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Via Trieste, 10
21040 Gerenzano

DIRETTORE RESPONSABILE: P. ROVELLINI
REDAZIONE: C. ZIGLIANI

REDAZIONE

Chiara Zigliani
risg@mi.camcom.it

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DIREZIONE E REDAZIONE
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A Systematic Review on Volatile Components and Therapeutic Properties of the Genus *Alpinia* (Zingiberaceae)

Anjelica Robert[1]
Wan Mohd Nuzul Hakimi Wan Salleh[1] ✉
Nurunajah Ab Ghani[2,3]
Abubakar Siddiq Salihu[1,4]
Farkhod Eshboev[5]

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

[2] Atta-ur-Rahman Institute for Natural Product Discovery (AuRIns),
Universiti Teknologi MARA
Bandar Puncak Alam
Selangor, Malaysia

[3] Faculty of Applied Sciences
Universiti Teknologi MARA
Shah Alam
Selangor, Malaysia

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

[5] Institute for Advanced Studies
New Uzbekistan University
Tashkent, Uzbekistan

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

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The genus *Alpinia* (Zingiberaceae) is a prolific source of aromatic and medicinal compounds, particularly through its essential oils, which are rich in monoterpenes, sesquiterpenes, and other bioactive volatiles. This systematic review consolidates current knowledge on the chemical composition, biological activities, and ethnopharmacological relevance of *Alpinia* essential oils. Following PRISMA guidelines, a comprehensive literature search was conducted across Scopus, PubMed, and Google Scholar. The essential oils of 37 *Alpinia* species primarily from Vietnam, India, and China were chemically characterized, with dominant compounds including β-pinene, 1,8-cineole, α-pinene, and γ-terpinene. Malaysian species were highlighted for their phytochemical richness and high oil yields. Pharmacologically, *Alpinia* essential oils exhibit a wide range of bioactivities, notably antioxidant, anti-inflammatory, antibacterial, antifungal, anticancer, and antidiabetic effects, aligning with their extensive traditional use in treating digestive, inflammatory, and infectious diseases. Despite growing interest, gaps remain in understanding the mechanisms of action, toxicity profiles, and clinical relevance of these oils. This review underscores the therapeutic promise of *Alpinia* essential oils and calls for further in vivo, mechanistic, and clinical studies to support their development as standardized phytotherapeutic agents.

Keywords: Zingiberaceae, *Alpinia*, essential oil, bioactive compounds, monoterpenes, antioxidant

1. INTRODUCTION

Essential oils, produced as secondary metabolites by aromatic plants, are complex mixtures of low-molecular-weight compounds that exhibit significant biological activities, including antioxidant, antimicrobial, antiviral, and anticancer properties [1]. These oils are predominantly composed of terpenoid compounds, primarily monoterpenes and sesquiterpenes, alongside various alcohols, aldehydes, ketones, ethers, esters, and phenols. These constituents not only contribute to the distinctive aroma and scent of essential oils but also play a vital role in the fragrance of spices and condiments. Moreover, essential oils are widely used for their repellent properties in the development of natural insecticides and herbicides [2]. Among the diverse plant families that produce essential oils, the Zingiberaceae family stands out as a rich source of bioactive constituents. Essential oils derived from different parts of Zingiberaceae species have been shown to possess a broad spectrum of pharmacological activities.

The Zingiberaceae family, commonly referred to as the ginger family, comprises over 50 genera and more than 1,600 species of perennial herbaceous plants characterized by creeping horizontal or tuberous rhizomes [3]. These plants are predominantly distributed across tropical regions of Asia, Africa,

and the Americas, with Southeast Asia serving as a major center of diversity. Within this region, the Malaysian floristic zone, which includes Brunei, Indonesia, Papua New Guinea, Peninsular Malaysia, the Philippines, and Singapore, hosts the highest concentration of Zingiberaceae genera and species [4]. Among the countries in this zone, Malaysia records the greatest richness, with at least 150 species across 21 genera, followed by Indonesia (113 species, 24 genera), Thailand (30 species, 11 genera), and Vietnam (26 species, 8 genera) [5]. Members of this family hold considerable economic and cultural significance. They are widely used as ornamental plants and culinary spices, most notably ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*), as well as in various traditional medicinal systems (Kress, 2008). Notable genera with established ethnopharmacological relevance include *Alpinia*, *Amomum*, *Curcuma*, *Elettaria*, *Hedychium*, *Kaempferia*, and *Zingiber*, which are frequently investigated for their therapeutic properties [6].

The genus *Alpinia* represents the largest and most extensively distributed genus within the Zingiberaceae family, comprising approximately 230 species [7]. Species within this tribe are evergreen herbs characterized by the absence of an abscission layer between the rhizome and the leafy shoots. The leaves are positioned in a plane that is transverse to the direction of rhizome growth. The flowers generally possess very small lateral staminodes, which may appear as slight swellings at the base of the labellum or be completely absent. Extrafloral nectaries are lacking, and the fruits are typically fleshy, spherical, and indehiscent. The natural range of this genus extends from Sri Lanka and the Western Ghats of India to regions such as China, Japan, Southeast Asia, and across the Pacific Islands, reaching as far as Fiji, Samoa, and the Caroline Islands, as well as northern New South Wales in Australia [8]. Among the numerous species within the Zingiberaceae family, several well-known examples include bitter ginger (*Zingiber zerumbet*), galangal (*Alpinia galanga*), ginger (*Zingiber officinal*), patumma or Siam tulip (*Curcuma alismatifolia*), and turmeric (*Curcuma longa*) [9].

Traditionally, species of *Alpinia* have been widely used in the treatment of various ailments, including bronchitis, eczema, colitis, ulcers, digestive disorders, and cholera, as well as for their antioxidant properties [10]. The traditional applications of various well-known *Alpinia* species vary significantly across regions. *A. galanga* is particularly renowned in Southeast Asian countries such as Indonesia, Thailand, and Malaysia. *A. zerumbet* has a long-standing history of use in the Ayurvedic medicinal system in India. In contrast, *A. oxyphylla* is prominently utilized in traditional Chinese medicine. Additionally, species such as *A. officinarum*, *A. calcarata* and *A. purpurata* are commonly employed in traditional practices across diverse regions, including Asia, Africa, and Latin America [11]. Due to their remarkably rich phytochemical composition, *Alpinia* species are consistently and extensively valued for their potent medicinal and diverse biological activities, primarily attributed to their highly aromatic and therapeutically significant essential oils.

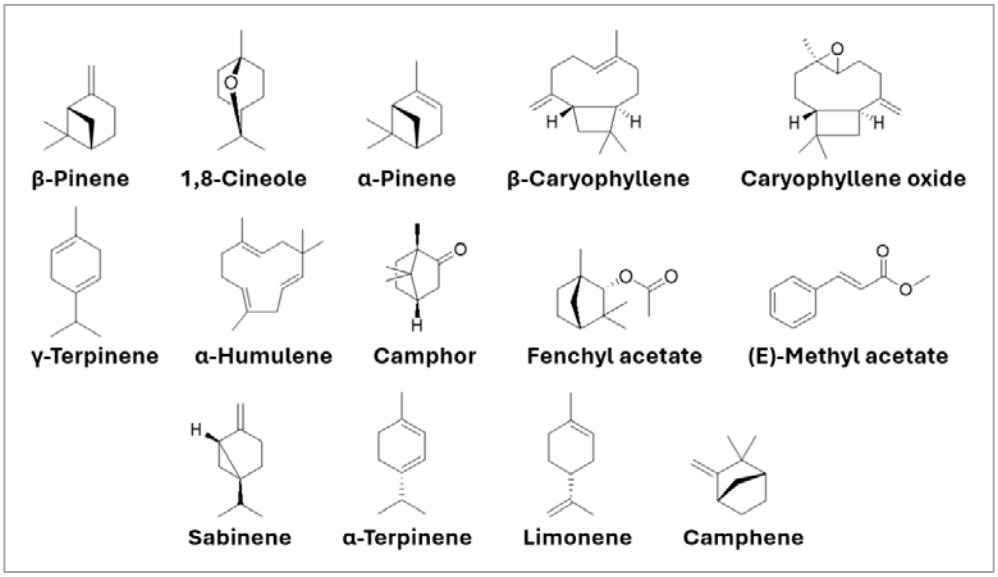

Figure 1. Chemical structures of major constituents found in *Alpinia* essential oils

Recently, essential oils and other aromatic compounds derived from plants have gained increasing attention for their potential use in alternative medicine. Therefore, a review on *Alpinia* essential oils is necessary to consolidate and simplify the available information. Relevant data were gathered through electronic searches of scientific databases, including Scopus, PubMed, and Google Scholar. This review aims to provide a comprehensive overview of published findings on the chemical composition, biological activities, and medicinal applications of essential oils extracted from various *Alpinia* species. While this review provides a global perspective, special attention is given to Malaysian species. Malaysia lies within the floristic center of Zingiberaceae diversity, yet many of its *Alpinia* species remain comparatively underexplored relative to those from Vietnam, India, and China. Highlighting the Malaysian representatives therefore adds regional significance, addresses a gap in the literature, and underscores their potential for both chemotaxonomic and pharmacological investigations.

2. SEARCH STRATEGY

The protocol for performing this study was developed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) [12]: (a) the first step was to exclude duplicate articles, (b) titles and abstracts were then read and the inclusion and exclusion criteria were applied, (c) all articles resulting from this stage were read in full, and the inclusion and exclusion criteria were applied again. Following this step, we reached the articles chosen for the present study. This systematic review was conducted through searches using Scopus, PubMed, and Google Scholar. The keywords used were *'Alpinia'*, 'essential oil', and 'biological activity' articles over the period from the beginning of the database until June 2025. In addition, as a second search strategy, we included studies obtained by a manual search of the reference lists of the included studies. Articles on the genus of *Alpinia* that reported traditional uses, essential oils, and their biological activities were included. The inclusion of articles considered the following criteria: (1) type of publication - original journal articles, (2) only articles in English (3), articles must present the chemical composition of essential oils, (4) articles must discuss the biological activity of the essential oils. The following were used as the exclusion criteria: (1) articles that did not show the search terms in the title and in the abstract; (2) review articles (3) full-text articles not found, (4) articles without one of the keywords, and (5) articles that did not show the composition of the essential oils.

3. MEDICINAL USES OF GENUS *ALPINIA*

The traditional medicinal applications of *Alpinia* species are deeply rooted in various ethnobotanical practices across Asia. Table I shows the medicinal uses of *Alpinia* species across different geographical regions, illustrating the genus's widespread ethnopharmacological significance. China emerges as the most frequently mentioned country. Several species such as *A. blepharocalyx*, *A. oxyphylla*, *A. katsumadai* and *A. kwangsiensis* are commonly used in Chinese traditional medicine for treating digestive ailments, urinary issues, oxidative stress, and kidney-related conditions [13-16]. Vietnam also holds a significant place, where species like *A. breviligulata*, *A. globosa*, *A. oxymitra*, and *A. tonkinensis* are traditionally used to relieve abdominal pain, nausea, indigestion, arthritis, and inflammation [17-19]. India demonstrates consistent use of *A. galanga* in Ayurvedic medicine, targeting eczema, bronchitis, gastrointestinal disturbances, and skin infections [20]. Other countries with notable ethnomedicinal practices involving *Alpinia* include Malaysia (*A. pahangensis*) [21], Indonesia (*A. malaccensis*) [22], Japan (*A. intermedia*) [23] and Taiwan (*A. japonica*, *A. nantoensis*) [24, 25].

Among the wide range of medicinal claims, the most consistently reported traditional use is for treating digestive-related disorders. Species such as *A. allughas*, *A. globosa*, *A. breviligulata*, *A. mutica*, *A. oxyphylla*, *A. suishaensis*, and *A. vietnamica* are frequently cited for their effectiveness in managing indigestion, stomach discomfort, vomiting, flatulence, and diarrhea [26, 27]. Inflammatory conditions and rheumatic pain are also commonly addressed using species like *A. jianganfeng* and *A. nantoensis* [25, 28]. Notably, the rhizome stands out as the most widely used plant part, featured in nearly every species documented. Rhizomes are traditionally employed to alleviate a broad spectrum of conditions, including digestive issues (*A. globosa*), respiratory ailments (*A. smithiae*), pain and inflammation (*A. chinensis*), and infections (*A. macroura*), likely due to their rich content of volatile oils and bioactive compounds.

In terms of historical documentation, the traditional uses of *Alpinia* species have been reported over a wide time span, reflecting both enduring traditional knowledge and growing modern scientific interest. Early records such as those of *A. chinensis* and *A. jianganfeng* date back to 1997 and 2002 respectively [29], while recent studies on species like *A. breviligulata* and *A. oxyphylla* [17, 30] indicate a resurgence of interest in these plants within the context of essential oil research and phytotherapy. Collectively, this ethnopharmacological diversity and the widespread use of *Alpinia* rhizomes reinforce their value as promising candidates for further investigation into their essential oil composition and bioactivity.

Table I - Medicinal uses of *Alpinia* species

Species	Part	Medicinal Uses
A. allughas	Rhizome	Traditionally used for the treatment of gout, colic, rheumatism, bronchial inflammation, digestive disorders, and respiratory issues, particularly in children [26]
A. blepharocalyx	Seed	Traditionally used in Chinese medicine to treat stomach disorders [13]
	Rhizome	Traditionally utilized to treat skin conditions [31]
A. blongifolia	Rhizome	Used to stimulate digestion and treat abdominal pain caused by cold, stomach aches, cough, respiratory inflammation, arthritis, and muscle pain [18]
A. breviligulata	Rhizome	Used in traditional Vietnamese medicine to treat abdominal pain by either drinking water infused with it or applying it topically to the abdomen [17]
A. calcarata	Rhizome	It is commonly used to treat cough, respiratory conditions, bronchitis, asthma, arthritis, and diabetes [32]
A. chinensis	Rhizome	Traditionally used asthma-relieving and analgesic (pain-relieving) properties [29]
A. conchigera	Rhizome	Traditionally utilized for post-partum care, managing fungal and skin infections [33]
A. elegans	Rhizome	Traditionally used to treat musculoskeletal diseases, haemoptysis (coughing up blood), headaches, stomach aches, and to prevent relapse in women [34]
A. galanga	Rhizome	Traditionally utilized in Ayurveda medicine for treating eczema, bronchitis, colds, measles, pityriasis versicolor, ear infections, gastritis, ulcers, and cholera [20]
A. globosa	Rhizome	Used in Vietnam as anti-nausea [18]
	Fruit	Used to treat stomach aches, indigestion, dysentery, abdominal pain, cholera, vomiting, and premature ejaculation [18]
A. hainanensis	Rhizome	Nutritional and dietary supplement for managing ulcerative colitis [35]
A. hankei	Rhizome	Traditionally used to treat fever and gastrointestinal discomfort [27]
A. intermedia	Rhizome	Traditionally used to relieve the symptoms of physical exhaustion, upper respiratory infection and other illnesses [36]
A. japonica	Root	Traditionally employed to dispel dampness, reduce swelling, pain relief, and improve blood circulation to clear meridian blockages. It has been used in treating dyspepsia, rheumatoid arthritis and traumatic injuries [24]
A. jianganfeng	Rhizome	Used in traditional Chinese medicine to treat rheumatism [28]
A. katsumadai	Seed	Markedly reducing oxidative stress and enhancing retinal function, it is also utilized in Chinese medicine to aid digestion, prevent nausea, soothe skin irritation, and treat gastric disorders [15]
A. kwangsiensis	Rhizome	In Chinese traditional medicine, it is used to treat abdominal pain, stomach cold-induced vomiting, and traumatic injuries [16]
A. latilabris	Rhizome	Traditionally used as a folk remedy for treating gastrointestinal diseases [37]
A. macroura	Rhizome	Used to treat wounds, reduce fever, and manage intestinal infections [38]
A. malaccensis	Fruit	Historically utilized to prevent nausea and vomiting [22]
	Leaf	Acts to freshen mouth and prevent vomiting [22]
	Rhizome	Used as hair oil and massage oil [22]
A. monopleura	Rhizome	Utilize to reduce body aches [39]
A. mutica	Rhizome	Traditionally utilized by locals to alleviate stomach gas issues [40]
	Fruit	Utilized to minimize inflammation [40]
A. nantoensis	Rhizome	Traditionally employed to ease pain, treat rheumatoid arthritis, support bruise healing, reduce toothache discomfort, and soothe stomach issues [25]
	Flower	Traditionally used to aid digestion, relieve gastric disorders, and counteract the effects of food or drug poisoning [25]
	Fruit	Traditionally used to dispel dampness, reduce vomiting, relieve diarrhea and nausea, and enhance appetite [25]
A. nigra	Root	Traditionally mixed with rice whisky and applied to the skin to treat fungal infections such as ringworm and melasma, it is also used in traditional medicine to manage gastric ulcers and jaundice [20]
	Rhizome	Used to treat obesity [41]
A. nutans	Rhizome	Widely used in folk medicine, primarily for treating indigestion, intestinal disorders, and high blood pressure [42]
A. officinarum	Rhizome	Involved in regulating nociception, respiratory defense mechanisms and gastrointestinal protection, it is also used for treating arthritis and microbial infections [43]
A. oxymitra	Flower	Used to treat arthritis [18]
	Rhizome	Traditionally used as a carminative and a remedy for diarrhoea [44]
A. oxyphylla	Rhizome	Helps enhance the effectiveness of nootkatone in preventing acute kidney injury [30]
	Fruit	Traditionally used to treat kidney deficiency, urinary issues, gonorrhea, digestive disorders, and excessive saliva production, it is also used for healing ulcers and managing dementia [14]
A. pahangensis	Rhizome	Used to treat flatulence [21]
A. pinnanensis	Rhizome	Used to treat coughs, fever, abdominal pain, flatulence, and stomach aches [38]
A. pricei	Rhizome	Possesses the ability to reduce and prevent hypercholesterolemia and is also used to alleviate abdominal discomfort, stimulate stomach secretions, and promote peristalsis [25]

Table I - Continues

A. purpurata	Inflorescence	It is consumed to relieve cough symptoms [45]
	Rhizome	Used to treat headaches, rheumatism, sore throat, and kidney infections [45]
A. rafflesiana	Rhizome	Utilized to treat nausea, vomiting, ulcers, and bacterial infections [46]
A. rugosa	Rhizome	Used to treat stomach ailments and nausea [27]
A. smithiae	Rhizome	Used in folk medicine for treating respiratory infections [27]
A. suishaensis	Rhizome	Used to help with digestive issues, improve circulation, and ailments related to wind or cold in traditional herbal formulation [28]
A. tonkinensis	Rhizome	Used to treat stomach aches, indigestion, and arthritis [18]
A. tonrokuensis	Rhizome	Used to treat fevers, muscle spasms, intestinal gas, and inflammation [27]
A. velutina	Rhizome	Used to treat digestive issues, respiratory conditions, and infections [27]
A. villosum	Rhizome	Protective substances for the digestive system [13]
A. vietnamica	Rhizome	The essential oil used for traditional, food, and medicinal purposes [27]
A. vitellina	Rhizome	Traditionally used to relieve stomach pain and indigestion [27]
A. zerumbet	Rhizome	Traditionally used in medicine to treat colds, flu, fever, flatulence, stomach issues, indigestion, cardiovascular diseases, high blood pressure, and inflammation, it also serves as an antispasmodic agent and plays a role in preventing atherosclerosis [47]

4. CHEMICAL COMPOSITION OF *ALPINIA* ESSENTIAL OILS

A comprehensive overview of the chemical compositions identified from the essential oils of 37 unique *Alpinia* species is provided in Table II, highlighting the genus's rich phytochemical diversity and the global interest in its bioactive constituents [48-96]. These species have been investigated across 11 countries, with Vietnam reporting the highest number (18 species), followed by India (8 species) and China (6 species). Additional studies have emerged from Indonesia and Brazil (3 species each), Taiwan (2 species), and one species each from Japan, Thailand, Sri Lanka, Egypt, and France. While certain species such as *A. galanga* and *A. zerumbet* were examined in multiple countries, each was counted only once in the total. This distribution underscores Southeast and South Asia, particularly Vietnam, India, and China, as key hotspots for *Alpinia* essential oil research, reflecting both regional biodiversity and longstanding ethnomedicinal significance.

The essential oil compositions are classified into six major chemical groups, which are monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, esters, and others. Among these, monoterpene hydrocarbons were the most frequently dominant across the *Alpinia* species. The most frequently reported compounds across the *Alpinia* species are dominated by monoterpene hydrocarbons and oxygenated monoterpenes. β-Pinene, a monoterpene hydrocarbon, was among the most common constituents and was found in *A. allughas* [48], *A. maclurei* [72], and *A. breviligulata* [17]. Another widely detected compound was 1,8-cineole, an oxygenated monoterpene, which occurred in multiple species including *A. nelumboides*, *A. vietnamica*, and *A. zerumbet* [78, 89, 91]. α-Pinene, also a monoterpene hydrocarbon, was reported in the oils of *A. breviligulata* and *A. macroura* [38], while limonene, from the same group, was detected in *A. napoensis* and *A. macroura* [38, 77]. Additionally, γ-terpinene, another frequently reported monoterpene hydrocarbon, was found in *A. napoensis*, *A. strobiliformis*, and *A. zerumbet* [31, 77, 93].

In terms of chemical richness, the rhizome essential oil of *A. officinarum* collected from China had the highest number of identified components, with 75 compounds accounting for 93.1% of its essential oil [81]. This was followed by the leaf essential oil of *A. nelumboides* from Vietnam, which contained 74 components comprising 88.1% of the oil [78] and the leaf essential oil of *A. nigra* from India, which consisted of 52 components with a concentration of 96.4% [79]. Regarding essential oil yield, the rhizome of *A. malaccensis* from Indonesia showed the highest yield at 1.22%, followed by the fruit of *A. monopleura* from Indonesia at 1.34%, and the fruit of *A. mutica* from India at 0.9% [22, 74, 75]. These findings highlight the commercial and therapeutic potential of selected *Alpinia* species, especially those with both high yield and diverse chemical constituents.

5. ANALYTICAL METHODS AND RELIABILITY

Volatile components were identified primarily by gas chromatography-mass spectrometry (GC-MS), using electron-impact spectra matched to commercial libraries (e.g., NIST/Wiley) in conjunction with calculated linear (Kovats) retention indices (RIs); where available, identities were confirmed by co-injection of authentic standards. GC-MS therefore served as the main qualitative tool and is regarded as the most widely used and reliable approach for volatile profiling in *Alpinia* oils summarized here. Relative abundances were typically obtained by GC-FID with peak-area normalization (semi-quantitative), which is appropriate for comparisons within a study but can vary across studies due to differing response factors. These practices underlie the species-level composition summaries and tables compiled in this review.

