi effettuate:

- Determinazione di amminoacidi **standard e fisiologici** liberi e totali dopo idrolisi
- Determinazione di amminoacidi **solforati** (metionina e cist(e)ina)
- Determinazione del **triptofano**



Per informazioni:

Liliana Folegatti
liliana.folegatti@mi.camcom.it

Stefania De Cesarei
stefania.decesarei@mi.camcom.it



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
riscg@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/riscg-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

Con il contributo di