Table II - Major components identified from *Alpinia* essential oils

Species	Locality (Part)	Total (%)[a]	Yield (%)	Group (%)[b]	Major components (%)
A. allughas	India (Rhizome)	16, 90.0	NM	MH, 68.5	β-Pinene (55.3%), α-pinene (9.7%) [48]
	India (Leaf)	18, 90.0	NM	MH, 32.1	β-Pinene (25.5%), α-pinene (5.4%) [48]
	India (Rhizome)	22, 85.2	NM	MH, 68.5	β-Pinene (55.3%), α-pinene (9.7%), 7-*epi*-α-eudesmol (4.1%) [49]
	India (Leaf)	42, 88.0	NM	MH, 31.3	β-Pinene (25.5%), 1,8-cineole (23.3%), α-humulene (9.7%) [49]
A. brephrocalyx	Vietnam (Leaf)	41, 93.3	0.15	SH, 49.3	β-Pinene (12.0%), τ-muurolol (4.0%), α-cadinol (3.0%) [31]
	Vietnam (Pseudostem)	38, 99.6	0.11	SH, 58.2	β-Pinene (10.4%), τ-muurolol (9.5%), α-cadinol (9.5%) [31]
	Vietnam (Rhizome)	37, 98.4	0.15	OS, 27.5	β-Pinene (9.5%), τ-muurolol (11.2%), α-cadinol (11.2%) [31]
A. breviligulata	Vietnam (Flower)	35, 87.9	0.30	MH, 68.2	α-Pinene (12.7%), β-pinene (20.2%), β-caryophyllene (14.0%) [50]
	Vietnam (Leaf)	NM, 92.4	0.31	OS, 31.9	α-Pinene (19.4%), caryophyllene oxide (15.1%), α-copaene (8.5%) [17]
	Vietnam (Stem)	NM, 97.4	0.12	OS, 62.4	Caryophyllene oxide (27.7%), humulene epoxide II (6.4%), α-copaene (5.4%) [17]
	Vietnam (Rhizome)	NM, 98.3	0.10	MH, 46.6	α-Pinene (5.5%), camphene (10.3%), limonene (4.5%) [17]
	Vietnam (Root)	NM, 89.6	0.11	MH, 29.3	α-Humulene (12.3%), camphene (10.3%), *endo*-fenchyl acetate (9.4%), β-caryophyllene (7.0%), α-pinene (5.5%) [17]
	Vietnam (Leaf)	40, 97.2	0.25	NM	Caryophyllene oxide (23.1%), α-pinene (17.7%) [50]
	Vietnam (Rhizome)	35, NM	0.08	NM	β-Pinene (11.1%), caryophyllene oxide (10.5%), β-caryophyllene (8.0%), α-humulene (7.9%), borneol (7.0%) [51]
	Vietnam (Root)	35, NM	0.05	NM	Caryophyllene oxide (13.0%), α-humulene (10.8%), fenchyl acetate (8.8%), β-caryophyllene (7.5%), 1,8-cineole (6.0%), borneol (5.5%) [51]
A. calcarata	India (Rhizome)	15, 83.1	0.48	NM	1,8-Cineole (25.7%), ethyl *iso*-allocholate (16.0%), fenchyl acetate (15.1%) [52]
	India (Root)	69, 98.3	0.88	OM, 66.9	Fenchyl acetate (49.8%) [53]
A. chinensis	Vietnam (Flower)	30, 99.0	0.86	S, 95	(*E*,*E*)-α-farnesene (26.5%), α-humulene (22.3%), β-bisabolene (17.1%), β-caryophyllene (13.1%) [54]
	Vietnam (Leaf)	40, 98.9	0.08	S, 69.7	β-bisabolene (47.9%), (*E*,*E*)-farnesyl acetate (6.6%), *trans*-β-farnesene (6.6%), β-pinene (5.1%) [55]
	Vietnam (Root)	19, 99.4	0.06	S, 51.2	Caryophyllene oxide (13.2%), β-bisabolene (10.4%), γ-selinene (8.6%), α-humulene (6.2%), β-caryophyllene (5.2%), valencene (5.2%), juniper camphor (4.4%) [56]
A. coriandriodora	China (Rhizome)	32, 92.0	NM	NM	β-Pinene (27.9%), 1,8-cineole (17.3%), *p*-Cymene (13.5%), camphene (7.3%), myrcene (5.4%) [57]
A. gagnepainii	Vietnam (Leaf)	46, 95.8	0.02	MH, 72.8	β-Pinene (27.0%), α-pinene (17.6%), limonene (6.8%) [58]
	Vietnam (Rhizome)	49, 98.3	0.01	MH, 54.2	β-Pinene (20.4%), α-pinene (13.6%), fenchone (13.0%) [58]
A. galanga	China (Flower)	57, 96.0	0.11	SH, 69.6	α-Farnesene (64.3%), farnesyl acetate (3.6%), eugenol acetate (3.2%), eugenol (3.1%), *E*-nerolidol (2.9%) [59]
	Vietnam (Leaf)	NM	0.11	NM	(*E*,*E*)-Farnesyl acetate (39.6%), β-caryophyllene (15.8%), caryophyllene oxide (7.4%), β-elemene (5.6%), (*E*)-phytol (4.5%) [60]
	Vietnam (Leaf)	NM	0.12	NM	(*E*,*E*)-Farnesyl acetate (33.1%), β-caryophyllene (20.0%), β-elemene (8.6%), germacrene D (5.6%), caryophyllene oxide (5.3%) [60]
	Vietnam (Rhizome)	NM	0.26	NM	1,8-Cineole (42.5%), indan-5-ol (20.2%), α-pinene (4.7%) [60]
	Vietnam (Root)	NM	0.13	NM	Caryophyllene oxide (20.3%), β-pinene (9.0%), 7-*epi*-α-selinene (7.2%), β-caryophyllene (5.7%), selin-11-en-4α-ol (4.7%) [60]

Table II - Continues

A. galanga	Thailand (Leaf)	25, 95.7	0.25	NM, 80.4	β-Caryophyllene (28.7%), aciphyllene (18.3%), α-bisabolene (10.7%) [61]
	Thailand (Rhizome)	27, 98.7	NM	NM, 94.8	*cis*-Ethyl cinnamate (47.3%), safrole (19.8%), *p*-vinylphenylisothiocyanate (11.5%) [61]
	India (Rhizome)	12, NM	0.30	NM	Myrcene (94.5%), β-ocimene (2.0%) [62]
	India (Leaf)	12, NM	0.12	NM	Myrcene (52.3%), β-ocimene (17.0%), α-pinene (9.0%) [62]
	India (Rhizome)	10, 98.5	0.04	OM, 73.7	1,8-Cineole (57.0%), geranyl acetate (10.2%), β-caryophyllene (5.4%), *n*-tricosanol (5.2%) [63]
	India (Rhizome)	53, NM	0.1	NM	Carotol (26.7%), 1,8-cineole (10.8%), β-caryophyllene (5.8%), fenchyl acetate (4.8%) [64]
	India (Root)	39, NM	0.08	NM	Fenchol (30.5%), cubenol (5.7%) [64]
	Sri Lanka (Rhizome)	16, 87.0	0.56	S, 50.8	Zerumbone (44.8%), *p*-cymene (6.5%), camphene (6.4%), 1,8-cineole (6.3%) [65]
A. globosa	Vietnam (Leaf)	70, 95.8	0.16	MH, 31.0	β-Pinene (12.1%), α-gurjunene (10.5%), (*Z*)-13-docosenamide (9.0%) [19]
A. guilinensis	China (Fruit)	14, 99.6	0.04	NM	β-Phellandrene (26.7%), 1,8-cineole (25.8%), β-pinene (15.7%), (*E*)-methyl cinnamate (10.4%), α-pinene (10.2%) [66]
	China (Leaf)	15, 99.8	0.06	NM	β-Pinene (49.7%), α-pinene (20.1%), β-phellandrene (19.9%) [66]
	China (Stem)	18, 93.3	0.03	NM	β-Pinene (34.2%), β-phellandrene (27.0%), α-pinene (13.4%) [66]
A. guinanensis	China (Leaf)	27, 97.4	0.04	MH, 47.9	1,8-Cineole (43.1%), α-phellandrene (17.1%), β-pinene (14.5%) [67]
A. hainanensis	China (Leaf)	34 93.6	0.14	NM	α-Ocimene (27.4%), β-pinene (10.1%), 9-octadecenoic acid (6.5%), *n*-hexadecanoic acid (5.8%), 9,12-octadecadienoic acid (5.4%) [68]
	China (Leaf)	34, 93.6	0.14	NM	α-Ocimene (27.4%), β-pinene (10.1%), 9-octadecenoic acid (6.5%), *n*-hexadecanoic acid (5.8%), 9,12-octadecadienoic acid (5.4%) [68]
	China (Flower)	36, 98.7	0.08	NM	α-Ocimene (39.8%), β-pinene (17.7%), α-terpinene (5.5%), terpinene-4-ol (4.9%), β-caryophyllene (4.9%) [68]
A. hongiaoensis	Vietnam (Leaf)	36, 89.9	0.25	M, 40.1	β-Pinene (32.8%), (*E*)-methyl cinnamate (15.8%), *cis*-eudesma-6,11-diene (4.5%) [69]
A. katsumadai	China (Seed)	14, 97.3	0.03	E, 64.2	(*E*)-Methyl cinnamate (64.2%), *cis*-4-decen-1-ol (7.3%), 2H-Inden-2-one, octahydro-, cis- (6.7%) [70]
	China (Leaf)	44, 96.8	0.11	NM	*p*-Menth-1-en-ol (22.0%), α-terpinene (19.0%), 4-carene (9.1%), 1,8-cineole (8.3%), camphor (5.6%) [68]
	China (Flower)	36, 98.5	0.10	NM	*p*-Menth-1-en-ol (21.3%), 1,8-cineole (20.2%), α-terpinene (12.6%), α-phellandrene (7.0%), 4-carene (6.4%) [68]
	Vietnam (Leaf)	27, 99.9	0.50	NM	Geraniol (25.0%), guaiol (23.0%), 1,8-cineole (14.4%), α-pinene (7.9%), α-phellandrene (6.8%) [71]
	Vietnam (Stem)	27, 89.7	0.05	NM	Fenchone (25.1%), geraniol (13.5%), 1,8-cineole (13.4%), β-pinene (11.6%) [71]
	Vietnam (Seed)	27, 95.3	0.19	NM	Geraniol (31.2%), linalool (11.4%), citronellol (10.5%), geranyl acetate (8.0%), decanol (6.8%) [71]
A. kwangsiensis	China (Rhizome)	31 92.4	0.16	M, 82.8	Camphor (17.5%), 1,8-cineole (15.1%), β-pinene (11.1%), α-pinene (10.5%), α-terpineol (7.2%), camphene (6.8%) limonene (5.2%) [16]
A. maclurei	Vietnam (Leaf)	30, 90.0	0.25	MH, 75.1	β-Pinene (53.0%), α-pinene (13.9%) [72]
	Vietnam (Stem)	42, 90.8	0.23	MH, 60.1	β-Pinene (47.1%), α-pinene (5.75%), caryophyllene oxide (5.7%), β-caryophyllene (5.5%) [72]
	Vietnam (Root)	46, 90.7	0.19	MH, 46.9	β-Pinene (22.1%), β-phellandrene (12.0%), fenchyl acetate (6.7%), bornyl acetate (6.0%) [72]
A. macroura	Vietnam (Leaf)	33, 95.2	0.21	MH 59.9	1,8-Cineole (17.7%), γ-terpinene (13.3%), β-pinene (11.4%), α-phellandrene (8.5%), α-pinene (7.8%), α-terpinene (6.4%) [39]
	Vietnam (Stem)	32, 93.6	0.15	MH, 71.9	γ-Terpinene (16.9%), β-pinene (16.4%), 1,8-cineole (11.2%), α-terpinene (9.4%), α-pinene (8.4%), α-phellandrene (5.9%) [39]
	Vietnam (Root)	46, 92.0	0.24	MH, 56.3	γ-Terpinene (13.9%), β-pinene (12.5%), 1,8-cineole (8.7%), α-terpinene (8.5%), fenchyl acetate (7.8%), α-pinene (6.5%) [39]

Table II - Continues

A. macroura	Vietnam (Fruit)	42, 93.5	0.18	MH, 35.9	β-Caryophyllene (18.1%), β-pinene (11.1%), 1,8-cineole (11.1%), caryophyllene oxide (7.1%), γ-terpinene (5.9%), α-pinene (5.8%), bornyl acetate (5.0%) [39]
	Vietnam (Flower)	35, 97.7	0.30	MH, 44.8	β-Caryophyllene (12.6%), sabinene (9.0%), β-pinene (8.8%), γ-terpinene (7.9%), bornyl acetate (7.2%) [39]
A. malaccencis	Indonesia (Rhizome)	16, NM	1.22	NM	(*E*)-Methyl cinnamate (64.4%), α-pinene (14.8%), β-pinene (13.5%), 1,8-cineol (10.4%), camphor (6.3%), α-terpineol (6.3%) [22]
	Indonesia (Stem)	16, NM	0.25	NM	(*E*)-Methyl cinnamate (30.2%), 1,8-cineole (16.6%), β-pinene (12.4%), α-pinene (13.1%), α-terpineol (6.2%), nerol (5.5%) [22]
	Indonesia (Leaf)	18, NM	0.70	NM	α-Pinene (30.6%), 1,8-cineole (21.4%), β-pinene (11.4%) [22]
A. manghaiensis	Vietnam (Rhizome)	23, 90.2	0.18	MH, 84.6	β-Pinene (46.5%), β-phellandrene (25.7%), α-pinene (8.5%) [73]
A. monopleura	Indonesia (Leaf)	19, 93.3	1.16	NM	Caryophyllene oxide (22.6%), β-caryophyllene (16.5%), α-pinene (14.7%), limonene (13.4%), β-pinene (10.2%), α-humulene (7.2%) [74]
	Indonesia (Fruit)	22, 96.4	1.34	NM	Caryophyllene oxide (24.1%), β-caryophyllene (18.3%), β-pinene (12.9%), limonene (11.5%), α-pinene (10.4%), α-humulene (7.6%) [74]
A. mutica	India (Rhizome)	47, 92.8	0.3	MH, 43.3	β-Pinene (20.2%), camphor (13.3%), 1,8-cineole (8.9%), camphene (7.9%), α-pinene (6.2%) [75]
	India (Fruit)	69, 97.8	0.9	OM, 36.7	1,8-Cineole (14.8%), camphor (11.7%), β-pinene (7.6%), camphene (4.8%) [75]
A. nantoensis	Taiwan (Leaf)	14, 77.9	0.11	NM	Camphor (21.3%), camphene (11.4%), β-pinene (8.2%), *p*-cymene (5.2%), α-pinene (4.4%), D-limonene (2.5%) [42]
	Taiwan (Rhizome)	15, 70.8	0.20	NM	Camphor (29.5%), camphene (10.4%), β-pinene (9.1%), α-pinene (4.2%), *p*-cymene (3.2%), D-limonene (2.4%) [42]
A. napoensis	Vietnam (Rhizome)	32, 95.8	0.26	MH, 49.8	1,8-Cineole (18.2%), γ-terpinene (12.5%), terpinene-4-ol (9.6%), β-pinene (9.2%), fenchyl acetate (7.7%) [76]
	Vietnam (Leaf)	25, 99.3	0.21	MH, 62.1	γ-Terpinene (23.2%), 1,8-cineole (21.8%), α-terpinene (11.7%), terpinen-4-ol (10.8%), o-cymene (9.1%) [77]
	Vietnam (Stem)	25, 94.5	0.17	MH, 61.4	γ-Terpinene (20.5%), 1,8-cineole (17.6%), α-terpinene (12.5%), o-cymene (8.4%), terpinen-4-ol (8.2%), β-pinene (5.3%), α-terpinolene (5.3%) [77]
	Vietnam (Root)	28, 94.3	0.25	MH, 64.6	γ-Terpinene (19.3%), α-terpinene (13.2%),1,8-cineole (10.7%), β-pinene (6.2%), α-terpinolene (5.1%) [77]
A. nelumboides	Vietnam (Rhizome)	14, 99.7	0.20	NM	1,8-Cineole (71.23%), geranial (6.45%), β-pinene (5.34%) [78]
	Vietnam (Leaf)	74, 88.1	0.22	NM	1,8-Cineole (32.8%), α-terpineol (8.1%), α-Pinene (4.2%) [78]
A. nigra	India (Seed)	44, 98.2	0.76	NM	β-Caryophyllene (49.0%), β-pinene (13.7%), α-humulene (7.8%), α-pinene (6.3%), caryophyllene oxide (4.5%) [79]
	India (Flower)	45, 98.3	0.06	NM	β-Caryophyllene (48.6%), β-pinene (14.4%), α-humulene (7.7%), α-pinene (6.6%), caryophyllene oxide (4.4%) [79]
	India (Leaf)	52, 96.4	0.23	NM	β-Caryophyllene (47.7%), β-pinene (13.8%), α-humulene (7.5%), α-pinene (6.4%), caryophyllene oxide (4.3%) [79]
	India (Rhizome)	46, 97.9	0.18	NM	β-Caryophyllene (48.7%), β-pinene (14.1%), α-humulene (7.7%), α-pinene (6.5%), caryophyllene oxide (4.4%) [79]
A. nutans	India (Aerial)	42, 97.1	0.76	MH, 27.8	Sabinene (27.8%), 1,8-cineole (17.4%), terpinen-4-ol (14.9%), *p*-cymene (5.2%), γ-terpinene (5.1%), *cis*-sabinene hydrate (3.3%) [44]
	India (Flower)	23, 88.9	0.51	OM, 41.5	Terpinen-4-ol (25.1%), γ-terpinene (19.4%), sabinene (14.2%), 1,8-cineole (10.8%) [44]
A. oblongfolia	Vietnam (Leaf)	38 99.1	0.18	MH 63.2	β-Pinene (17.9%), α-phellandrene (3.5%), limonene (11.1%), (*E*)-methyl cinnamate (16.7%), *p*-cymene (9.6%), camphene (9.3%) [80]
	Vietnam (Rhizome)	52, 98.1	0.12	MH, 80.7	β-Pinene (26.2%), α-phellandrene (11.7%), limonene (14.5%), (*E*)-methyl cinnamate (4.1%) [80]
A. officinarum	China (Rhizome)	75, 93.1	0.60	OM, 40.3	1,8-Cineol (22.0%), α-terpineol (11.8%), γ-cadinene (8.6%) [81]
A. oxyphylla	China (Fruit)	74, 83.9	0.9	SH, 56.2	Valencene (19.0%), calamenene (10.1%), β-caryophyllene (5.5%), α-panasinsen (4.8%), α-selinene (4.0%) [82]
A. pinnanensis	Vietnam (Leaf)	NM, 97.9	0.41	OM, 31.6	1,8-Cineole (20.5%), 4-phenyl-2-butanol (19.5%), α-phellandrene (10.8%), β-pinene (5.5%) [39]
	Vietnam (Stem)	NM, 96.0	0.11	SH, 42.3	1,8-Cineole (10.0%), β-elemene (8.7%), α-gurjunene (7.6%), β-pinene (7.3%), (*E,E*)-farnesol (7.2%) [39]

Table II - Continues

A. pinnanensis	Vietnam (Root)	NM, 89.0	0.15	SH, 27.3	(*E,E*)-Farnesol (8.4%), α-gurjunene (6.2%), camphene (5.6%), fenchyl acetate (5.4%), linalool (4.6%), β-pinene (4.6%) [126]
	Vietnam (Fruit)	NM, 96.1	0.23	OS, 37.9	α-Cadinol (18.1%), β-caryophyllene (11.4%), (*E,E*)-farnesol (6.3%), β-pinene (6.1%), β-elemene (5.6%) [39]
A. purpurata	Brazil (Flower)	42, 97.6	0.05	SH, 41.5	β-Caryophyllene (18.2%), β-pinene (13.9%), linalool (9.6%), 7-*epi*-α-selinene (8.2%), α-pinene (6.2%) [83]
	Brazil (Leaf)	30, 98.9	0.44	MH, 49.7	β-pinene (34.7%), α-pinene (11.8%), *trans*- β-guaiene (6.0%) [84]
A. speciosa	India (Leaf)	24, 90.6	NM	OM, 59.3	Terpinen-4-ol (28.4%), 1,8-cineole (19.2%), sabinene (8.2%), *p*-cymene (8.0%), γ-terpinene (5.7%) [50]
	India (Flower)	25, 86.2	NM	OM, 68.9	Terpinen-4-ol (26.0%), 1,8-cineole (24.4%), linalool (6.1%), sabinene (11.3%) [50]
	India (Rhizome)	66, 96.0	0.69	MH, 61.2	Terpinen-4-ol (15.4%), 1,8-cineole (11.1%) [85]
	Taiwan (Seed)	38, 97.0	0.33	NM, 58.3	Camphor (19.3%), sabinene (15.1%), β-ocimene (7.9%), 1,8-cineole (5.6%), terpinen-4-ol (5.3%), α-pinene (5.1%) [86]
	Taiwan (Leaf)	39, 98.7	0.14	NM, 61.1	Camphor (31.6%), sabinene (9.4%), γ-terpinene (8.0%), terpinen-4-ol (6.6%), α-terpineol (5.5%) [86]
	Egypt (Leaf)	33, 93.7	0.12	M, 87.1	Sabinene (10.2%), γ-terpinene (11.1%), *p*-cymene (5.9%) [87]
	Egypt (Rhizome)	33, 93.0	0.10	M, 86.2	Sabinene (9.8%), γ-terpinene (9.3%) [87]
	Egypt (Stem)	33, 87.1	0.05	M, 75.2	Sabinene (7.5%), γ-terpinene (8.2%) [87]
	Brazil (Leaf)	17, 79.4	0.25	S, NM	Terpinen-4-ol (20.4%), 1,8-cineole (14.8), γ-terpinene (9.4%), *p*-cymene (9.3%), α-thujene (4.5%) [88]
A. strobiliformis	Vietnam (Leaf)	39, 94.9	0.18	MH, 47.2	1,8-Cineole (25.5%), β-pinene (10.7%), γ-terpinene (12.5%) [31]
	Vietnam (Pseudostem)	47, 96.1	0.13	MH, 55.8	1,8-Cineole (14.1%), β-pinene (15.0%), γ-terpinene (11.0%) [31]
	Vietnam (Rhizome)	34, 98.1	0.20	MH, 56.9	1,8-Cineole (16.5%), β-pinene (12.5%), γ-terpinene (13.6%) [31]
A. tonkinesis	Vietnam (Leaf)	51, 97.4	0.21	MH, 74.8	β-Pinene (33.5%), β-ocimene (9.6%), γ-terpinene (9.2%), α-pinene (8.4%) [19]
A. vietnamica	Vietnam (Rhizome)	36, 98.0	NM	MH, 62.5	1,8-Cineole (29.0%), geraniol (15.2%), β-pinene (12.8%), α-pinene (7.5%) [89]
	Vietnam (Leaf)	45, 95.3	NM	MH, 60.4	1,8-Cineole (25.7%), β-pinene (21.3%), geraniol (9.0%), α-pinene (6.1%) [89]
A. vittata	Brazil (Leaf)	20, NM	NM	MH	β-Pinene (35.3%), α-pinene (10.1%), *epi*-cubenol (5.0%) [90]
A. zerumbet	Japan (Leaf)	NM	0.07	NM	1,8-Cineole (18.8%), camphor (11.9%), (*E*)-methyl cinnamate (7.5%), cryptone (6.6%) [91]
	Brazil (Leaf)	16, 96.5	0.17	OM, 69.4	Terpinene-4-ol (32.9%), 1,8-cineole (21.4%), γ-terpinene (10.0%), sabinene (7.1%) [92]
	France (Flower)	71, 97.8	0.25	M, 51.5	1,8-Cineole (17.4%), terpinen-4-ol (14.7%), sabinene (7.0%), γ-terpinene (6.9%) [93]
	Japan (Leaf)	17, 100	0.07	NM	*p*-Cymene (28.0%), 1,8-cineole (17.9%), terpinen-4-ol (11.9%), limonene (6.3%), camphor (5.2%) [94]
	Brazil (Leaf)	14, 100	NM	NM	Terpinen-4-ol (37.4%), caryophyllene oxide (7.5%), *trans*-sabinene hydrate (6.6%), 1,8-cineol (4.2%) [95]
	Brazil (Leaf)	13, 89.1	0.44	OM, 52.5	Terpinen-4-ol (43.6%), bornyl acetate (13.8%), caryophyllene oxide (11.3%), α-humulene (10.7%) [96]

[a]Total components identified in the oil and their percentage
[b]Major group components identified in the oil and their percentage
NM-not mentioned; MH-monoterpene hydrocarbon; SH-sesquiterpene hydrocarbon; OS-oxygenated sesquiterpene; OM-oxygenated monoterpene; AM-aromatic monoterpene; E-ester; S-sesquiterpene; M-monoterpene

Table III - Major components identified from Malaysian *Alpinia* essential oils

Species	Locality (Part)	Total (%)[a]	Yield (%)	Group (%)[b]	Major components (%)
A. aquatica	Malaysia (Rhizome)	49, 79.2	0.10	MH, 34.7	β-Pinene (11.7%), α-humulene (8.9%), aromadendrene (8.7%), sabinene (7.7%) [97]
	Malaysia (Leaf)	36, 83.6	0.09	MH, 40.5	Germacrene D (21.3%), β-pinene (15.6%), sabinene (12.1%) [97]
	Malaysia (Pseudostem)	37, 80.4	0.02	SH, 60.8	α-Humulene (19.8%), germacrene D (15.2%), β-caryophyllene (8.7%) [97]
A. conchigera	Malaysia (Leaf)	40 63.6	0.30	S 36.2	β-Bisabolene (15.3%), β-pinene (8.2%), β-sesquiphellandrene (7.6%), chavicol (7.5%), β-elemene (6.0%) [98]
	Malaysia (Pseudostem)	33, 66.1	0.04	S, 51.1	β-Bisabolene (19.9%), β-sesquiphellandrene (11.3%), β-caryophyllene (8.8%), β-elemene (4.7%) [98]
	Malaysia (Rhizome)	39, 65.1	0.32	S, 31.8	1,8-Cineole (17.9%), β-bisabolene (13.9%), β-sesquiphellandrene (6.8%), β-elemene (4.0%) [98]
A. galanga	Malaysia (Rhizome)	40, 88.2	0.15	M, NM	α-Pinene (10.2%), *trans*-β-farnesene (18.2%), β-bisabolene (16.2%), α-bergamotene (10.7%), 1,8- cineole (5.5%), geranyl acetate (5.1%) [99]
A. latilabris	Malaysia (Fruit)	44, 97.7	0.05	M, 92.1	1,8-Cineole (35.9%), β-pinene (19.0%), α-pinene (8.8%), camphor (8.8%), camphene (5.8%) [100]
A. ligulata	Malaysia (Rhizome)	9, NM	0.80	AM, 67.5	(*E*)-Methyl cinnamate (36.4%), α-humulene (5.8%) [101]
A. murdochii	Malaysia (Rhizome)	37, 71.0	0.48	SH, 26.8	γ-Selinene (15.5%), (*E,E*)-farnesyl acetate (6.5%), terpinene-4-ol (5.5%), α-terpineol (5.0%) [102]
	Malaysia (Leaf)	40, 94.3	0.09	MH, 72.5	β-Pinene (23.8%), sabinene (23.7%), terpinene-4-ol (10.4%) [102]
A. nieuwenhuizii	Malaysia (Rhizome)	9, NM	0.90	AM, 81.2	(*E*)-Methyl cinnamate (67.8%), β-pinene (3.1%) [101]
A. pahangensis	Malaysia (Leaf)	38, 86.9	0.18	M, 90.5	β-Pinene (36.6%), α-pinene (7.5%), limonene (4.8%) [103]
	Malaysia (Rhizome)	38, 75.2	0.09	M, 28.1	(*E,E*)-Farnesyl acetate (8.6%), α-terpineol (6.3%) [103]
A. rafflesiana	Malaysia (Leaf)	47, 97.3	0.03	SH, 44.0	*β*-caryophyllene (32.6%), caryophyllene oxide (8.6%), (2*E*,6*Z*)-farnesol (4.9%), α-terpineol (4.2%) [104]
	Malaysia (Pseudostem)	40, 99.2	0.01	OM, 46.8	1,8-Cineole (32.2%), myrcene (13.6%), β-caryophyllene (9.8%) [104]
	Malaysia (Rhizome)	30, 97.9	0.05	OS, 38.3	Tetracosane (42.6%), τ-cadinol (7.4%), α-terpineol (6.7%) [104]
	Malaysia (Fruit)	37, 95.0	0.07	OS, 47.9	Tetracosane (13.3%), caryophyllene oxide (8.0%), 1,8-cineole (4.6%) [104]
A. scabra	Malaysia (Rhizome)	41, 70.9	0.16	NM	γ-Selinene (33.4%), α-selinene (3.6%), α-terpineol (3.5%) [102]
	Malaysia (Leaf)	40, 86.6	0.02	NM, 73.0	β-Pinene (63.3%), α-pinene (6.5%) [102]
	Vietnam (Pseudostem)	47, 96.1	0.13	MH, 55.8	1,8-Cineole (14.1%), β-pinene (15.0%), γ-terpinene (11.0%) [31]
	Vietnam (Rhizome)	34, 98.1	0.20	MH, 56.9	1,8-Cineole (16.5%), β-pinene (12.5%), γ-terpinene (13.6%) [31]
A. zerumbet	Malaysia (Fruit)	48, 97.0	0.30	S, 77.4	(*E,E*)-Farnesol (51.2%), α-farnesene (8.0%) [105]

[a]Total components identified in the oil and their percentage
[b]Major group components identified in the oil and their percentage
NM-not mentioned; MH-monoterpene hydrocarbon; SH-sesquiterpene hydrocarbon; OS-oxygenated sesquiterpene; OM-oxygenated monoterpene; AM-aromatic monoterpene; E-ester; S-sesquiterpene; M-monoterpene

6. EXTRACTION METHODS AND SOURCES OF VARIABILITY

Essential oils in the included studies were most often obtained by hydrodistillation or steam distillation (Clevenger-type), with yields reported where available alongside composition. Beyond the extraction route, between-study variation in identities and relative abundances can arise from plant factors (species/chemotype, organ, phenology), geography/season, and analytical conditions (stationary phase/column length, carrier-gas flow, oven program, peak deconvolution and normalization). Accordingly, we anchor identifications in GC–MS with retention indices (RI) and, where available, authentic standards, and we report relative percentages exactly as provided in the source studies rather than meta-normalizing across datasets.

7. CHEMICAL COMPOSITIONS OF MALAYSIAN *ALPINIA* ESSENTIAL OILS: A REGIONAL PERSPECTIVE

A detailed account of the major components identified from the essential oils of 12 *Alpinia* species collected in Malaysia is presented in Table III [97-105]. The analysis covered various plant parts, including rhizomes, leaves, pseudostems, and fruits, with rhizomes and leaves being the most frequently examined. Notably, species such as *A. aquatica*, *A. conchigera*, *A. murdochii*, *A. rafflesiana*, and *A. scabra* were studied for more than one plant part, offering deeper insights into intra-species chemical variation. The inclusion of multiple anatomical parts underscores the diverse phytochemical potential of Malaysian *Alpinia* species. The essential oil constituents were classified into eight major chemical groups, with monoterpene hydrocarbons being the most frequently dominant, particularly in the leaf and rhizome oils of *A. aquatica*, *A. murdochii*, and *A. scabra*. The highest number of identified components was observed in *A. rafflesiana* pseudostem oil, with 47 compounds accounting for 99.2% of the total oil [104]. Other species with high component richness include *A. zerumbet*, *A. latilabris*, and *A. aquatica*. In terms of essential oil yield, the highest was recorded in the rhizome of *A. nieuwenhuizii* (0.90%), followed by *A. ligulata* (0.80%) and *A. murdochii* (0.48%) [101, 102]. Among the most frequently reported compounds, β-pinene (monoterpene hydrocarbon) was consistently identified in *A. aquatica*, *A. murdochii*, *A. scabra*, *A. latilabris*, and *A. pahangensis* [97, 100, 102, 103]. 1,8-Cineole, an oxygenated monoterpene, was dominant in *A. latilabris*, *A. rafflesiana*, and *A. conchigera* [100, 104]. Other commonly detected compounds include α-pinene, γ-terpinene, caryophyllene oxide, β-caryophyllene, and β-bisabolene, highlighting consistent chemical signatures across species.

8. BIOLOGICAL ACTIVITIES OF *ALPINIA* ESSENTIAL OIL

The essential oils of *Alpinia* species have been extensively investigated for their diverse biological activities [105-125]. A summary of these activities for several *Alpinia* essential oils is presented in Table IV. Among the most frequently investigated properties is antioxidant activity. For instance, *A. gagnepainii* leaf and rhizome oils exhibited significant antioxidant effects, with IC_{50} values of 2446 and 1519 µg/mL in the ABTS assay, respectively [58]. Additionally, *A. katsumadai* seed oil showed remarkable antioxidant potential with IC_{50} values of 1.6 µg/mL in the DPPH assay [106]. Similarly, *A. malaccensis* leaf oil demonstrated IC_{50} values of 18 µg/mL and 20 µg/mL in the DPPH and ABTS assays, respectively [108]. In addition to antioxidant properties, *Alpinia* essential oils have also shown significant anti-inflammatory effects. For example, *A. oxyphylla* fruit oil inhibited nitric oxide (NO) production, with an IC_{50} value of 10 µg/mL [14]. Furthermore, *A. galanga* flower oil exhibited notable anti-inflammatory activity through the inhibition of tyrosinase and other enzymes, with IC_{50} values of 2.3 µg/mL [112].

The antibacterial properties of *Alpinia* essential oils have also been extensively studied, revealing significant effects against both Gram-positive and Gram-negative bacteria. *A. oxyphylla* fruit oil demonstrated strong antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, with MIC values of 0.295 mg/mL, 1.18 mg/mL, and 1.18 mg/mL, respectively [14]. Additionally, *A. katsumadai* seed oil showed significant antibacterial effects, particularly against *Campylobacter jejuni* and *Campylobacter coli*, with MIC values ranging from 0.256 to 4.103 mg/mL [106]. Moreover, *A. galanga* flower oil exhibited antibacterial activity against *S. aureus*, *Bacillus subtilis*, *P. aeruginosa*, and *P. vulgaris* with MIC values of 3.13 mg/mL [59].

Cytotoxicity studies have demonstrated that *Alpinia* essential oils possess significant anticancer potential. *A. coriandriodora* rhizome essential oil shows in-vitro anticancer activity in A549 lung carcinoma cells, comparable to an elemene-injection positive control, via G1 arrest and mitochondrial-pathway apoptosis and with reduced migration/invasion [126].

The antifungal properties of *Alpinia* oils have also been explored. For example, *A. zerumbet* essential oil showed activity against *Acremonium brasiliensis* with an MIC value of 0.4 µg/mL [115]. Similarly, *A. galanga* oil demonstrated inhibition of *Malassezia*, with inhibition zones ranging from 16 mm to 34 mm [117]. In

Table IV - Biological activities of several *Alpinia* essential oils

Bioactivities	Essential oils	Description
Antioxidant	*A. gagnepainii*	The leaf and rhizome oil showed IC_{50} value of 2446 and 1519 µg/mL in ABTS assay [58]
		The leaf and rhizome oil showed IC_{50} value of 107 and 295 µg/mL in DPPH assay [58]
	A. oxyphylla	The fruit oil showed IC_{50} value of 188.9 µg/mL in DPPH assay [14]
		The fruit oil showed IC_{50} value of 40.1 µg/mL in ABTS assay [14]
	A. katsumadai	The seed oil showed IC_{50} value of 1.6 µg/mL in DPPH assay [106]
		The seeds oil showed IC_{50} value of 10.4 µg/mL in DPPH assay [106]
	A. officinarum	The rhizome oil showed IC_{50} value of 58 µg/mL in DPPH assay [107]
		The rhizome oil showed radical scavenging activity towards hydrogen peroxide with IC_{50} value of 4.5 µg/mL [107]
	A. coriandriodora	The rhizome oil showed inhibition percentage of 85% in DPPH assay 85% [57]
	A. malaccenis	The leaf oil showed IC_{50} value of 18 µg/mL (DPPH) and 20 µg/mL (ABTS) assays [108]
Anti-inflammatory	*A. oxyphylla*	The fruit oil showed activity against NO production and β-hexosaminidase with IC_{50} value of 10 and 141 µg/mL, respectively [14]
		The fruit oil showed activity against NO production and β-hexosaminidase with IC_{50} value of 5.5 and 158 µg/mL, respectively [14]
		The fruit oil inhibited the release of PGE2 with IC_{50} value of 90 µM [23]
		The fruit oil inhibited COX-1, COX-2 enzyme and 5-LOX activity with IC_{50} values of 299, 199, and 170 µg/mL, respectively [109]
		The fruit oil strongly reduced the IL-6 and TNF-α levels in the range of 87.2 to 94.1% and 37.8 to 37.9%, respectively [110]
	A. zerumbet	The rhizome oil showed inhibitory activity against NO production with IC_{50} ranging from 3.62 to 8.33 µM [111]
		The seed oil showed the highest inhibitory activity against tyrosinase, pancreatic lipase (PL), 15-lipoxygenase (15-LOX), and LDL oxidation, with IC_{50} values of 2.3, 5.0, 1.2, and 15.4 µg/mL, respectively [112]
	A. japonica	The seeds oil inhibited NO production with IC_{50} value of 10 µM [23]
Antibacterial	*A. oxyphylla*	The fruit oil showed activity against *S. aureus* with MIC value of 0.295 mg/mL [14]
		The fruit oil showed activity against *E. coli* with MIC value of 1.18 mg/mL [14]
		The fruit oil showed activity against *P. aeruginosa* with MIC value of 1.18 mg/mL [14]
	A. officinarum	The rhizome oil showed strong activity against *M. tuberculosis* and *M. bovis* with IC_{50} values of 4.2 and 4.7 µg/mL, respectively [23]
		The rhizome oil showed activity against *B. cereus*, *B. subtilis*, and *S. aureus* with MIC ranging from 16 to 32 µg/mL [23]
		The rhizome oil caused cell death in *E. coli* and *S. paratyphi* with MIC value of 50 µg/mL [23]
	A. katsumadai	The seeds oil showed strong effect against *C. jejuni* and *C. coli* with MIC ranging from 0.256 to 4.103 mg/mL [106]
		The seeds oil showed effects on *M. smegmatis* with MIC less than 64 mg/L [106]
		The seed oil demonstrated inhibitory effects on *S. aureus, S. epidermidis*, and *E. coli*, with MIC values ranging from 0.12 to 2.55 mg/mL [106]
	A. galanga	The flower oil exhibit strong activity against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *P. vulgaris* with MIC values of 3.13 mg/mL [59]
		The rhizome oil showed strong activity against *B. subtilis*, *S. aureus*, and *S. typhimurium* with MIC (0.04 to 1.28 mg/mL) and MBC (0.08 to 2.56 mg/mL) [113]
	A. guilinensis	The fruit oil showed strong activity *S. aureus*, *B. subtilis*, and *E. coli* with MIC value of 1.25 to 2.5 mg/mL [66]
		The leaves oil has strong inhibitory effect on *E. coli* with MIC value of 2.5 mg/mL [66]
		The stem oil showed moderate activity against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli* with MIC value of 2.5 to 5 mg/mL [66]
	A. latilabris	The fruit oil showed activity against *E. coli* with MIC value of 5.0 mg/mL [100]
		The fruit oil showed activity against *S. aureus* and *B. subtilis* with MIC value between 2.50 to 5.0 mg/mL [100]
	A. mutica	The fruit oil showed activity against *S. aureus* and *B. subtilis* with MIC value 2.50 mg/mL [100]
	A. pahangensis	The rhizome oil exhibits activity against *S. aureus* with MIC value <1.0 µg/µL [103]
	A. malaccensis	The root oil showed activity against *E. aerogenes*, *E. coli*, *P. aeruginosa*, and *S. dysenteriae* with MIC value of 60 µM [23]

Table IV Continues

Cytotoxicity	*A. officinarum*	The rhizome oil inhibited the growth of gastric carcinoma (SGC-7901), human breast cancer (MCF-7), and cervical carcinoma (CaSki) with IC_{50} value of 11, 15, and 15 µM, respectively [23]
	A. mutica	The rhizome oil inhibited the UCK2 enzyme and reduced the proliferation of HT-29 cells with IC_{50} values of 28and 44 µM, respectively [23]
	A. katsumadai	The rhizome oil suppressed the proliferation of human gastric cancer cells (MGC803) with IC_{50} value of 16 µM [23]
	A. nelumboides	The rhizome oil showed activity on NCI-H460 and HepG2 with IC_{50} value of 98.2 and 189.8 µg/mL, respectively [78]
Antidiabetic	*A. galanga*	The rhizome oil enhanced the viability of human umbilical vein endothelial cells impaired by high glucose by 83% [23]
		The flower oil displayed significant α-glucosidase activity with IC_{50} value of 0.16 mg/mL [59]
	A. nelumboides	The leaves oil inhibited α-glucosidase with IC_{50} value of 2.42 mg/mL [78]
	A. pahangensis	The rhizome oil showed inhibitory activity against α-amylase and α-glucosidase with IC_{50} values of 114.8 and 153.8 µM, respectively [114]
Antifungal	*A. zerumbet*	The essential oil showed activity against *A. brasiliensis* with MIC value of 0.4 µg/mL [115]
	A. purpurata	The essential oil inhibits the growth of *Candida krusei* by 7.33 to 11.0 mm inhibition zone diameter [116]
	A. galanga	The essential oil showed activity against *Malassezia* with inhibition zone ranged from 16 mm to 34 mm [117]
	A. conchigera	The rhizome oil showed activity against *C. albicans, M. canis,* and *T. rubrum* with MIC values of 62.5, 156, and 156 µg/mL, respectively [118]
Anticancer	*A. oxyphylla*	The rhizome oil suppresses the growth of HepG2, A549, and NCI-H460 cells with IC_{50} values of 52, 48, and 44 µM, respectively [14]
		The seeds oil exhibits activity against HL-60 leukemia cells and A549 cells with IC_{50} values of 58.4 and 4.27 µM, respectively [14]
		The seed oil suppresses the proliferation of SCC25 and A431 cells with IC_{50} values of 18.8 and 13.3 µM, respectively [14]
	A. galanga	The flower oil showed significant activities against A549, PC-3, NCI-H1299, L929 cells with IC_{50} values ranging from 41.5 to 127.3 µg/mL [59]
		The rhizome oil reduced the viability of A549 lung cancer cells, CRL2522 fibroblasts, and MCF-12A breast cancer cells, exhibiting IC_{50} values of 100 µg/mL [119]
		The rhizome oil suppresses the melanogenesis process by inhibiting tyrosinase activity, with an IC_{50} value of 7.3 µg/mL [120]
Antiproliferative	*A. katsumadai*	The seeds oil exhibited significant effects on NCI-H460, HeLa, SMMC-7721, and HCT-116 cell lines with IC_{50} values ranging from 12.7 to 42.2 µM [106]
		The seeds oil showed significant inhibition of SMMC-7721 cells with IC_{50} values of 4.8 µM [106]
	A. galanga	The rhizome oil showed IC_{50} value of 170 µg/mL against MCF-7 [121]
Antiviral	*A. katsumadai*	The seeds oil inhibited rotavirus infection with EC_{50} values of 8.4 µg/mL [106]
		The seeds oil showed inhibitory activity against porcine rotavirus, G5P with EC_{50} values ranging from 0.7 to 33.7 µg/mL [122]
Insecticidal	*A. oxyphylla*	The fruit oil showed activity against *D. melanogaster* with LC_{50} value of 11.50 µmol/mL [14]
	A. galanga	The rhizome oil showed activity against *H. longicornis* nymphs by inhibiting the AChE with IC_{50} value of 60 µg/cm^2 [23]
Tyrosinase	*A. pahangensis*	The flower oil exhibited a moderate effect with IC_{50} value of 0.62 mg/mL [59]
Antiangiogenic	*A. oxyphylla*	The fruit oil showed inhibitory activity against blood vessel formation by 31% [123]
Antidiarrhoea	*A. oxyphylla*	The fruit oil showed effect on carbachol-induced contraction by reducing 25.5% [124]
Anticholinesterase	*A. galanga*	The flower oil exhibited activity against AChE and BChE with IC_{50} values of 2.49 and 10.14 mg/mL, respectively [59]
Toxicity	*A. calcarata*	The rhizome oil showed LC_{50} value of 9.64 mg/cm^2 against *T. castaneum* [54]
		The rhizome oil showed LC_{50} value of 0.86 mg/cm^2 against *L. serricorne* [54]
		The rhizome oil showed LC_{90} value of 1.19 mg/cm^2 against *C. chinensis* [54]
	A. galanga	The rhizome oil showed activity against *C. gestroi* with LD_{50} value 5407 mg/kg [125]
		The rhizome oil showed activity against *C. curvignathus* with LD_{50} value 3456 mg/kg [125]

addition to the bioactivities discussed above, *Alpinia* oils have been reported to exhibit several other beneficial effects, such as anticancer [14], antiproliferative [106], antiviral [122] and insecticidal activities [23]. Furthermore, these oils have shown antiangiogenic [123] and antidiarrheal effects [124], making them suitable for a diverse range of applications in both medicinal and industrial contexts.

9. BIOACTIVITY MECHANISMS AND SYNERGISM

Across the included studies, whole *Alpinia* essential oils (EOs) exert their effects through well-described mechanisms, which include antioxidant activity via hydrogen-atom/electron transfer radical scavenging; anti-inflammatory effects through suppression of NO/iNOS and down-modulation of NF-κB/MAPK signaling; antibacterial/antifungal actions, primarily via membrane permeabilization, ion leakage, and biofilm inhibition; and cytotoxicity through cell-cycle arrest and mitochondrial apoptosis (Δψm loss, Bax/Bcl-2 shift, caspase-9/-3 activation, PARP cleavage). Notably, *A. coriandriodora* rhizome EO induces G1 arrest and mitochondrial apoptosis in A549 cells, illustrating these pathways in a whole-oil context [14, 59, 106, 126]. Given that EOs are mixtures of terpenes and oxygenated terpenes, synergism among major and minor constituents is likely and may explain why whole oils can outperform single compounds at isoeffective doses; accordingly, we report EO activities in µg/mL or mg/mL and avoid extrapolating µM data from isolated constituents.

10. CONCLUSION

This review highlights the considerable phytochemical diversity and bioactivity of essential oils obtained from *Alpinia* species. Rich in monoterpenes, sesquiterpenes, and other volatile constituents, these oils demonstrate a wide spectrum of biological activities, including antioxidant, antibacterial, antifungal, anti-inflammatory, cytotoxic, and antidiabetic effects. Such findings corroborate the extensive traditional uses of *Alpinia* in ethnomedicine across Asia and other regions. Notably, variations in chemical composition associated with species differences, plant parts, environmental conditions, and geographic origin underscore the importance of standardization in future research and potential therapeutic applications. The Malaysian *Alpinia* species have emerged as promising sources of bioactive compounds with significant chemotaxonomic and pharmacological relevance. Despite the growing body of evidence supporting the medicinal potential of *Alpinia* essential oils, further studies are necessary to clarify their mechanisms of action, evaluate their safety and efficacy through rigorous in vivo and clinical investigations, and assess their feasibility for development into standardized natural products. Addressing these gaps will be critical to fully realize the therapeutic and commercial potential of *Alpinia* essential oils in modern phytotherapy and pharmaceutical applications.

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Evaluation of Physicochemical Properties, Phytochemical Composition, Antioxidant Activity, Mineral Content, and Sensory Characteristics of Crackers Fortified with Olive Pomace

Kadriye Altay[1] ✉
Arda Akdoğan[2]
Selma Lubabe Erdoğan[3]
Gülşah Çalışkan Koç[4]

[1] Olive Research Institute
Bornova, İzmir, Türkiye

[2] Gümüşhane University
Engineering and Natural Sciences Faculty
Food Engineering Department
Gümüşhane, Türkiye

[3] Alanya University
Vocational School Hotel
Restaurant and Catering Services
Department
Culinary Program
Alanya, Antalya, Türkiye

[4] Uşak University
Eşme Vocational School
Food Processing Department
Food Technology Program
Eşme, Uşak, Türkiye

✉ CORRESPONDING AUTHOR
Ministry of Agriculture and Forestry
Olive Research Institute
University Street, No:43, 35100
Bornova/Izmir, Türkiye
Tel.: +902324627073,
Fax: +902324357042
e-mail: kadriye_ergun555@hotmail.com

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Olive pomace (OP), a by-product of olive oil production, is a rich source of various bioactive compounds. OP can be used as a value-added product for the development of functional food. The research aimed to produce functional crackers incorporating different levels of OP powder (ranging from 0% to 20%). Subsequent analysis involved evaluating the physicochemical and phytochemical compositions, antioxidant activity, mineral content, and sensory characteristics of all samples. In light of the findings, the samples containing OP powder are rich in phytochemicals (total phenolic and flavonoid contents), with higher antioxidant activities (total antioxidant content, DPPH radical scavenging activity, ABTS radical scavenging activity, and ferric reducing power), as well as a high amount of minerals compared to the control sample ($P<0.05$). Moreover, as the concentration of OP increased, there was a decline in the moisture content, water activity, and color values of the crackers. Conversely, the ash, fat, total flavonoid, total phenolic, and mineral contents, as well as antioxidant activity and hardness values, exhibited an increase. Sensory analyses revealed that the addition of 10% OP powder produced highly acceptable crackers compared to the higher concentration of OP (15 and 20%). Overall results indicated that functional crackers can be produced by the addition of powder and a maximum of 10% OP powder fortification is recommended for consumer acceptability.

Keywords: Olive pomace; cracker; phytochemical; antioxidant activity; mineral composition; sensory properties

1. INTRODUCTION

Olive pomace (solid waste from olive oil extraction) and olive mill wastewater (aqueous discharge of the process) are by-products of the olive oil industry. The amount of OP produced varies depending on the method used to extract olive oil, but the average is 35-40 kg OP /100 kg of processed olives [1,2]. OP, which contains fragments of skin, pulp, kernel pieces, and oil, is one of the main by-products of the olive oil industry [3]. OP contains valuable components from olive oil. More than 98% of olive oil is made up of acylglycerols, and triacylglycerols (TAGs) make up the majority of these acylglycerols. TAG composition is a crucial factor in the quality of fats and oils since it can influence both their physiological and nutritional features, such as sensitivity to lipase-hydrolysis and melting point and crystallization [4]. In addition, olive oil contains sterols [5]; fatty alcohols and waxes [6]; phenols [7]; alkanes and alkenes [8]; carotenoids, and chlorophylls [9] in its structure. OP is rich in polysaccharides (cellulose) and lignin, fatty acids, minerals (potassium, calcium, magnesium, etc.), polyalcohols, pigments, polyphenols, and a quantity of proteins that can be considered a promising source of value-added products or antioxidants [3,10-12]. Natural antioxidants have health advantages because they can stop biological

deterioration from causing damage [13]. In OP obtained from the areas of Perdifumo and Acquamela di Casalvelino (Salerno, Italy), the predominant phenolic compounds have been identified as tyrosol, caffeic acid, and gallic acid. [14]. Researchers reported that the environmental, agronomic, and technological properties of virgin olive oil production have a significant impact on the concentration of these chemicals [5, 9, 14]. Olive tree irrigation can change the phenolic composition of virgin olive oil depending on the cultivar, ripening stage, and geographic origin of the olive. In other studies, it was reported that the origin of the fruit, olive tree irrigation, geographic origin of the olive, cultivation, harvest date, extraction method, ripening stage, climate, and rainfall all have an impact on biosynthesis within the fruit, and, consequently, the composition and quality of the oil [4, 8, 9, 15].

Researchers stated that the phenolic extracts and isolated polyphenols from olive processing by-products such as pomace can also be used as a natural antioxidant and antimicrobial in food products [12, 16]. For example, the biological characteristics of different olive powder extracts were produced using conventional heating and ohmic heating-assisted extraction and they reported using a green technique such as ohmic heating to recover the bioactive chemicals found in olive powder [12]. Sinrod et al. [16] reported that the use of drum drying at low speed resulted in the lowest reduction in the antioxidant activity of extract when compared to other drying methods (freeze, hot air, and drum drying), even though all drying methods decreased the antioxidant activity relative to extract derived from fresh pomace.

OP is generally used as an animal feed additive, fertilizer, soil strengthener, alternative fuel, etc. [17-19]. In addition, studies related to the production of lipase enzyme-activated carbon and biodiesel from OP are available [20, 21]. Moreover, there are studies in which pomace is used as a food additive. It was stated that pomace is used to increase the phenolic and fiber content, as well as antioxidant activity, in cereal products such as biscuits, pasta, bread, and breadsticks [22-25]. There are numerous investigations on phenolic compound recovery using various technologies [26, 27].

Pomace drying is an important process in terms of waste evaluation. Drying increases the durability of the pomace and makes it easier to process. Furthermore, in order to obtain more oil from the pomace, it is necessary to dry the pomace. For the mentioned reasons, the literature includes several studies on the drying of 2-phase or 3-phase OPs [28-31].

Fruits, vegetables, or by-products are used to develop functional products with higher added value than the cereal products that are consumed extensively all over the world [22-25; 32-35]. It is important for the evaluation of OP, which has a low cost and can be obtained in high quantity as a by-product during olive oil production, while pomace oil or powder can be used as a functional food ingredient in food formulations. Crackers, one of the snack foods consumed by consumers of all age groups [36, 37], were preferred in this study.

The objective of this investigation was to create crackers by incorporating varying concentrations (0-20%) of OP powder. The study aimed to assess how the incorporation of OP powder influences the physical, chemical, and phytochemical characteristics, antioxidant activity, and mineral content of crackers. Additionally, the research examined the effect of substituting OP powder on the sensory properties and overall acceptance of the crackers by consumers.

2. MATERIALS AND METHODS

2.1. MATERIALS

The wheat flour (Söke Milling Industry and Trade Inc.), yeast (Dr. Oerker Food Ind. and Commerce Inc.), sunflower oil (Kucukbay Oil Inc.), and salt (Billur Boiled Salt Trade Inc.) were obtained from a local market in Alanya, Antalya, Turkey.

The OP was obtained during the production of olive oil (dual-phase decanter centrifugation system) from different olive varieties harvested in the 2021/2022 crop season from the Bornova Olive Research Institute garden (38° 27′ 45.9936” and 27° 22′ 11.0964”). The location, which belongs to the governorate of İzmir, is located in the west of Turkey. Its climate is characterized by hot, dry summers and mild, rainy winters. The average annual temperature and precipitation of the location (Bornova) are 16.0°C and 742 mm, respectively.

The fresh OP was dried at 55°C in an oven (Memmert UN110, Schwabach, Germany) for 24 hours. The dried pomace was ground using a grinder (Premier, PRG259, Turkey) and subsequently subjected to a sieving process (<1000 μm) to remove olive pit fragments. To ensure the effectiveness of this procedure, sieving was performed three consecutive times, and the retained fractions were visually examined to verify the complete elimination of residual stone particles. Only the fine powder passing through the sieve was collected and used in subsequent analyses. This multiple-step approach has been reported as an effective strategy for reducing the presence of stone residues in two-phase olive pomace (De Leonardis et al., 2022). In addition, before incorporation into the formulations, the sieved pomace powder was carefully inspected to guarantee homogeneity and the absence of hard fragments, thus ensuring its safety and suitability for food applications. The dried samples were stored at 4°C for further analysis.

2.2. METHODS

2.2.1 Cracker production

Crackers were prepared in the bakery kitchen at Alanya University. The following procedure was adopted after several preliminary trials (in 100 g): wheat flour (62.15 g/100 g), salt (1 g/100 g), OP powder (0 (Control), 5, 10, 15, and 20% substitution for wheat flour), and yeast (0.55 g/100 g) were weighed, placed in a bowl, and mixed. Sunflower oil (6 g/100 g) and drinking water (30.3 g/100 g) were then added to it and kneaded with a mixer (Electrolux Mixer 5 lt, Sweden) for 10 min to obtain a homogeneous mixture. The dough obtained was placed in a holding container, covered, and fermented at 30±2°C for 15 min. At the end of the fermentation period, the dough was rolled out in 1.5 cm thickness using a rolling pin, cut into 1x1 cm pieces, and placed on a baking tray lined with cooking paper at intervals. It was then baked in a preheated oven at 180±2°C (Air-o-convect bakery, Electrolux, Sweden) for 10 min. The baked products were removed from the oven and crackers were obtained.

2.2.2 Physicochemical composition

The moisture, ash, and fat contents, as well as pH analyses, were performed according to American Association of Cereal Chemists methods [39]. The water activity value of the cracker samples was determined using a water activity measuring device (LabTouch, Novasina, Switzerland) with an accuracy of ±0.001. In addition, the moisture content and water activity of wheat flour and OP powder were determined.

2.2.3 Extraction Method and Total Flavonoid, Phenolic, and Antioxidant Content Analysis

Five-gram samples and 25 ml methanol were mixed for 24 h in a mechanical shaker (IKA, Germany). The mixture was filtered and used for flavonoid, phenol, and antioxidant analyses. Antioxidant activity was measured using three different methods: DPPH assay, ABTS assay, and FRAP assay. All analyses were made in triplicate.

Total flavonoid content

Using the aluminum chloride method, the total flavonoid content of crackers was determined according to Kasangana et al. (2015)'s method, where catechin was used as the standard [40]. Sample (500 µl), methanol: water (70:30 by volume) solution (3200 µl), 0.5 M NaNO2 (150 µl), and 0.3 M AlCl3 (150 µl) were mixed in a test tube. NaOH (1000 µl 1 M) was added after 5 min incubation. The same procedure was applied for standard catechin solutions (dissolved in ethanol; 25, 50, 100, 200, and 400 mg/kg) and blank samples (distilled water, 39). A UV-vis spectrophotometer (Shimadzu Spectrophotometer UV-1700 PharmaSpec, Japan) was used to measure the absorbance of crackers at 506 nm. The total flavonoid contents of samples were calculated using the calibration curve (y = 0.0005x + 0.0098 (R2 = 0.990)). The obtained results are expressed in mg CE/100 g.

Total phenolic content

The total phenolic content of the crackers was measured according to the Folin-Ciocalteu method studied by Kasangana et al. [40]. The sample (300 µl), distilled water (3.4 ml), methanol (500 µl), and Folin-Ciocalteu's reactive (200 µl) were mixed in a glass tube. Na2CO3 (600 µl, 10% weight/volume) solution was added to the mixture after 10 min incubation at room temperature. The absorbance of samples was measured at 760 nm using a UV-vis spectrophotometer (Shimadzu Spectrophotometer UV-1700 PharmaSpec, Japan) after 120 min incubation. The same procedure was applied for standard gallic acid (GA) solutions (20, 40, 60, 80, 120, and 160 mg/kg) and blank samples (distilled water, 39). The total phenolic content of the crackers (mg GAE/100 g) was calculated with the calibration curve (y = 0.0063x + 0.00002 (R2 = 0.999)). The results were presented as mg GAE/ 100 g.

Total antioxidant content

The total antioxidant analysis was performed according to Prieto et al. [41]. Sample (500 µl), distilled water (2.5 ml), and molybdate reactive (1 ml, 28 mM NaH-2PO4.H2O, 4mM H24Mo7N6O24.4H2O) were mixed in the screw-capped tubes. The samples were placed into the water bath (Memmert GmbH + Co. KG, Germany, 95°C for 90 min) for incubation. The incubation tubes were then cooled at 20-22°C. The same procedure was applied for standard ascorbic acid (AA) solutions (25, 50, 100, 150, 200, 400, and 800 mg/kg) and blank samples (distilled water). The absorbance of the mixtures was measured at 695 nm using a UV-vis spectrophotometer (Shimadzu Spectrophotometer UV-1700 PharmaSpec, Japan, *40*). The total antioxidant content of the samples (mg AAE / 100g) was calculated using the calibration curve (y = 0.0035 x -0.075 (R2 = 0.995)).

DPPH assay

The antioxidant activity was assessed using the DPPH assay following the method of Ahmed et al. [42]. The stock solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) (10 mM) was prepared by dissolving in methanol and kept in a refrigerator until further use. The DPPH solution was diluted in methanol until the absorbance of the solution reached 0.980±0.02 at 517 nm using a UV-vis spectrophotometer (Shimadzu Spectrophotometer UV-1700 PharmaSpec, Japan). 100 µl sample and 3 ml DPPH were mixed in a test tube. The same procedure was applied for standard ascorbic acid solution (dissolved in methanol) (20, 50, 100, 150, 200, and 400 mg/kg) and blank samples

(methanol). The absorbance of samples was measured at 517 nm using a UV-vis spectrophotometer. Measurements were carried out in triplicate and the results are given in mg AAE / 100 g.

ABTS Assay

Antioxidant activity was determined using the ABTS assay as described by Ahmed et al. [42]. The ABTS solution (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) was prepared by mixing 9.5 mL ABTS (7 mM) with 245 µL potassium persulfate (100 mM). The mixture was kept in the dark at room temperature for 18 h. The resulting solution was diluted with potassium phosphate buffer (0.1 M, pH 7.4) until its absorbance reached 0.706±0.001 at 734 nm, measured with a UV-vis spectrophotometer (Shimadzu UV-1700 PharmaSpec, Japan). A volume of 150 µL of sample was mixed with 2850 µL of ABTS solution in a test tube. The same procedure was applied to ascorbic acid standards (dissolved in methanol; 2, 5, 10, 15, 20, 50, and 100 mg/kg) and ethanol blanks. Measurements were conducted in triplicate, and the results were expressed as mg AAE/100 g.

FRAP Assay

The ferric reducing antioxidant power (FRAP) of the samples was measured according to the method described by Benzie and Strain [43]. One milliliter of sample extract was mixed with 1 mL of FRAP reagent, which was prepared by combining 30 mM acetate buffer (pH 3.6), 10 mM TPTZ (in 40 mM HCl), and 20 mM $FeCl_3 \cdot 6H_2O$. A total of 250 µL of sample was mixed with 2750 µL of the FRAP reagent. Standard solutions ($FeSO_4 \cdot 7H_2O$; 5, 10, 25, 50, 75, and 100 mg/kg) and distilled water blanks were used for calibration. Absorbance was recorded at 593 nm using a UV-vis spectrophotometer (Shimadzu UV-1700 PharmaSpec, Japan). FRAP values were expressed as mg $FeSO_4$/100 g, calculated using the standard curve equation (y = 0.0127 x -0.0716, R^2 = 0.993).

2.2.4 Mineral composition

The mineral content (Fe, Ca, Zn, Mg, Cu, Al, Mn, and K) of the cracker samples was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, Varian Vista, Australia), following a modified method by Bubert and Hagenah [44]. Cracker samples (0.5 g) were placed in Teflon perfluoroalkoxy vessels and digested in a microwave oven (Berghoff Instruments, Germany) with 5 mL HNO_3 and 1 mL H_2SO_4, sequentially heated at 180°C, 200°C, and 220°C for 5 minutes each. After digestion, the solution was diluted with distilled water to 50 mL and then topped off to final volume with 5% HCl. Measurements were done in triplicate and results were expressed as mg/kg.

2.2.5 Hardness and color measurements

The hardness of the crackers was determined using a Texture Analyzer (Brookfield CT3, USA) equipped with a TA7 probe, with a trigger load of 5 g and a test speed of 3.0 mm/s. Pre- and post-test speeds were set to 10.0 mm/s. Surface color values (*L**, *a**, *b**) were measured using a Konica Minolta CR-400 Chroma Meter (Konica Minolta Sensing Inc., Japan). The total color change (ΔE) was calculated using the following equation:

$$\Delta E = \sqrt{\left(L^* - L_0\right)^2 + \left[\left(a^* - a_0\right)^2 + \left(b^* - b_0\right)\right]^2} \quad (2)$$

where L_0, a_0, and b_0 represent the color parameters of the control sample.

2.2.6 Sensory properties

Sensory evaluation was conducted by a panel of 20 trained individuals (12 females and 8 males; 3 smokers and 17 non-smokers) aged between 20-35 years from Alanya Hamdullah Emin Paşa University, Alanya, Antalya, Turkey. The panelists were trained to assess six sensory attributes (color, appearance, flavor, taste, texture, and overall acceptability) using a 5-point hedonic scale (1 = strongly dislike, 5 = strongly like).

2.2.7 Statistical analysis

Cracker production was carried out in duplicate and all analyses were performed in triplicate. Statistical analyses were conducted using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Differences among samples were evaluated by one-way ANOVA and Duncan's multiple range test at $P<0.05$. The effect of oil pomace on phytochemical content and antioxidant activity was modelled using linear ($y = ax + b$), power ($y = ax^b$), logarithmic ($y = a \ln(x) + b$), and quadratic ($y = ax^2 + bx + c$) regression equations in Microsoft Excel 2019. Goodness of fit was assessed using the coefficient of determination (R^2).

3. RESULTS AND DISCUSSION

3.1. CHEMICAL COMPOSITION, WATER ACTIVITY AND PH VALUES OF CRACKERS

The moisture content and water activity of the wheat flour and dried olive pomace powder were found as 11.76±0.16 % (wet basis, wb) and 0.322±0.010 and 2.45±0.21% (wb) and 0.233±0.010, respectively. It was stated that the moisture content of pomace samples dried with a fluidized bed, drum, combined (microwave+convectional oven), roller, and tray dryers varied between 4.6-8% [45-49]. It is thought that the reason why the moisture content value of the OP in our study is lower than in other studies may be due to many different factors such as geographical area, environmental conditions, the origin of the olive, cultivar,

harvest date, stage of ripening, climate, method of extraction, and drying conditions [4,5,8,9].

The moisture, ash, and fat contents, water activity, and pH values of crackers are given in Table I. Compared to the control, the moisture content and water activity values of the OP powder-added crackers were found to be low ($P<0.05$). This may be due to the lower moisture content of OP (2.45%) compared to wheat flour (11.76%). Lekjing and Venkatachalam [50] reported that the ingredients significantly affect the final moisture content of the crackers. The moisture content (2.59-3.06%) and water activity (0.185-0.192) values of breadsticks with 5 and 10% red grape pomace reported by Rainero et al. [33] were compatible with our results. The moisture content of crackers with OP was found to be lower than green gram flour crackers (5.76%) [50]. This may be due to different cracker formulations and production processes. The addition of the OP to the cracker formulation resulted in a significant decrease in the moisture content value of the samples up to a 10% OP powder substitution level ($P<0.05$). However, the further increase did not cause a significant decrease in the moisture content of the crackers ($P>0.05$).

According to the ash analysis results, it can be said that the ash content of the crackers significantly increased with the addition of OP powder ($P<0.05$). The ash content of OP was reported as 4.55±0.22 g/100 g (dry weight, dw) [12]. The high ash content of the OP may be the reason for the high ash content of crackers with OP. The highest fat content (11.26±0.07 %, dw) was determined in the cracker sample with 20% OP. With the addition of pomace, the fat content of the crackers generally increased ($P<0.05$). It has been stated that this is due to the residual oil in the pomace [25]. Since olive pomace is rich in oil, it is expected that increased OP powder concentration will increase the fat content of crackers. Supporting our findings, the same study observed a significant increase in fat content with an increase in the amount of OP in the breadsticks.

Water activity values of the cracker samples were found to be lower than 0.254 and the crackers can be considered microbiologically stable. The water activity values of the crackers significantly decreased with the addition of OP ($P<0.05$). This is expected due to the lower water activity value of OP than wheat flour and the low moisture content of OP-added crackers. The pH values of the cracker samples ranged between 5.02-5.17. With the addition of OP, the pH value of the cracker samples decreased. However, this decrease was found to be insignificant up to the 20% OP powder replacement ratio ($P>0.05$). Similar to our results, Rainero et al. [33] reported that increasing the amount of red grape pomace decreased the pH value of breadsticks and the acidic character may cause a more tenacious and less extensible structure in the dough. In addition, previous studies have reported that high pH values cause weakening of gluten, a decrease in consistency, and undesirable brown spots [51,52].

3.2. PHYTOCHEMICAL CONTENT AND ANTIOXIDANT ACTIVITY OF CRACKERS

Antioxidants may react differently to various radical or oxidant sources. The mechanisms of action of all radical sources and antioxidant chemicals in a complex system cannot, therefore, be accurately reflected by a single technique. Nowadays, it is crucial to assess the antioxidant content of food. To determine the overall antioxidant potential of any food matrix, estimation of the total antioxidant using several assays is crucial. Using three in vitro assays—DPPH, ABTS and FRAP—the total antioxidant activity of the food products was measured. Many researchers have investigated the antioxidant activities of bakery products using these methods [25,53–55].

The total flavonoid, total phenolic, and total antioxidant contents, as well as antioxidant activities of crackers are given in Table II. With the addition of OP to the cracker dough, total flavonoid, phenolic, and antioxidant contents and antioxidant activity (DPPH, ABTS, and FRAP) of cracker samples significantly increased compared to the control sample ($P<0.05$). This is thought to be due to the fact that pomace is

Table I - Proximate composition of crackers

Sample (%)	Moisture Content (%, Wet Basis)	Ash Content (%, Dry Basis)	Fat Content (%, Dry Basis)	Water Activity	pH
0 (Control)	3.18±0.49 ^{c}	1.75±0.03 ^{a}	7.68±0.21^{a}	0.254±0.00^{d}	5.17±0.01^{b}
5	2.12±0.35 ^{b}	2.23±0.04 ^{b}	8.91±0.10^{b}	0.252±0.00^{d}	5.13±0.01^{b}
10	1.55±0.32 ^{a}	2.42±0.02 ^{c}	9.12±0.15^{b}	0.241±0.00^{c}	5.11±0.01^{b}
15	1.47±0.17^{a}	2.44±0.06 ^{c}	10.98±0.03^{c}	0.228±0.00^{b}	5.10±0.01^{b}
20	1.47±0.13 ^{a}	2.57±0.02 ^{d}	11.26±0.07^{c}	0.217±0.00^{a}	5.02±0.01^{a}

Mean ± SD (n=3). Different letters (a-e) in the columns represent the statistical difference ($P<0.05$).

rich in phytochemicals, which contribute to its antioxidant activities [3,10,11]. With the addition of 5% OP to the crackers, the total phenolic content and antioxidant activity (DPPH and ABTS) of the samples increased approximately two times, while the total flavonoid content increased four times compared to the control. It can also be said that the addition of OP most affects the amount of total flavonoid content, and least affects the total antioxidant content and FRAP.

The total flavonoid ($y = 1.0705x^2 + 7.4377x + 42.751$, $R^2 = 0.9866$), total phenolic ($y = -0.0729x^2 + 3.7371x + 15.688$, $R^2 = 0.9956$), and total antioxidant ($y = 0.0913x^2 + 0.4861x + 70.984$, $R^2 = 0.9815$) contents, and ABTS ($y = -0.0947x^2 + 4.6929x + 21.214$, $R^2 = 0.9467$, graphs were not given) of crackers followed a quadratic trend depending on the increase of OP concentration, whereas the antioxidant activities of the crackers followed a linear trend (DPPH AA Eq.: $y = 2.082x + 12.732$, $R^2 = 0.9949$, and FRAP: $y = 3.91x + 57.052$, $R^2 = 0.97$, graphs were not given).

Similarly, Simonato et al. [24] reported that an increase in the OP percentage increased both the total phenolic content and antioxidant activity of the cooked and uncooked pasta samples. In addition, it was also claimed that DPPH ($R^2 = 0.90$) and ABTS ($R^2 = 0.99$) assay strictly correlated to the OP percentage. Simsek and Sufer [25] also reported that an increase in the replacement ratio of OP with wheat flour increased the total phenolic content and antioxidant activity (DPPH and FRAP) in breadsticks fortified with OP.

Phenolic compounds such as flavonoids, phenolic acids, and diterpenes contribute to the antioxidant activity of the foods. As a result of the study, in order to determine the relationship between total flavonoid and total phenolic contents and antioxidant capacity, the correlation graphs were drawn and shown in Figure 1. It can be stated that both the total flavonoid and phenolic contents showed a linear relationship with antioxidant activity. The correlation coefficient values were generally found higher than 0.90, except for the ABTS-total flavonoid and ABTS-total antioxidant graphs. It can also be stated that the antioxidant activity of the crackers can be expressed with their total flavonoid and total phenolic contents. Antioxidant activity may also come from the presence of volatile oils, carotenoids, vitamins, etc. [56].

3.3. MINERAL COMPOSITION OF CRACKERS

Table III provides the mineral composition of cracker

Table II - Total flavonoid, total phenolic, and total antioxidant contents and antioxidant activity (DPPH, ABTS, and FRAP) of cracker samples with OP (Wet basis)

Sample (%)	TFC (mg CE /100 g)	TPC (mg GAE /100 g)	TAC (mg AAE /100g)	DPPH (mg AAE /100 g)	ABTS (mg AAE /100 g)	FRAP (mg $FeSO_4$ Eq. /100 g)
0 (Control)	30.38±4.98^a	15.02±0.40^a	69.36±1.07^a	13.38±0.29^a	20.62±2.13^a	51.54±0.58^a
5	125.50±5.00^b	33.73±1.60^b	78.93±1.80^b	21.86±1.16^b	46.01±2.14^b	83.62±0.10^b
10	242.00±7.50^c	46.24±0.80^c	85.02±0.36^c	33.35±0.58^c	51.14±0.46^c	99.30±2.01^c
15	352.60±14.90^d	53.54±0.00^d	95.53±1.42^d	45.62±0.87^d	76.64±0.76^d	110.40±0.10^d
20	638.00±17.50^e	62.10±0.60^e	118.87±0.35^e	53.55±3.20^e	75.27±0.61^d	135.90±2.00^e

Mean ± SD (n=3). Different letters (a-e) in the columns represent statistical differences (P<0.05).
TFC: Total Flavonoid Content, CE: Catechin Equivalent,TPC: Total Phenolic Content, GAE: Gallic Acid Equivalent, TAC: Total Antioxidant Content, AAE: Ascorbic Acid Equivalent, DPPH: 1,1-diphenyl-2-picrylhydrazyl, ABTS: 2,2-Azino-Bis (3-Ethylbenzthiazoline-6-Sulfonic Acid), Diammonium Salt, FRAP: Ferric Reducing Antioxidant Power Assay, Eq: Equivalent.

Table III - Mineral composition of cracker samples with OP (Wet basis)

Sample (%)	Minerals (mg/kg)							
	Fe	Ca	Zn	Mg	Cu	Al	Mn	K
0 (Control)	49.52±0.65^a	386.46±2.15^a	8.18±0.23^a	342.74±2.80^a	3.56±0.36^a	14.39±1.11^a	9.48±0.47^a	1852.12±12.23^a
5	49.52±1.33^a	421.93±2.31^b	8.67±0.29^a	369.94±2.64^b	4.34±0.52^b	14.48±0.43^a	9.77±0.12^a	2853.75±8.25^b
10	55.19±0.43^b	552.17±6.90^c	14.93±0.42^c	420.17±2.39^c	9.65±0.31^c	20.38±0.58^b	9.64±0.32^a	3777.55±18.45^c
15	58.28±1.20^c	805.43±1.84^d	8.60±0.33^a	436.65±2.22^d	9.80±0.19^c	23.66±1.03^c	9.61±0.44^a	4721.40±17.11^d
20	59.29±0.45^c	832.72±1.79^e	11.13±0.25^b	485.49±2.53^e	9.75±0.32^c	48.46±1.20^d	9.65±0.46^a	5888.50±6.01^e

Mean ± SD (n=3). Different letters (a-e) in the columns represent the statistical difference (P<0.05).

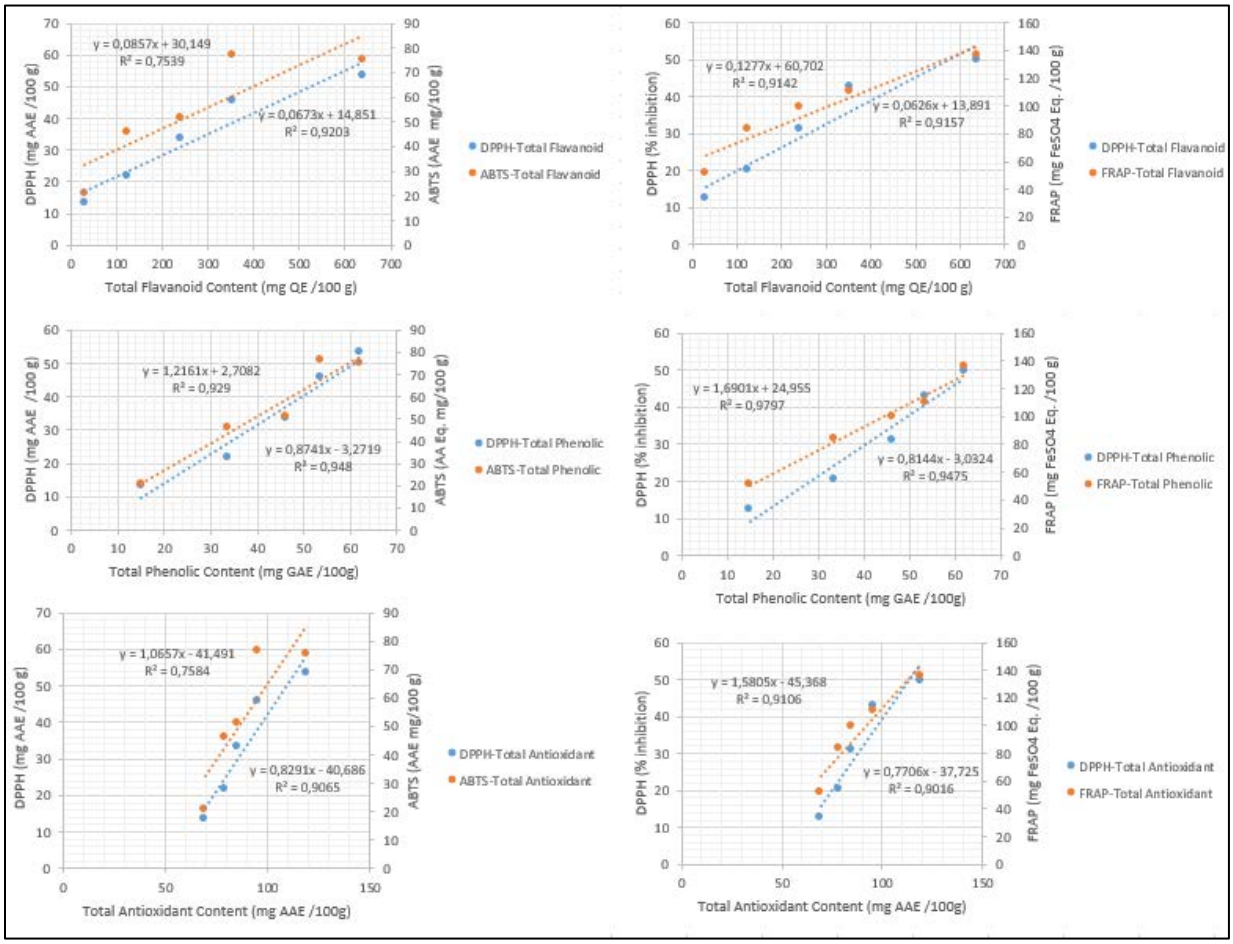

Figure 1 - Linear correlation between total flavonoid, phenolic, and antioxidant contents and antioxidant activity of the crackers

samples containing OP. The addition of OP powder influenced the mineral composition of the resulting crackers ($P<0.05$). When examining the mineral content of the samples, there was no statistically significant difference between the control and the sample enriched with 5% OP in terms of iron (Fe), zinc (Zn), aluminum (Al), and manganese (Mn) content ($P>0.05$). Generally, the addition of OP at concentrations of 10% and above led to a statistically significant increase in mineral content ($P<0.05$). However, manganese (Mn) content was not found to be dependent on the concentration of OP ($P>0.05$).

It has been reported that OP is rich in potassium (2798.44±29.64 mg/kg dw), calcium (352.03±23.01 mg/kg dw), and magnesium (145.41±16.54 mg/kg dw). It also contains iron (12.24±2.71 mg/kg dw), sodium (16.42±0.67 mg/kg dw), aluminum (16.93±2.08 mg/kg dw), manganese (1.56±0.22 mg/kg dw), zinc (2.92±0.36 mg/kg dw), copper (2.73±0.29 mg/kg dw), boron (4.02±0.52 mg/kg dw), and barium (0.18±0.03 mg/kg dw) [12].

Similarly, our findings demonstrated that the incorporation of OP powder into crackers resulted in increased content of several minerals, including Fe, Ca, Zn, Mg, Al, and K. The elevated mineral levels may be attributed to the inherent richness of OP in mineral compounds compared to wheat flour. Studies in the literature also support these results. For example, high dietary fiber biscuits enriched with OP exhibited a calcium content of 731.15±5.32 mg/kg and a magnesium content of 301.16±3.45 mg/kg [23]. Another study involving muffins enriched with olive pomace flour, extra virgin olive oil, and hydrolysed soy protein reported an improved mineral composition of 1.09±0.01%, which was attributed to the composition of both olive pomace flour (3.02±0.08%) and hydrolyzed soy protein (9.87±0.25%). The study also suggested that interactions between fibers and certain proteins may result in the formation of complexes with minerals, thereby increasing the micronutrient content in the final product [57].

3.4. HARDNESS AND COLOR VALUES OF CRACKERS

Hardness is a crucial quality parameter in crackers, as consumers generally prefer a crisp texture. The hardness values of the crackers exhibited an upward trend with the incorporation of higher levels of OP (Figure 2). The increase was evident up to a 15% OP powder inclusion level; beyond this concentration, no further increase in hardness was observed. A similar trend was

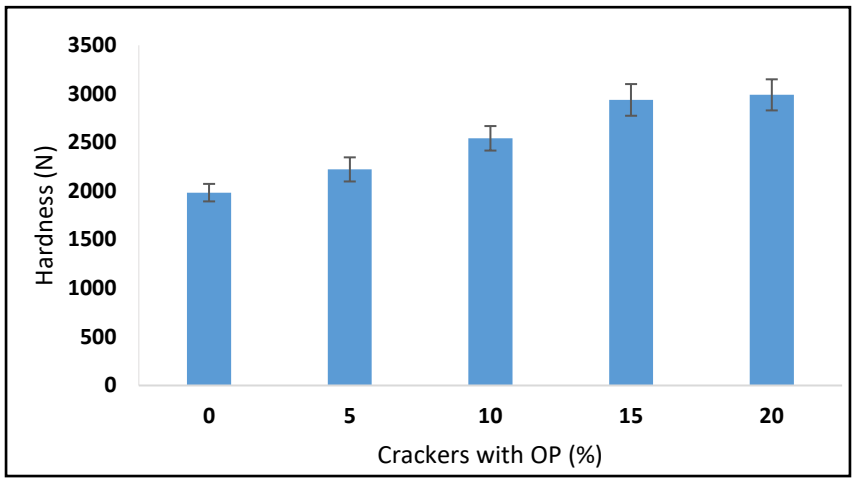

Figure 2 - Hardness levels of crackers Mean ± SD (n=3)

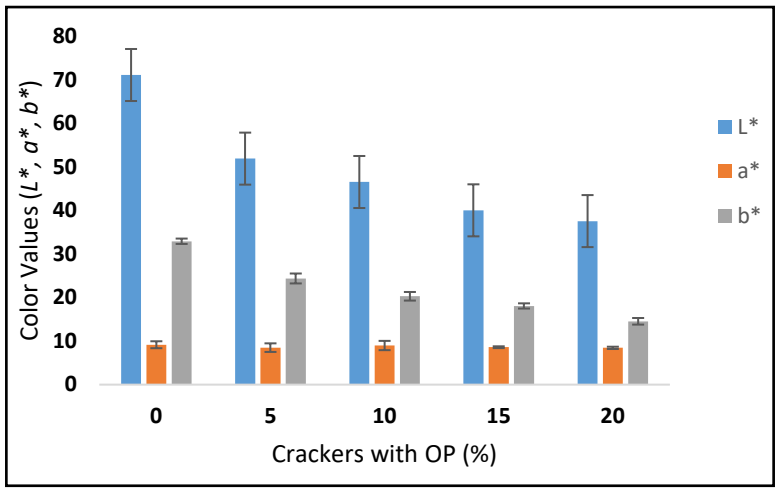

Figure 3 - Color values of crackers Mean ± SD (n=3)

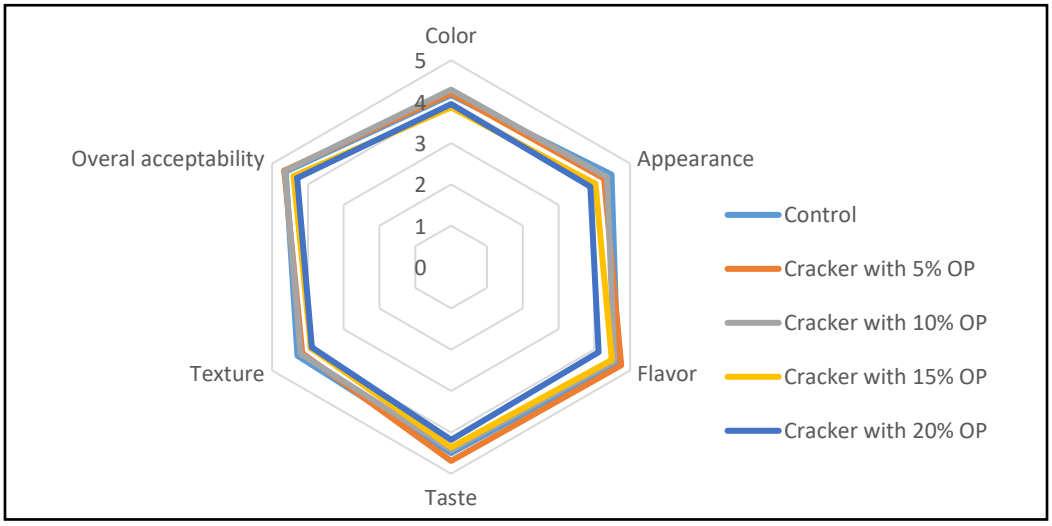

Figure 4 - The radar chart of consumer preference scores of crackers with OP Mean ± SD (n=3)

reported by Drisya et al. [58] in cookies fortified with dried Murraya koenigii leaves. The researchers noted that higher amounts of these leaves (0 to 15%) diluted the gluten by increasing the protein, dietary fiber, calcium, and iron content, resulting in a harder cookie dough with less stickiness and springiness compared to the control.
Similarly, in our investigation, increased OP levels correlated with higher mineral content, which may have contributed to the enhanced hardness. The inclusion of substantial amounts of OP could have diluted the gluten structure, leading to a more rigid cracker texture. Additionally, elevated pH values associated with OP may have weakened gluten networks, contributing further to the formation of harder crackers [51,52]. Collar et al. [59] also stated that fiber addition can affect the mechanical properties of dough by increasing hardness and decreasing cohesiveness.
Figure 3 illustrates the color values of crackers at various OP powder ratios. The L* (lightness) and b* (yellowness) values exhibited significant variations with the addition of OP ($P<0.05$), while there were no statistically significant differences in a* (redness) values among the OP-containing samples ($P>0.05$), except for the control. The L* values decreased with increasing OP content, likely due to the naturally dark color of OP powder. The a* values also declined in the OP-added samples, and b* values decreased in parallel with increasing OP concentrations. As OP ratio increased, the ΔE (total color difference) values rose significantly ($P<0.05$), ranging from 21.04±0.98 in crackers with 5% OP to 38.28±2.43 in those with 20%.
Similar to our findings, Simsek and Sufer [25] reported a significant reduction in L* and b* values with increasing OP content in breadsticks. In another study, lower L* and b* values were also reported for OP-enriched cooked pasta compared to control samples [24]. Furthermore, Qadri et al. [60] highlighted that Maillard reactions, caramelization, and thermal processing at high temperatures can lead to surface color changes in dough products.

3.5. SENSORY PROPERTIES OF CRACKERS

Figure 4 displays the sensory characteristics of crackers manufactured with varying OP ratios, along with the radar chart depicting consumer preference scores. The control samples and crackers containing 5% and 10% OP exhibited similar sensory attributes. Notably, the crackers enriched with 10% OP attained the highest overall acceptability, receiving a score of 4.66 (±0.36) in comparison to the control. Crackers with 20% OP received the lowest acceptability score among all other samples. In this recipe, in terms of sensory attributes including color, appearance, texture, and overall acceptability, crackers were found well accepted up to a ratio of 10% OP incorporation, while in terms of flavor and taste properties, crackers were found acceptable up to a ratio of 15% OP incorporation. The previous study has demonstrated that high-fiber biscuits with a 15% incorporation level with OP had the highest overall acceptability (4.58±0.50, *23*). In order to develop products with health benefits, Padalino et al. [61] produced functional spaghetti from durum wheat semolina that was enhanced with olive paste powder (10-15%), a by-product of olive oil. It was emphasized that it may be better to add optimal olive paste powder to avoid a negative impact on sensory qualities and the overall quality of the pasta. Overall, the lowest percentage of olive paste (10%) was found to be better in terms of sensory and textural aspects. In our study, OP incorporation crackers with the lowest ratios (5-10%) were found to be superior in terms of sensory properties.

4. CONCLUSIONS

This study showed that OP, which is a by-product of olive oil industries, could be used as a source of functional components to produce a high-value-added cracker. OP-fortified crackers are rich in ash, fat, flavonoid, and phenolic contents, antioxidant activities, and mineral composition compared to crackers made with only wheat flour (control). These valuable components in the crackers increased with the increase in the amount of OP (0 to 20%). With the increase in the amount of OP, the moisture content, water activity, and color values of the crackers decreased. Among the cracker samples, those incorporated with 10% OP powder had the highest overall acceptability score. These findings show that OP is a valuable functional ingredient for producing crackers high in flavonoids, phenols, minerals, and antioxidants, as well as crackers with good sensory attributes. Furthermore, there was a high correlation between phenolic and flavonoid content and antioxidant activities. In addition, functional crackers prepared using OP powder fortification could find a place in the functional food product market.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Author's contribution
Kadriye Altay: performed the experimental work, contributed to the formatting and writing of the manuscript.
Arda Akdoğan: conducted the literature review, performed the experimental work, and carried out the statistical analysis.
Selma Lubabe Erdoğan: conducted the literature

review, performed the experimental work, and carried out the statistical analysis.
Gülşah Çalışkan Koç: conceived the research idea, supervised the analytical work, contributed to the statistical analysis, and took the lead in formatting and writing the manuscript.

ORCID ID
K. Altay: https://orcid.org/0000-0002-1629-6841
A. Akdoğan: https://orcid.org/0000-0002-8537-5914
S.L. Erdoğan: https://orcid.org/0000-0002-4248-001X
G. Çalışkan Koç: https://orcid.org/0000-0002-6542-3093

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Effects of Harvesting Time and Isolation Techniques on The Physicochemical Characteristics of Grapefruit (*Citrus paradisi,* Macfadyen), Bitter Orange (*Citrus aurantium,* Linnaeus) and Kumquat (*Fortunella margarita,* Swingle) Essential Oils

Muharrem Gölükcü[1]
Burcu Bozova[1]
Haluk Tokgöz[1]
Demet Yıldız Turgut[1]
Orçun Çınar[1]
Ertuğrul Turgutoglu[1]
Angelo Maria Giuffrè[2] ✉

[1] Republic of Türkiye Ministry of Agriculture and Forestry Batı Akdeniz Agricultural Research Institute [BATEM] Food Technology Department 07112 Antalya, Türkiye

[2] Department AGRARIA University of Studies "Mediterranea" of Reggio Calabria 89124 Reggio Calabria, Italy

✉ CORRESPONDING AUTHOR
amgiuffre@unirc.it
Tel.: +39-327-7022840

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The essential oil (EO) yield and physicochemical parameters of four grapefruit cultivars, a bitter orange, and a kumquat cultivar were evaluated in relation to harvest time and isolation technique. EO samples were obtained by hydrodistillation (HD) and cold pressing (CP). The chemical composition of the EOs was determined by gas chromatography-mass spectrometry (GC-MS/FID). EO yield across the samples ranged from 1.24% to 2.83%. The isolation method significantly influenced the relative density, optical rotation, and refractive index of the oils. The cultivar also had a statistically significant effect on refractive index and optical rotation. Oils obtained by CP had higher relative densities (0.8473-0.8524) compared to those obtained via HD (0.8385-0.8413). CP oils also exhibited higher refractive index values (1.4749-1.4765) than HD oils (1.4721-1.4733). In contrast, HD-isolated oils had higher optical rotation values (95.48-99.13°) than CP (91.55-94.50°). While some variation in EO composition was observed based on cultivar, harvesting time, and extraction method, these differences were mostly insignificant. Limonene was identified as the major component, ranging from 93.7% (Rio Red as grapefruit) to 96.0% (Nagami as kumquat). Cultivar and harvest time effects on limonene content were statistically significant, whereas the effects of the isolation method were not ($p>0.05$). β myrcene was the second main component, ranging from 1.9% to 2.1%. While harvest time and isolation method had a significant impact on its concentration, the cultivar effect was not important. The study demonstrated a small variation in the composition of these citrus essential oils. Harvest time appears to be a less critical factor for these specific properties.

Keywords: citrus essential oil, cultivar, harvest time, isolation method, limonene.

1. INTRODUCTION

World citrus production has shown significant increases in recent years. Production, which was 105,930,647 tons across 7,449,973 hectares of land in 2000, reached 169,388,810 tons across 10,554,846 hectares of land in 2023. China is the main citrus producing country (28%), followed by Brazil, Türkiye, Spain, and Egypt. Türkiye accounted for 7,877,982 tons of global production in 2023 [1]. A significant portion of citrus production consists of oranges, mandarins and lemons. Additionally, citrus such as grapefruit, sour orange and kumquat are also produced. Citrus are used both for fresh consumption and, significantly, in the food process. During the evaluation of citrus fruits in the food industry, representing approximately 50-65% of the fruit weight, waste is generated, and a significant portion of this is peel [2]. Notably, citrus waste is rich in several beneficial components, including flavonoids, carotenoids, dietary fiber, soluble sugars, and essential oils [3, 4].
Aromatic compounds known as essential oils are occurring in large quantities within oil sacs located at different depths in the flavedo part of the peel.

Citrus peel oils contain many bioactive components, the main one being limonene [5, 6], which has various functional properties [7]. Citrus oils are used in many areas such as pathogen inhibition, insect control, medicine, cosmetics and food because they are Generally Recognized as Safe (GRAS) [8, 9]. Consequently, citrus peel oils find applications in numerous sectors such as the food industry [10, 11], cosmetics, perfumery, pharmaceuticals, and the production of cleaning products [12,13,14]. A key characteristic of these oils is their high limonene content, which, in citrus essential oils from peels of orange, mandarin, bergamot, and bitter orange, can range from 36.54% to 96.10% [15]. While the primary components of essential oils largely determine their biological activities, minor components can also play a significant role through synergistic, antagonistic, or additive interactions [16,17]. Limonene (p-mentha-1,8-diene) itself is industrially important and used extensively in food, medicine, and cosmetics. Its aromatic properties and high absorption make it valuable in the pharmaceutical and cosmetic industries. Due to its aroma, limonene is also commonly used in the food industry and is GRAS-listed by the FDA [18, 19]. It is important to note that the essential oil composition of citrus peel oils can change by citrus type and cultivar, as well as the isolation method employed (cold press, hydrodistillation) [20, 21].

While most research on citrus essential oils concentrates on oranges, relatively few studies specifically examine grapefruit, bitter orange, and kumquat. In Türkiye, which possesses significant raw material potential, the production of essential oils from these and various citrus fruits holds importance for both the global and national economy. The purpose of this research was to reveal certain quality properties and volatile oil compositions of peel essential oils isolated from four grapefruit, a bitter orange and a kumquat cultivar using two different methods across four different harvest periods. Therefore, the compositional data of these oils, extracted through both cold-pressing and hydrodistillation, can offer valuable insights for researchers, producers, and the industry. Furthermore, evaluating citrus peels, which are currently considered waste, can contribute to the growth of both producers and the processing industry. It is important to note that the quality of volatile oil is affected by factors such as the citrus cultivars used, the harvest time of the material, and the isolation method employed.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

Citrus fruits used in the research were harvested from the experimental area of the Aksu-Central Unit of the Batı Akdeniz Agricultural Research Institute (Antalya, Türkiye), the Ministry of Agriculture and Forestry between 2021-2023 production seasons. Mature fruits for each cultivar of grapefruit (*Citrus paradise*; Red Blush, Rio Red, Star Ruby, Marsh Seedless), bitter orange (*Citrus aurantium*) and kumquat (*Fortunella margarita*) were harvested in four different harvest periods (Table I). Approximately 15-20 fruits were collected from four sides of every tree. Fruits were taken to the laboratory on the same day.

Upon arrival at the laboratory, the initial analysis focused on fruit weight and peel ratio. For each of the three repetitions, ten fruits were individually weighed, and their peels were also weighed separately, both with a precision of 0.01 g. The reported fruit fresh weight and peel/fruit ratio represent the average values calculated from these ten fruits within each repetition.

2.2. HYDRODISTILLATION (HD) AND COLD PRESS (CP) PROCESS

The essential oil in the samples was extracted using hydrodistillation with a Clevenger-type apparatus, following the ISO 6571 standard [22]. The resulting essential oil amount is expressed as a volume percentage in relation to the fresh fruit peel weight (mL/100 g, %). For the collection of essential oil via cold pressing, the method described by Bozova et al. [23] was followed. The grated citrus peel was squeezed manually with a kitchen-type apparatus. The water-oil extract was centrifuged (15294 × g at 20°C for 20 min) to obtain cold pressed oil. Essential oil yield was determined from 100 g of fresh peel (mL/100 g, %).

Table I - Grapefruit, bitter orange and kumquat cultivars and harvest times

Species	Cultivars	1th Harvest	2nd Harvest	3th Harvest	4th Harvest
Citrus paradisi	Red Blush	01 December	20 December	10 January	30 January
	Rio Red	01 December	20 December	10 January	30 January
	Star Ruby	10 November	30 November	20 December	10 January
	Marsh Seedless	01 December	20 December	10 January	30 January
Citrus aurantium	Yerli Turunç	20 January	10 February	02 March	20 March
Fortunella margarita	Nagami	20 December	10 January	30 J January	20 February

2.3. PHYSICOCHEMICAL PROPERTIES OF THE OIL SAMPLES

Physicochemical characteristics are crucial indicators of the quality and purity of essential oils. Relative density, optical rotation, and refractive index, all of which are fundamental attribute parameters outlined in the European Pharmacopoeia. Relative density was determined according to ISO 279 [24], while refractive index was determined at 20°C by a refractometer (A. Krüss Optronic, DR6000 model) following ISO 280 [25]. The optical rotation values were specifically determined for citrus peel essential oils by a polarimeter (Optical Activity Ltd., PolAAR 31 model) according to ISO 592 [26].

2.4. ESSENTIAL OIL COMPOSITION

Gas chromatography–mass spectrometry (GC-MS) analysis was performed with an Agilent 7890A system coupled with a mass spectrometer (Agilent 5975C) and a flame ionization detector (FID), following the methodology of Özek et al. [27]. For analysis, samples were diluted as 1:50 using hexane. Capillary column (HP Innowax, 60.0 m × 0.25 mm × 0.25 µm) was used in the analysis. Helium was used as the carrier gas (0.8 mL/min). The sample was injected as 1 µL with a split ratio of 50:1, and an injector temperature was adjusted to 250°C. The column temperature was kept at 60°C for 10 minutes, increased to 250°C at a rate of 20°C/minute, and kept at 250°C for 10.5 minutes, resulting in a total analysis time of 60 minutes. The mass detector operated with a scanning range of m/z 35-500 and electron bombardment ionization at 70 eV. The essential oil components were identified by comparing their mass spectra with those in the Wiley and OIL ADAMS GC/MS Libraries. Relative Retention Indices (RRI) were calculated by C8-C40 alkane series (Sigma, USA) as standard samples. The relative percentage of the separated compounds were detected by analyzing FID chromatograms.

2.5. STATISTICAL ANALYSES

The research employed a randomized parcel trial design with three replications [28]. The analyzes were repeated twice and the obtained data were tested according to variance analysis (ANOVA) and the Duncan Test by the SAS statistical program. Data are presented as mean±standard error (SE).

3. RESULTS AND DISCUSSION

The ANOVA and Duncan results for the fruit weight, peel ratio of the fruit, and peel essential oil content of the four grapefruit, a bitter orange and a kumquat cultivar were evaluated within the scope of the study given in Table II and Table III. The influence of the cultivar (C) on the fruit weight of the samples determined was significant, while the influence of harvest time (HT) and C×HT interaction was insignificant. The Marsh Seedless cultivar had the highest average fruit weight among the grapefruit cultivars examined within the scope of the research. Kumquat and bitter orange fruit weights are considerably lower than grapefruit cultivars (Table III). The average fruit weights of the citrus cultivars generally increased from the first harvest to the last harvest time except for Nagami (kumquat). The influence of the cultivar on the peel ratio was also important, while the impact of HT and C×HT was insignificant. It was observed that the peel ratios of Star Ruby were lower than other grapefruit cultivars. Bitter orange and kumquat had a higher peel ratio than grapefruit.

The variation in the essential oil yield of the samples according to the cultivar and harvest time were subjected to statistical evaluation based on the hydrodistillation method data. While the effect of the cultivar and C×HT interaction on the essential oil content of the samples was statistically important, the influence of the harvest time remained insignificant. Within the cultivars, the peel's essential oil ratios of Yerli Turunç as bitter orange was higher than grapefruit cultivars and Nagami as kumquat. Among the analyzed cultivars, the one with the lowest essential oil content was Marsh Seedless. When the effect of harvest time is examined on a cultivar basis, these differences can reach significant levels. For instance, in Star Ruby, the amount of essential oil, which was 1.80% at the beginning of the harvest, decreased by approximately 30% to 1.24% in the fourth harvest period. Fruit weights, peel quantities and essential oil content in the peel are taken into consideration, showing that it is more appropriate to harvest Mars Seedless and Red Blush in the fourth harvest period, Rio Red, Yerli Turunç and Nagami in the third harvesting period and Star Ruby in the first three harvest periods. Hydrodistillation yielded a considerably larger amount of essential oil, ranging

Table II - ANOVA results for physical properties of fruits and essential oil content

	Fruit weight		Peel ratio		Essential oil content	
	F value	p value	F value	p value	F value	p value
Cultivar (C)	149.05	0.0001	201.09	0.0001	7.44	0.0001
Harvest time (HT)	6.50	0.0022	0.83	0.4893	0.44	0.7230
C×HT	0.90	0.5748	1.36	0.2416	2.58	0.0066
Coefficient of variation	12.9351		6.7376		14.7538	

from 4.24 to 7.87 times the quantity obtained through cold pressing. Kaanin-Boudraa et al. [29] found the essential oil ratio to be 0.33% for grapefruit. Ghani et al. [30] determined the essential oil content of grapefruit to be between 0.14-0.43%, depending on harvesting time. In the research carried out by Miya et al. [31], the essential oil yields of three different cultivars were determined to be in the range of 0.21-0.34%. Farahmandfar et al. [32] determined the essential oil content for fresh bitter orange peel to be 1.0%. Sicari and Poiana [33] found the essential oil content of oval kumquat to be 0.95% using the hydrodistillation method. Lakache et al. [34] determined the essential oil ratio to be 0.30% using the hydrodistillation method for fresh oval kumquat. Our study findings differ from the literature findings, and it is thought that this is due to the difference in the materials studied.

The variance and the Duncan results for physical characteristics of grapefruit cultivars, bitter orange and kumquat based on harvesting times and extraction techniques are presented in Table IV, and Table V. Relative density, refractive index and optical rotation values of essential oils are of critical importance for industrial application, quality control, and chemotaxonomy. These physical and chemical properties provide fundamental information about an oil's purity, quality, and botanical identity. The relative density values of the citrus cultivars in relation to harvest times and isolation techniques ranged between 0.8385-0.8524. The variance analysis revealed that the isolation method was the only factor with a significant effect on the relative density of the volatile oils. Specifically, hydrodistillation yielded samples with lower relative density values compared to those obtained through the cold press method. Cold-pressed oils may contain less volatile, heavier molecules (such as waxes, coumarins, carotenoids, fatty acids) or a higher concentration of compounds that rotate light less, compared to distilled oils. This strongly indicates that the two methods yield different chemical profiles. In ISO standards, the

Table III - Duncan test results of physical properties of fruits according to cultivar and harvesting time (mean±SE)

C	HT	Fruit weight (g/fruit)	Peel ratio (%)	Essential oil yield (CP, %)	Essential oil yield (HD, %)	Essential oil (HD, mL/whole fruit)
Star Ruby	1	262.35±26.59	24.07±1.415	0.26±0.120	1.80±0.084	1.14
	2	268.95±27.98	22.65±0.120	0.23±0.090	1.81±0.155	1.10
	3	307.15±19.10	22.76±1.135	0.24±0.130	1.62±0.027	1.13
	4	334.09±9.94	24.22±0.160	0.26±0.130	1.24±0.038	1.00
	mean	**293.13^{b}±14.38**	**23.43^{d}±0.440**	**0.25±0.045**	**1.62^{bc}±0.080**	**1.09**
Marsh Seedless	1	355.89±52.12	27.16±1.215	0.23±0.070	1.45±0.127	1.40
	2	341.87±20.94	28.15±0.960	0.28±0.105	1.48±0.113	1.42
	3	331.57±35.18	29.62±1.815	0.22±0.065	1.45±0.079	1.42
	4	368.13±21.01	28.29±0.335	0.24±0.020	1.60±0.188	1.67
	mean	**349.37^{a}±40.00**	**28.30^{c}±0.563**	**0.24±0.028**	**1.50^{c}±0.060**	**1.48**
Red Blush	1	283.34±25.31	28.78±2.495	0.29±0.130	1.65±0.023	1.35
	2	268.38±14.22	25.39±0.445	0.28±0.155	1.32±0.193	0.90
	3	345.41±5.55	26.71±0.870	0.28±0.125	1.50±0.046	1.38
	4	370.90±14.48	27.19±0.225	0.29±0.100	1.84±0.058	1.86
	mean	**317.01^{b}±18.59**	**27.02^{c}±0.685**	**0.29±0.049**	**1.58^{bc}±0.073**	**1.37**
Rio Red	1	293.98±41.87	28.74±0.875	0.34±0.135	1.70±0.177	1.44
	2	280.22±18.55	29.36±1.465	0.37±0.145	1.86±0.074	1.53
	3	345.15±3.65	27.01±3.350	0.37±0.125	1.92±0.043	1.79
	4	342.34±4.49	26.39±2.095	0.38±0.155	1.61±0.063	1.45
	mean	**315.42^{b}±36.89**	**27.87^{c}±0.934**	**0.36±0.053**	**1.77^{b}±0.058**	**1.55**
Yerli Turunç	1	132.16±3.28	36.32±0.050	0.49±0.060	2.22±0.110	1.07
	2	124.40±10.93	42.89±0.275	0.46±0.040	1.98±0.238	1.06
	3	149.81±19.43	40.66±1.005	0.48±0.020	2.83±0.260	1.72
	4	164.05±7.33	40.28±2.010	0.47±0.000	2.07±0.211	1.37
	mean	**142.61^{c}±20.79**	**40.04^{b}±0.992**	**0.49±0.015**	**2.04^{a}±0.098**	**1.30**
Nagami	1	10.21±0.15	49.67±2.875	0.26±0.150	1.33±0.140	0.07
	2	12.09±0.18	53.83±1.215	0.25±0.145	1.32±0.193	0.09
	3	11.53±1.14	55.45±3.024	0.29±0.140	1.89±0.227	0.12
	4	9.84±1.24	53.89±0.435	0.26±0.090	1.88±0.125	0.10
	mean	**10.92^{d}±1.34**	**53.21^{a}±1.178**	**0.26±0.051**	**1.61^{bc}±0.114**	**0.09**

A **significant difference** ($p<0.05$) between cultivar means is denoted by different letters in the same column.

relative density value for grapefruit peel essential oil extracted by CP was stated as 0.852-0.860 [35], for bitter orange as 0.840-0.860 [36], and for kumquat as 0.842 [37]. The relative density value for grapefruit essential oil isolated by CP was determined as 0.85 by Viudo-Martos et al. [38]. In parallel, density value of the peel oil of Citrus paradise isolated using the cold press technique was 0.8573 g/mL [39]. Similarly, relative density values of grapefruit peel oil isolated by hydrodistillation and microwave extraction were 0.856, 0.862, respectively [40]. While the relative density values of samples extracted by CP aligned with limit values, those derived from hydrodistillation were generally lower than literature values. This difference is thought to stem from various factors including cultivar, harvest time, climate, and cultural process differences, in addition to the oils' chemical composition.

The refractive index for the samples varied from 1.4721 to 1.4766. For HD-obtained oils, the refractive index values across cultivars are very narrow (1.4723 - 1.4731), suggesting that these properties are relatively similar among the cultivars. The values across the four harvest times are generally very close to each other, often within the average error range. This indicates that harvest time (maturation periods) alone does not have a large and consistent effect on the physical properties examined. The isolation method significantly affected the refractive index value of the volatile oil. The refractive index value of CP oil was higher than that obtained by HD. This is likely to be on account of different non-volatile chemical components such as waxes [41], carotenoids [42], and coumrains [43] in the sample instead of EO composition. Indeed, the oils produced by these two methods exhibit observable sensory differences, such as color. According to ISO standards, cold-pressed grapefruit peel oil has a refractive index limit value of 1.474-1.479 [35]. Vasek et al. [39] determined the refractive index value of grapefruit peel essential oil to be 1.4723. Conversely, Kamal et al. [44] determined the refractive index of grapefruit peel essential oil obtained by hydrodistillation as 1.4627-1.4635, depending on drying methods. In another study, the refractive index values of red grapefruit and oval kumquat peel essential oils obtained by hydrodistillation were determined to be 1.463 and 1.475, respectively [37]. The research findings revealed that, in this regard, the analyzed cultivars were generally consistent with standard data but showed partial differences from literature values. This may be due to differences in the characteristics of the cultivar studied in the literature and the region where it is grown.

Each pure essential oil has a characteristic range of optical rotation. This quality could be used as a parameter in the authentication of essential oils. The effect of cultivar (C), isolation method (IM) and C×IM interaction on optical rotation values of these citrus volatile oils was found to be statistically significant. Yerli Turunç (bitter orange) had the highest optical rotation value with respect to the cultivar. On the other hand, like other physical parameters, difference in optical rotation according to isolation method was more obvious than other factors. HD essential oils consistently show significantly higher optical rotation values than CP oils for all cultivars and harvest times. The reference range specified in the ISO standards for grapefruit peel essential oil varies between +91° and +96° values [38], and for bitter orange peel essential oil, it varies between +88° and +95° values [35]. Vasek et al. [39] determined the optical rotation value for grapefruit peel essential oil isolated by the cold press method as +90.25°. While all the essential oil obtained from grapefruit and bitter orange cultivars by CP was compatible with these limit values, the data determined for HD essential oils obtained by HD were higher than these limit values. It is thought that this difference may be due to chemical composition (carotenoids, coumarins, waxes etc.), except for the essential oil component. In fact, literature values have also shown that there may be differences in this sense.

The essential oil composition variation of Star Ruby, Marsh Seedless, Red Blush, Rio Red (grapefruit), Yerli

Table IV - ANOVA results for relative density, refractive index and optical rotaion values

	Relative density		Refractive index		Optical rotation	
	F value	p value	F value	p value	F value	p value
Cultivar (C)	1.51	0.2044	6.41	0.0001	2.42	0.0490
Harvest time (HT)	0.92	0.4405	1.35	0.2702	0.06	0.9825
Isolation method (IM)	1109.08	0.0001	2139.44	0.0001	211.02	0.0001
C×HT	0.50	0.9312	0.68	0.7876	0.86	0.6111
C×IM	1.93	0.1065	19.18	0.0001	1.02	0.4148
HT×IM	1.46	0.2365	1.24	0.3055	0.48	0.7008
C×HT×IM	1.35	0.2119	0.55	0.8993	0.49	0.9361
Coefficient of variation	0.1623		0.0235		1.5186	

Table V - Mean values for relative density, refractive index and optical rotation values of peel volatile oils by cultivar, harvesting time and isolation method (±standard error)

C	HT	Relative density		Refractive index		Optical rotation (°)	
		HD	CP	HD	CP	HD	CP
Star Ruby	1	0.8408±0.0005	0.8487±0.0012	1.4723±0.0001	1.4757±0.0001	96.45±0.250	93.68±0.275
	2	0.8410±0.0005	0.8488±0.0013	1.4721±0.0003	1.4758±0.0001	97.88±0.075	92.60±0.300
	3	0.8404±0.0022	0.8504±0.0005	1.4724±0.0002	1.4762±0.0001	99.13±0.325	93.38±0.275
	4	0.8403±0.0008	0.8502±0.0010	1.4724±0.0001	1.4760±0.0000	96.28±0.225	92.30±0.100
	mean	**0.8406±0.0005**	**0.8495±0.0005**	**1.4723±0.0001**	**1.4759±0.0001**	**97.43±0.447**	**92.99±0.231**
Marsh Seedless	1	0.8397±0.0004	0.8524±0.0004	1.4725±0.0002	1.4760±0.0004	97.28±0.175	91.58±0.480
	2	0.8398±0.0015	0.8500±0.0008	1.4723±0.0001	1.4762±0.0000	96.38±1.075	92.33±1.375
	3	0.8390±0.0005	0.8499±0.0004	1.4728±0.0005	1.4759±0.0004	95.88±1.875	92.73±0.980
	4	0.8398±0.0005	0.8507±0.0003	1.4721±0.0002	1.4761±0.0002	96.88±1.475	93.43±0.125
	mean	**0.8396±0.0003**	**0.8507±0.0004**	**1.4724±0.0001**	**1.4760±0.0001**	**96.60±0.534**	**92.52±0.418**
Red Blush	1	0.8395±0.0005	0.8507±0.0007	1.4722±0.0001	1.4759±0.0003	97.95±0.550	91.88±0.525
	2	0.8413±0.0005	0.8487±0.0007	1.4724±0.0002	1.4760±0.0002	97.53±0.625	91.58±1.425
	3	0.8399±0.0014	0.8482±0.0021	1.4724±0.0002	1.4756±0.0003	96.38±1.775	91.73±0.475
	4	0.8404±0.0008	0.8473±0.0008	1.4723±0.0000	1.4764±0.0002	96.75±1.150	92.26±0.460
	mean	**0.8403±0.0004**	**0.8487±0.000**	**1.4723±0.0001**	**1.4759±0.0001**	**97.15±0.489**	**91.86±0.327**
Rio Red	1	0.8399±0.0002	0.8484±0.0010	1.4723±0.0002	1.4762±0.0003	96.83±1.025	92.03±0.880
	2	0.8407±0.0007	0.8488±0.0015	1.4724±0.0001	1.4765±0.0003	97.05±1.050	91.85±0.250
	3	0.8398±0.0015	0.8487±0.0024	1.4725±0.0004	1.4765±0.0003	96.73±1.325	91.55±0.050
	4	0.8385±0.0007	0.8508±0.0005	1.4724±0.0001	1.4766±0.0002	96.55±0.550	92.33±0.075
	mean	**0.8397±0.0004**	**0.8491±0.0007**	**1.4724±0.0001**	**1.4764±0.0001**	**96.79±0.394**	**91.94±0.204**
Yerli Turunç	1	0.8396±0.0001	0.8493±0.0013	1.4723±0.0001	1.4751±0.0000	98.13±1.425	93.98±2.075
	2	0.8385±0.0002	0.8495±0.0013	1.4722±0.0003	1.4750±0.0003	98.05±0.250	94.18±1.775
	3	0.8407±0.0003	0.8476±0.0005	1.4723±0.0002	1.4749±0.0004	98.20±0.800	94.35±1.750
	4	0.8394±0.0001	0.8475±0.0013	1.4727±0.0003	1.4755±0.0007	95.83±0.225	93.83±1.325
	mean	**0.8397±0.0002**	**0.8485±0.0006**	**1.4724±0.0001**	**1.4751±0.0001**	**97.55±0.491**	**94.08±0.666**
Nagami	1	0.8399±0.0014	0.8507±0.0005	1.4727±0.0005	1.4753±0.0002	96.83±0.575	92.48±0.975
	2	0.8409±0.0002	0.8485±0.0010	1.4733±0.0003	1.4751±0.0002	95.48±1.875	93.45±0.850
	3	0.8401±0.0007	0.8482±0.0004	1.4733±0.0002	1.4750±0.0002	96.98±0.375	93.33±1.125
	4	0.8390±0.0012	0.8488±0.0003	1.4731±0.0001	1.4751±0.0004	98.53±0.275	94.50±2.100
	mean	**0.8400±0.0004**	**0.8490±0.0004**	**1.4731±0.0001**	**1.4751±0.0001**	**96.95±0.559**	**93.44±0.580**

For each factor, different letters in the same column indicate a significant difference between the mean values ($p<0.05$).

C: Cultivar, HT: Harvest Time, HD: Hydrodistillation, CP: Cold-pressed

Turunç (bitter orange) and Nagami (kumquat) cultivars for three harvest times and two isolation methods are shown in Table VI.

Partial differences were examined in the essential oil composition of the Star Ruby, Marsh Seedless, Red Blush and Rio Red cultivars based on harvesting time and isolation technique. The amount of limonene varied between 93.7% and 95.1% in these grapefruit cultivars. These data indicate that these cultivars are a valuable source of limonene. β-myrcene ranked second proportionally (1.9-2.0%), followed by sabinene (0.3-0.9%) and α-pinene (0.5-0.6%) for these grapefruit cultivars (Table VI). Sabinene is a significant monoterpene hydrocarbon that is especially present in grapefruit cultivars. Among the samples evaluated in the scope of the research, Rio Red peel oil had the highest number of identified components, with 19 components. Citronellal, (E)-α-farnesene, and β-sinensal were detected only in Rio Red among the grapefruit cultivars.

Table VI - Essential oil composition (%) of the citrus cultivars.

Component	RRI	SR	MS	RB	RR	YT	N
α-pinene	1030	0.5-0.6	0.5-0.6	0.5-0.6	0.5-0.6	0.5-0.6	0.4-0.5
β-pinene	1122	-	-	-	-	0.1-0.3	-
Sabinene	1132	0.5-0.8	0.4-0.7	0.4-0.6	0.3-0.9	0.1-0.2	0.1-0.1
β-myrcene	1170	1.9-2.0	2.0-2.0	1.9-2.0	1.9-2.0	1.9-2.0	1.8-2.0
Limonene	1214	94.4-95.0	94.1-94.8	94.3-95.0	93.7-95.1	95.4-95.9	95.4-96.0
β-phellandrene	1223	0.3-0.3	0.3-0.3	0.3-0.3	0.3-0.3	0.3-0.3	0.2-0.3
γ-terpinene	1260	0.2-0.3	0.2-0.3	0.1-0.3	0.1-0.3	0.1-0.2	-
Octanal	1304	0.3-0.4	0.3-0.4	0.3-0.4	0.3-0.4	0.1-0.1	-
Citronellal	1481	-	-	-	0.1-0.1	-	0.1-0.1
α-copaene	1491	udl-0.1	0.1-0.2	0.1-0.2	0.1-0.1	-	-
Decanal	1501	0.1-0.3	0.3-0.3	0.1-0.2	0.3-0.3	udl-0.1	-
Linalool	1549	0.1-0.2	0.1-0.1	0.1-0.1	0.1-0.1	0.1-0.4	-
1-Octanol	1557	0.1-0.1	udl-0.1	udl-0.1	udl-0.2	-	-
Linalyl acetate	1561	-	0.1-0.3	udl-0.1	-	udl-0.5	-
β- caryophyllene	1596	0.3-0.4	0.1-0.3	0.2-0.4	0.1-0.2	-	-
(*E*)-α-farnesene	1663	-	-	-	0.1-0.2	-	-
α-terpineol	1709	udl-0.1	0.1-0.3	udl-0.2	udl-0.1	udl-0.1	-
β-bisabolene	1746	udl-0.1	0.1-0.1	0.1-0.1	0.1-0.1	-	0.9-1.5
Geranial	1748	udl-0.1	udl-0.1	-	0.1-0.1	-	-
δ-cadinene	1751	0.1-0.1	0.1-0.2	0.1-0.2	0.1-0.1	-	-
Geranyl acetate	1767	-	-	-	-	0.1-0.1	0.2-0.3
Citronellol	1776	-	-	-	-	-	0.1-0.2
Nerolidol	2047	-	-	-	-	udl-0.1	-
β-sinensal	2221	-	-	-	udl-0.2	-	-
Unidentified		0.1-0.4	0.1-0.4	0.1-0.6	0.0-0.5	0.2-0.5	0.0

SR: Star Ruby, MS: Marsh Seedless, RB: Red Blush, RR: Rio Red, YT: Yerli Turunç, N: Nagami

RRI: Relative Retention Index, udl: under detection limit

Fifteen compounds were identified in Yerli Turunç (*Citrus aurantium*) essential oil from the peel, also known as bitter orange. The limonene content, which is the main component in bitter orange peel essential oils, varied between 95.4% and 95.9% based on the harvest time and isolation method. As can be understood from the findings, no significant variation occurred in this regard. The β-myrcene content showed a variation between 1.9% and 2.0%. Among the samples evaluated in the scope of the research, Nagami (kumquat) peel essential oil had the lowest number of identified components. In total, nine different components were identified. This indicates that kumquat peel oil has a simpler chemical profile compared to the other samples. In these samples, limonene was again detected as the main component, with its concentration ranging from 95.1% to 96.0%. As in many other samples analyzed, β-myrcene proportionally followed limonene in these samples. The third-ranked component, unlike in many other species and cultivars, was β-bisabolene, and its amount varied between 0.9% and 1.5%, depending on the harvest time and extraction method. Nagami also contains components like citronellol, which are absent in the others. Partial differences were also observed in the percentages of other identified components depending on the harvest time and essential oil extraction method.

The ANOVA and Duncan Test results for the ratios of β-myrcene and limonene, which are found at higher levels than other components in the peel volatile oil composition of the samples, are presented in Table VII and Table VIII. β-myrcene content is significantly influenced by both the harvest time and isolation method, and, crucially, the effect of harvest time varies depending on the specific cultivar (Table VII). The lowest β-myrcene content was detected in the Yerli Turunç (bitter orange) peel oil during the first harvest period, whereas, conversely, it was found in the Nagami (kumquat) during the fourth harvest period. Among the grapefruit cultivars, the lowest β-myrcene content was found in the fourth harvest period for all cultivars except for Marsh Seedless. When evaluating the β-myrcene content based on isolation methods, it will be observed that essential oils obtained via HD (hydrodistillation) are higher than those obtained via CP (cold pressing). While the cultivar and harvesting time had a significant effect on the content of the main component, limonene, the influence of the

isolation method and all interactions remained insignificant. The cultivars with the lowest limonene content were Rio Red (94.41%) and Marsh Seedless (94.42%). The average limonene content was determined to be 94.73% for Star Ruby and 94.76% for Red Blush. The limonene content of Yerli Turunç (bitter orange) and Nagami (kumquat) peel oils was higher compared to grapefruits. While not as strong as the cultivar effect, it implies that harvest time can influence limonene levels to some extent. The highest limonene content, based on average data, was observed in the second harvest period sample.

Miya et al. [31] studied the composition of three grapefruit peel volatile oils extracted by hydrodistillation. Their research findings revealed that the essential oil composition varied among the different cultivars. In all three cultivars, limonene was identified as the main component, distributed within a range of 86.70-89.90% in the samples. It was also found that myrcene, which was present in higher proportions compared to other components, varied between 2.43-3.57%. Kırbaşlar et al. [45] performed essential oil component analyses of fully maturated grapefruit (unknown cultivar, harvested in November) peel oils obtained by cold press. In the study, it was determined that the ratios of the main components limonene, myrcene and β-pinene were 92.5%, 2.6% and 0.6%, respectively. Ghani et al. [30] investigated the effect of harvesting time on the peel essential oil composition of grapefruit (Red Blush), obtained by hydrodistillation. Within the scope of the research, fruits were harvested at four different periods: September 26 (green stage), October 28, December 5, and February 3 (pinkish yellow stage). The study findings showed that the contents of limonene and myrcene varied between 90.92-93.82% and 1.22-1.88%, respectively, depending on the harvest time. Ou et al. [46] studied the effect of the isolation method on grapefruit peel volatile oil. Their research findings demonstrated that the isolation method significantly impacted the essential oil's composition. The essential oil obtained via hydrodistillation had limonene and myrcene contents of 96.06% and 2.06%, respectively. In contrast, the sample obtained by cold press contained 91.83% limonene and 3.06% myrcene, in the same order. Sutour et al. [47] studied on the composition of volatile oils obtained from five kumquat species by hydrodistillation methods. Limonene was detected as the main constituent in oval kumquat as 93.1%, followed by germacrene D (2.4%) and (1.4%) myrcene. Sicari and Poiana [33] obtained oval kumquat peel oil

Table VII - ANOVA results for β-myrcene and limonene

	β-myrcene		Limonene	
	F value	**p value**	**F value**	**p value**
Cultivar (C)	1.54	0.1490	25.93	0.0001
Harvest time (HT)	4.93	0.0046	2.89	0.0448
Isolation method (IM)	4.60	0.0371	0.66	0.4199
C×HT	4.01	0.0001	1.25	0.2731
C×IM	0.14	0.9833	0.85	0.5194
HT×IM	0.03	0.9927	0.36	0.7793
C×HT×IM	0.34	0.9875	0.28	0.9953
Coefficient of variation	1.9438		0.4599	

Table VIII - Mean percentages of β-myrcene and limonene contents (±SE)

	β-myrcene	**Limonene**
Star Ruby	1.95^{ab}±0.009	94.73^{b}±0.114
Marsh Seedless	1.97^{a}±0.005	94.42^{c}±0.104
Red Blush	1.96^{ab}±0.006	94.76^{b}±0.104
Rio Red	1.97^{a}±0.009	94.41^{c}±0.156
Yerli Turunç	1.94^{b}±0.007	95.62^{a}±0.0063
Nagami	1.96^{ab}±0.023	95.60^{a}±0.072
1^{th} HT	1.97^{a}±0.008	94.84^{b}±0.150
2^{nd} HT	1.96^{a}±0.006	95.11^{a}±0.095
3^{th} HT	1.97^{a}±0.009	94.97^{ab}±0.102
4^{th} HT	1.93^{b}±0.012	94.77^{b}±0.171
HD	1.97^{a}±0.006	94.96±0.105
CP	1.95^{b}±0.007	94.89±0.085

Different letters within the same column (for each factor) indicate a significant difference between means ($p<0.05$).

by hydrodistillation, solvent and supercritical carbon dioxide extraction methods. Limonene and β-myrcene were detected as the main components in the samples and it was determined that these components were distributed in the ranges of 96.08-96.91% and 1.63-1.77%, respectively. Another study by Peng et al. [48] studied the effect of distillation and cold press methods on the essential oil composition of kumquat peel. The study found that volatile oil isolated by steam distillation had limonene and myrcene content of 94.71% and 1.88%, respectively. In contrast, the sample obtained by cold press contained 95.06% limonene and 2.02% myrcene. The findings showed that these extraction methods showed similarities with regards to volatile oil composition. Farahmandfar et al. [32] also investigated the impact of drying treatments applied to the peel on the composition of bitter orange peel essential oil. Limonene was detected as the highest constituent in all citrus samples, followed by myrcene. The proportions of these components ranged from 81.19-88.7% and 3.07-3.81%, respectively. Bourgou et al. [49] also determined that there could be significant differences in bitter orange peel essential oil composition based on harvest time. In their study, limonene, the main component of the bitter orange peel volatile oil extracted by hydrodistillation, was determined to have a wide distribution, ranging from 67.90% to 90.95%. For the study, fruits were collected during three periods: pre-ripening (green), semi-ripe (yellow), and ripe (orange). The origin, climate, and extraction method are all key factors that can cause substantial variations in the compositions of volatile oils. This variability is also observed in oils extracted from variable cultivars of citrus fruits [50]. The limit values of limonene and β-myrcene in ISO standards were stated as 92-96% and 1.5-2.5%, respectively for CP grapefruit essential oil [35], and 93-95% and 1,5-3%, respectively for Mediterranean type CP bitter orange essential oil [36]. Evaluation of the limit data showed that the study's cultivars were incompatible with the established limit values.

4. CONCLUSIONS

As a result of the research, when the analysis findings obtained were evaluated, it was determined that the citrus peel essential oil ratio showed significant differences depending on the cultivar and C×HT interaction. The highest essential oil yield was found as 2.83% for bitter orange at the third harvest time. The cultivar contributes to variations in refractive index and optical rotation. The isolation method (HD vs. CP) has a consistent and significant impact on all three physicochemical properties. While CP oils have higher relative density and refractive index, HD oils have higher optical rotation. This is a crucial finding, indicating that the chosen isolation technique profoundly affects the physical characteristics of the volatile oil. Conversely, the cultivar, isolation method, and harvest time do not appear to significantly influence the relative density, refractive index, or optical rotation values. While the volatile oil composition showed slight variations based on cultivar, harvest time, and extraction method, these variations were generally statistically insignificant. For all samples analysed within the scope of the research, limonene was the dominant component, followed by □-myrcene at an average proportion of 2%. The research findings indicated that essential oils with high limonene content (93.7-96.0%) could be produced from these citrus cultivars across four different harvest periods using two different isolation methods. Cold press application is a widely preferred method in citrus peel essential oil on an industrial scale. Furthermore, evaluating the two isolation methods, particularly concerning limonene, a key functional component of citrus oils, revealed that producing these essential oils via hydrodistillation did not negatively affect their limonene content. On the other hand, the existing standards for citrus peel oils were established for essential oils obtained by the cold-press method. However, in non-integrated facilities, essential oil production is also carried out from citrus peels using the hydrodistillation method. Consequently, the study findings have revealed the necessity of creating an international standard for citrus peel oils obtained through hydrodistillation, especially considering their physical properties.

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MATERIALI A CONTATTO CON ALIMENTI

TEST e ANALISI

CONFORMITÀ BfR XXXVI Carta fibra vergine e riciclata e DGCCRF *MCDA n°4(V02-01/01/2019)*

- Determinazione della ***formaldeide*** in estratto acquoso - (UNI EN 1541:2002)
- Determinazione del contenuto di ***gliossale*** - (DIN 54603:2008)
- ***Imbiancanti ottici*** migrabili - (UNI EN 648:2019)
- Migrazione specifica della somma delle ***ammine aromatiche primarie*** (UNI EN 13130-1:2005+BVL LFGB §64 L 00.00-6:1995/Cor:2002)
- Determinazione e quantificazione degli ***ftalati*** - (metodo interno)
- ***Bisfenolo A*** - (UNI EN 17497:2020)
- Determinazione di ***diisopropilnaftalene*** (DIPN) mediante estrazione con solvente - (UNI EN 14719:2005)
- ***Cadmio***, ***piombo*** e ***alluminio*** in estratto acquoso - (UNI EN 12498:2019 + metodo interno)

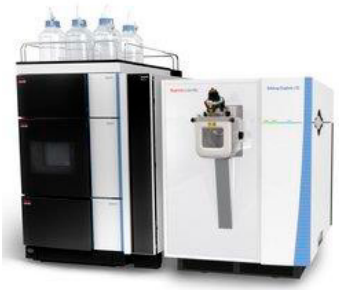
RICERCA e SVILUPPO

Sviluppo di ***nuove metodiche analitiche*** per la determinazione e quantificazione di contaminanti o molecole di interesse

ENTE ITALIANO DI NORMAZIONE · DG CCRF · BfR Bundesinstitut für Risikobewertung · cen EUROPEAN COMMITTEE FOR STANDARDIZATION

NIAS *Non Intentionally Added Substances*

"impurità presente nelle sostanze utilizzate, intermedio di reazione formatosi durante il processo produttivo o prodotto di reazione o di decomposizione"
Reg. UE N. 10/2011 Considerando 18-20, articolo 3



VALUTAZIONE

della conformità ai requisiti riportati nell'**articolo 3 del Regolamento CE N. 1935/2004** sui materiali e gli oggetti destinati a venire a contatto con gli alimenti (**MOCA**)

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Riferimenti:
PIERANGELA ROVELLINI
Responsabile Team Chimica e Sicurezza alimentare
pierangela.rovellini@mi.camcom.it

Removal Of Chemical Oxygen Demand (COD) From Olive Oil Mill Wastewater Using Activated Carbon

Kamelia Ziane[1]
Soumia Djeziri[1]
Zoubida Taleb[1] ✉
Salima Choukchou Braham[1]
Hadja Mebarka Djellouli[1]

[1] Laboratory of Materials & Catalysis
Faculty of Exact Sciences
Djillali Liabes University
Sidi Bel Abbes, Algeria.

The objective of this study is the production of an economical (inexpensive) activated carbon based on agro-food waste from the olive industry (olive pits). This activated carbon was applied in the treatment of olive oil mill wastewater (vegetation water) in order to reduce its high chemical oxygen demand (COD) and thus limit its harmful effects on the environment. The AC was prepared by the valorization of agro-food waste in the form of olive pits (calcination followed by chemical activation using phosphoric acid chosen from among other agents such as sodium hydroxide, potassium chloride, and zinc chloride, but these gave less yield.). A range of analyses was carried out for the characterization of the used adsorbent, including: iodine value, moisture content and ash content. The effect of several parameters such as contact time, amount of adsorbent and pH of the solution was studied. The best adsorption conditions are simple to achieve: ambient temperature and direct use of the olive oil mill wastewater (V = 100 mL, pH 2.05) with 3 g mass of AC adsorbent. The adsorption experiments demonstrated a significant reduction in chemical oxygen demand (COD), confirming the efficiency of the prepared activated carbon.

Keywords: Activated Carbon, COD, Olive Pits, Kinetic Adsorption, Olive Oil Mill Wastewater.

✉ Laboratory of Materials & Catalysis
Faculty of Exact Sciences
Djillali Liabes University
BP 89, Sidi Bel Abbes, Algeria
mail: zoubidataleb@yahoo.fr
phone: 00213 561694080

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1. INTRODUCTION

The olive oil industry, in addition to its main production, which is virgin olive oil, leaves two by-products: both liquid (olive oil mill wastewater OMW: margins) and solid (pomace and leaves) [1,2]. These effluents have so far had little economic value in Algeria. Liquid waste (margins) poses serious environmental problems [3]. It is too loaded with organic matter, minerals and polyphenols. It is often discharged into sewers, stored in evaporation ponds or spread directly on the ground without any prior treatment. This results in a negative impact on the environment, leading to soil clogging, pollution of surface and ground water, and the release of nauseating odors. In addition, the presence of polyphenols, responsible for phytotoxic and antimicrobial effects, makes biological treatment ineffective [4].
Current research has shown that activated carbon derived from olive pits (and other olive waste) offers superior adsorption efficiency and lower costs compared to commercial activated carbon, thanks to the use of abundant waste from the olive industry and the absence of expensive conventional precursors [5,6].
On the other hand, olive pits and other olive-growing residues (pomace, mill solids, stones) are widely recognized as excellent, cost-effective precursors for activated carbons used to remove phenolic compounds and dyes from wastewater from olive mills and other effluents [7,8].
In addition, various processes for treating vegetable waters have been developed: physical, chemical, and even biological processes and current trends should aim

at integrating various technologies to treat vegetable waters at low cost. The different types of vegetable water treatment studied so far only solve part of the problem. Indeed, most of the proposed processes remain insufficient and incomplete, or even limited.
Activated carbon absorption is among the processes used for the successful treatment of margins. The transformation of olive pits into activated carbon is an opportunity to produce inexpensive adsorbents using local renewable resources [9].
This study aims to prepare from olive pits an activated carbon that is chemically activated using phosphoric acid. Some adsorption parameters were studied such as time contact, the used mass of AC and the initial pH of the solution.

2. EXPERIMENTAL PART

2.1. SAMPLING

The sampling of olive oil mill wastewater and olive pits was carried out in the region of Sidi Bel Abbes (West Algeria) from an oil mill extraction system by centrifugation in three phases during the olive campaign 2021-2022. The olive oil mill wastewater taken from the storage tank was homogenized and transported in 5-liter bottles. These bottles were stored away from light at 4°C for later use. This temperature is critical for preserving the characteristics of the wastewater from the olive oil mill [10-12].

2.2. DETERMINATION OF THE CHEMICAL OXYGEN DEMAND (COD)

COD was determined using potassium dichromate. The principle basis of this method is boiling oxidation (150 °C for 2 h) of the reducing materials in an acid medium (H_2SO_4) with an excess of potassium dichromate in the presence of silver sulfate as a catalyst and mercury sulfate as a complex agent of chlorides. The optical density of the sample was obtained by spectrophotometry at a wavelength of 620 nm.

2.3. SAMPLING ACTIVATED CARBON PREPARATION FROM OLIVE PITS

Firstly, the olive pits were washed with distilled water several times, dried, crushed and sieved. The dried sample was carbonized at a temperature of 700°C for 1 hour. The activation was carried out chemically using H_3PO_4 acid. This agent has been widely used for activation of carbons [13]. Next, the sample was immersed in a volume of the acid under agitation. The liquid was then separated by simple filtration and dried at 105°C for 24 h. After drying, the activated carbon was washed again with distilled water until reaching a pH of 6 in the residual water. Finally, the activated carbon was put in the oven at 110°C for 24 hours and packed in ready-to-use boxes. This is a standard protocol, widely cited in research on activated charcoal and olive-based precursors, applied to olive pits/residues and other lignocellulosic waste [14-16].

2.4. CHARACTERIZATION OF PREPARED ACTIVATED CARBON

2.4.1 Moisture content

The moisture content represents the amount of water physically not bound to the activated carbon. Moisture is a ratio expressed as a percentage; it is determined by drying the adsorbent in an oven at 105°C until its weight remains constant. It is calculated using Equation 1 [17]:

$$H\% = \frac{m_0 - m_1}{m_0} \times 100 \qquad \text{(Equation 1)}$$

Where *H%* is moisture content (%) and m_0 and m_1 (g) are the mass of the AC adsorbent before and after drying, respectively.

2.4.2 Ash content

This is the inorganic, inert, amorphous and unusable part present in the material [17,18]. 1g of adsorbent was placed in a crucible. This crucible was moved to the oven and heated for one hour up to 1000°C. After cooling, the crucible was weighed. The ash content is calculated using Equation 2, as follows:

$$\%Ash = \frac{p_0 - p_1}{p_0} \times 100 \qquad \text{(Equation 2)}$$

Where *%Ash* is the percentage of ash rate content (%) and P_0 and P_1 (g) are the weight of the filled crucible before and after carbonization, respectively.

2.4.3 Iodine Value

The iodine index (iodine number) is a microspore content measure of an adsorbent material. The iodine number is an indicator of the porosity of an activated carbon [18,19] [8,9].
The iodine adsorption capacity of AC material was determined according to the following procedure: 10 ml of a 0.01 N iodine solution was titrated with a 0.1 N sodium thiosulfate solution, with a few drops of a starch solution as an indicator, until the color disappeared. In the second step, 0.05 g of activated carbon was added to 15 mL of a 0.1 N iodine solution with 5 min of stirring. Then, the obtained iodine solution was filtered and titrated with 0.1 N sodium thiosulfate solution with two drops of starch solution. The iodine value (*Id*) was calculated using Equation 3, as follows:

$$Id = \frac{(V_b - V_s) \cdot N \cdot 126.9 \cdot 15/10}{M} \qquad \text{(Equation 3)}$$

Where (V_b -V_S) in mL is the difference between the required volumes of sodium thiosulfate for the blank (V_b) and the sample (V_s), *N* is the normality of the sodium thiosulfate solution in (mol/L), *126.9* g/mol is the

atomic mass of iodine and *M* (g) is the mass of the AC adsorbent.

2.5. THE POINT OF ZERO CHARGE (PH_{PZC})

The point of zero charge (pH_{PZC}), the pH at which the adsorbent is neutral in aqueous suspension, was determined following the procedure of Altenor et al. [20]. In this method, 50 mL of 0.01M NaCl solution was placed in closed erlenmeyer flasks at room temperature. Then, the pH of the solutions was modified to values from 2 and 12 by adding 0.1M HCl or 0.1M NaOH solutions. These pHs were mentioned as the initial pH ($pH_{initial}$). Finally, 0.1g of solid adsorbent (AC) was added to each flask and the final pH (pH_{final}) was measured after 24 h. The pH_{final} was plotted against the $pH_{initial}$ and the pH_{PZC} is the point where the curve pHfinal versus pHinitial intersects the first bisector.

2.6. ADSORPTION EXPERIMENTAL PROCEDURE

Adsorption experiments were carried out by varying the time of the solution, adsorbent mass and pH of the solution at ambient temperature by magnetic agitation. For each experiment, 100 mL of OMW was treated using the prepared activated carbon. After stirring, the solution was filtered and the filtrate was analyzed to obtain the residual chemical oxygen demand (COD). The removal yield (% Y) was calculated using the following expressions:

$$Y(\%) = \frac{COD_0 - COD_e}{COD_0} \times 100 \qquad \text{(Equation 4)}$$

Where COD_0 is the initial chemical oxygen demand (134.3 gO_2/L) and COD_e (gO_2/L) is the equilibrium chemical oxygen demand [21-23].

3. RESULTS AND DISCUSSION

3.1. CHARACTERIZATION OF ADSORBENT

The results of the moisture content, ash content and iodine value of the prepared activated carbon are shown in Table 1. The low ash content (0.05%) implies that the activated carbon essentially consists of organic matter, therefore of the carbon element [24]. The prepared activated carbon also has a low moisture content (0.0022%), which highlights a low water content conservation. For the iodine value, the prepared activated carbon gives a high iodine value 959.33 mg/g that clearly illustrates the microporosity of an activated carbon.

Table I - Activated Carbon Properties

Characteristics	Value
Moisture content	0.05 %
Ash content	0.0022%
Iodine value	959.33 mg/g

3.2. ADSORPTION STUDIES

3.2.1 Effect of contact time

This study was carried out in order to determine the fixed quantities of the pollutant since its contact until a time t, in order to know the equilibrium time. For this, a solution for 100mL, with an initial chemical oxygen demand (134.3 gO_2/L) of OMW, was put in contact with a mass of activated carbon (1 g) at the pH of the solution (pH 4.5). The mixture obtained was immediately agitated at different contact times varying from 60 to 300 minutes.
Figure 1 shows the time effect of adsorption on activated carbon. The adsorption yields (%) of COD from olive oil mill wastewater increase exponentially with time, reaching their maximum value after 120 minutes. Beyond that comes the state of adsorption equilibrium. It thus becomes clear that activated carbon has an adsorption capacity (28.66%) of the initial COD of OMW.

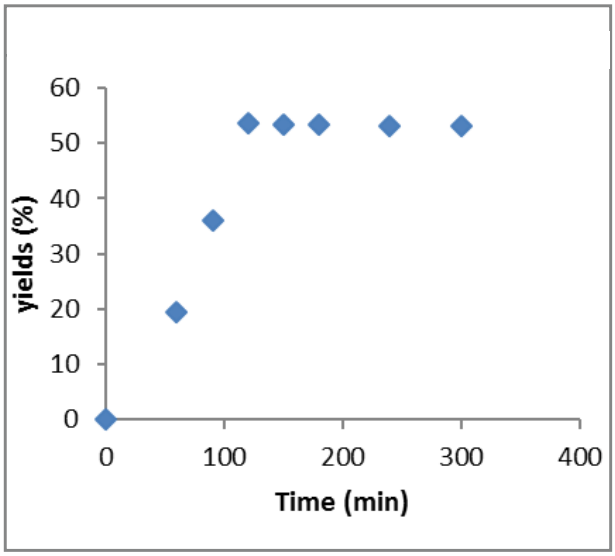

Figure 1 – Effect of time on adsorption

3.2.2 Effect of AC mass on adsorption

In this set of experiments, different masses of the AC adsorbent chosen from 0.5 to 5 g were treated with 100 mL solution of initial COD of OMW at the optimum time obtained from the effect of contact time experiments (120 minutes). Figure 2 shows the percentage of adsorption as a function of the AC adsorbent mass. In all cases, the percentage yields of this pollutant removal increases when the mass of the AC adsorbent increases until total saturation. This is due to the presence of large numbers of active sites on the surface [25]. The curve in Figure 2 shows that 3 g of carbon mass can adsorbate a maximum of COD (53.66%) from OMW. In the next step, the optimal AC mass of 3 g will be taken to study the pH effect on adsorption from OMW.

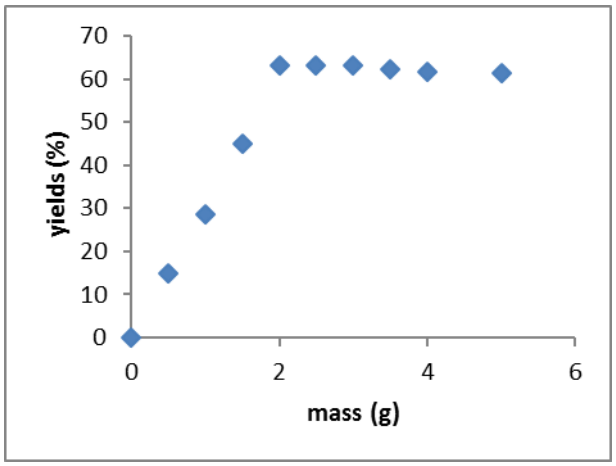

Figure 2 – AC mass effect on adsorption

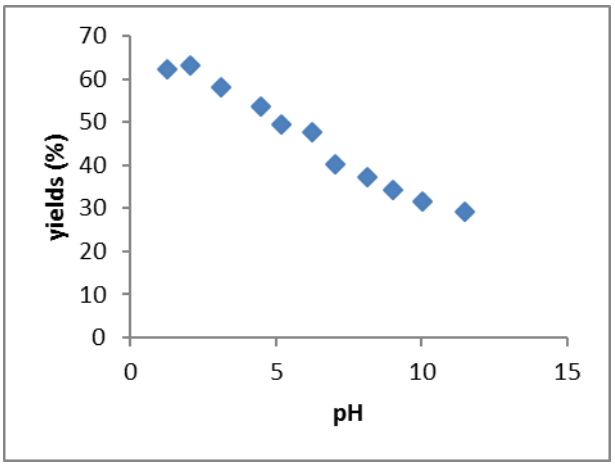

Figure 3 – Effect of pH on adsorption

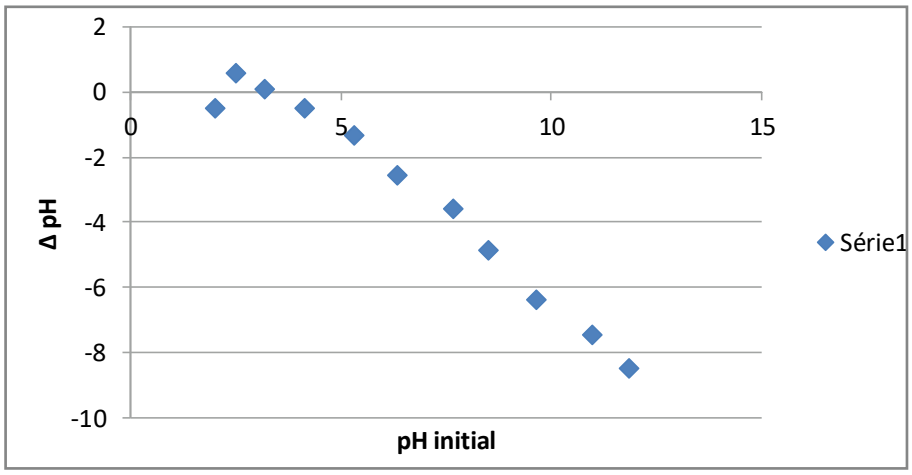

Figure 4 – The pH point of zero charge (pHpzc)

3.2.3 Effect of pH on adsorption

The pH of the solution has a very significant role in the adsorption process [26]. The pH of the OMW solution was chosen between 1.25 and 11.5 and a contact time was fixed from previous results at 120 min. The obtained results (Figure 3) show a considerable influence of the pH value on the adsorption by the activated carbon: the adsorption yields decrease with the increase of the pH values. The lower adsorption yields in an alkaline medium can be explained by an excess of OH ions in the solution, which leads to competition with this effluent on the adsorption sites of the activated carbon. Indeed, the high concentration and high mobility of OH ions would promote their adsorption relative to the pollutant [27]. The optimum pH allowing maximum adsorption of OMW on activated carbon (Y% = 63.33) is pH 2.05 under the study conditions.

3.2.4 The point of zero charge (pHpzc)

The pH_{pzc} is an important parameter based on determining the pH sensitivity range and allows for the prediction of the active surface area and adsorption capacities [28]. The pHpzc value of AC found was 3.2 (Figure 4).

This pH shows that at pH values below this value, the activated carbon surface is positively charged, which promotes attractive electrostatic interactions and improves adsorption. This result confirms the best adsorption yield (Y% = 63.33) obtained at pH 2.05 during the previous session (session 3.2.3).

4. CONCLUSIONS

The discharge of olive oil mill wastewater from olive oil-producing industries is a major problem, especially for countries in the Mediterranean basin. They contain a large organic fraction and cause several types of pollution.

In this study, activated carbon was successfully used for the elimination of the organic fraction from olive oil mill wastewater. The activated carbon was prepared from olive pits using carbonization and a chemical activation process. The material was characterized using different techniques: ash content, moisture content and iodine value. Under the operating conditions, the results of adsorption on activated carbon show that the prepared AC can adsorb close to 63.33% of

pollutant using 3 g and 100 mL volume of solution at 120 min and pH 2.05.
The obtained results indicate the possibility of valorization of olive pits in the form of activated carbon, which could have significant socio-economic impacts.

Acknowledgment
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Short note

Determination of Mineral Oil Aromatic Hydrocarbons (MOAH) in vegetable oils and fats - Comparison between RP-HPLC- Fluo and LC-GC-FID method

Antonio Perna
Pierangela Rovellini ✉

Innovhub - Stazioni Sperimentali per l'Industria
Via Giuseppe Colombo 79
Milano, Italy

This study provides an update on the application of the RP-HPLC-FLUO method for MOAH determination, following the previously published work on its validation and applicability [1]. A total of 161 edible oil samples from 10 different matrices were analysed in 2024 and compared with LC-GC-FID results [2]. The methods showed good alignment, particularly in sunflower oils, while grapeseed and rice oils confirmed higher contamination levels. Five additional samples from interlaboratory circuits validated the method's accuracy. RP-HPLC-FLUO is suggested for use as a valuable semi-quantitative tool for pre-screening analysis, suitable for routine monitoring and high-throughput control strategies.

Keywords: MOAH, HPLC-FLUO, Contaminants, LC-GC-Flm

1. INTRODUCTION

Mineral oil aromatic hydrocarbons (MOAH) are a class of complex aromatic compounds that originate from petroleum refining. MOAH have known concerns due to their potential genotoxic and carcinogenic effects, especially those containing multiple aromatic rings. Their presence in food matrices, particularly edible oils, has drawn increasing regulatory and scientific attention due to possible contamination during processing, storage, and packaging. While the reference-standard method for MOAH determination - on-line LC-GC-FID as per EN ISO 16995 and its subsequent revision method UNI EN ISO 20122:2024 [2] - offers high sensitivity and specificity, it remains analytically demanding, requiring dedicated instrumentation, advanced purification steps, and significant operational costs. The JRC technical report [3] points out that the analytical method used must be sufficiently sensible to ensure that food is not contaminated with potentially carcinogenic MOAH and that any other technique is acceptable only if it provides equivalent results to LC-GC-FID. In response to these challenges, an alternative method using reverse phase high-performance liquid chromatography coupled with fluorescence detection (RP-HPLC-FLUO) was recently proposed and validated as a rapid, cost-effective, and accessible screening technique for routine MOAH monitoring [1]. This method leverages the intrinsic fluorescent properties of aromatic compounds to offer semi-quantitative estimation with sufficient sensitivity and repeatability for preliminary risk assessment, as validated through international proficiency testing and inter-laboratory comparisons.
The present work provides an extensive update on the application of the HPLC-FLUO method to a large and diversified sample set (n=161) collected during 2024. A total of 10 distinct matrices were considered, including crude and refined oils from sunflower, grapeseed, rice, and other seeds. For each sample, MOAH levels were determined using both HPLC-FLUO and LC-GC-FID, allowing direct method comparison.

✉ CORRESPONDING AUTHOR
Pierangela Rovellini
e-mail: pierangela.rovellini@mi.camcom.it
Tel:+39 02 8515 3571

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The results demonstrate that HPLC-FLUO yields MOAH quantifications in close alignment with those from the reference LC-GC-FID method across most matrices, particularly in sunflower seed oil, which represented approximately half of the dataset. Notably, grapeseed and rice oils consistently showed the highest MOAH levels, corroborating findings from previous studies and highlighting these matrices as particularly prone to contamination. Some matrix-dependent deviations were observed, reflecting differences in chemical complexity and possible interferences, without compromising the screening utility of HPLC-FLUO.
Furthermore, this study evidenced a consistent alignment between the results obtained by HPLC-Fluo and those achieved by LC-GC-FID, as corroborated through their comparison across multiple proficiency tests.
Overall, this comparative study reaffirms the applicability of the HPLC-FLUO method as a reliable semi-quantitative tool for MOAH pre-assessment, supporting its integration into routine monitoring frameworks where rapid and broad-scale evaluation is required.

2. MATERIALS AND METHODS

2.1. CHEMICALS AND STANDARDS

All solvents used were of HPLC grade. The standard solution for external calibration included 4-ethyl-toluene CAS 99 620-14-4 purchased form Sigma Aldrich (Merck Life Science Milan-Italy) (for monoaromatics, Channel 1) and Chiron AS (Stiklestadvn, 1 N-7041 Trondheim Norway) standard mix (20 MOAH compounds, for polyaromatics, Channel 2). Naphthalene CAS 91-20-3, pyrene CAS 129-000, and coronene CAS 191-07-1 were used as retention time markers for C-fraction identification.

2.2. SAMPLE PREPARATION

Samples of well-homogenized vegetable oil or fats (0.5 g) were dissolved in isopropyl alcohol (5 ml) and mixed using vortex mixing and an ultrasonic bath when necessary. The solutions were filtered through a 0.45 µm nylon membrane into HPLC vials. Solid or highly viscous fats were preheated to ensure complete dissolution, followed by refrigerated clarification at 4°C for 5 hours before filtration.

2.3. HPLC-FLUO ANALYSIS

Analyses were performed on a Shimadzu HPLC system equipped with a C18 reverse-phase column (ReproSil 80 ODS-2, 250 mm × 4.0 mm, 3 µm). A gradient elution was used (from 60:40 to 2:98 water/acetonitrile over 50 min, held for 40 min). The flow rate was 0.4 mL/min, and injection volume was 20 µL.
Fluorescence detection was performed using dual channels:

- Channel 1: λ_{ex} = 254 nm / λ_{em} = 280 nm (monoaromatics)
- Channel 2: λ_{ex} = 254 nm / λ_{em} = 430 nm (polyaromatics)

2.4. QUANTIFICATION AND CALIBRATION

Quantification was based on external calibration using 4-ethyl-toluene and Chiron standards, with results expressed in mg/kg. C-fractions were assigned based on the retention times of naphthalene, pyrene, and coronene, though not fully aligned with JRC GC standards, as in this case the range is real for the MOAH fraction and not related to the MOSH external standard.

2.5. VALIDATION

The method was previously validated for linearity, LOQ (0.5 mg/kg), recovery (83-106%), and repeatability (CV% ~1.4%). Participation in international proficiency tests supported its accuracy and robustness [1].

3. RESULT AND DISCUSSION

A comparative analysis of MOAH quantification using HPLC-FLUO and LC-GC-FID across various edible oil matrices reveals a generally good alignment between the two methods, with matrix-dependent variations. Tables I, II, III, IV, V, VI, VII, VIII, IX, X, XI report the comparison data obtained. The complete Tables dataset including sample identification codes, matrices and analytical results is available at:
https://docs.google.com/spreadsheets/d/1OHcx8oeQThcvF7J8rpzh8UQtVZ5hI9ty/edit?usp=sharing&ouid=102913661671723803508&rtpof=true&sd=true.
For most matrices, HPLC-FLUO demonstrated satisfactory consistency with LC-GC-FID, supporting its suitability as a semi-quantitative pre-screening tool. However, discrepancies in absolute values were observed for certain matrices, likely due to differences in matrix complexity and analytical selectivity. In sunflower seed oil, which constituted the largest subset of the dataset, the two methods showed excellent agreement, confirming the robustness of HPLC-FLUO in routine monitoring of MOAH in this matrix. Conversely, grapeseed oils (both raw and refined) exhibited higher MOAH levels across both methods, with LC-GC-FID occasionally detecting slightly lower concentrations. This can be attributed to multiple contamination factors along the production chain. Firstly, grape seeds, as a by-product of winemaking, may be exposed to various sources of mineral oil contamination, including lubricants and processing aids used in harvesting and pressing.
Secondly, the extraction process - often solvent-based (e.g., using hexane) - can lead to co-extraction of aromatic hydrocarbons, especially when industrial equipment is not thoroughly maintained or cleaned.
While refining is intended to remove impurities, it may not be fully effective in eliminating MOAH, particularly when these compounds are strongly retained in the lipid matrix or are transformed during high-temperature steps such as deodorization.

Finally, contamination can also occur during storage or transport, particularly in non-dedicated or improperly cleaned containers.
These findings highlight the relevance of continuous monitoring and the importance of implementing strict controls throughout the entire production chain to minimize MOAH contamination, as well as the importance of having easy pre-screening methods available. Rice oils (crude and refined) were among the most contaminated matrices, with both techniques showing consistent trends but differing in absolute quantification. These variations could stem from matrix interferences or differential recoveries during sample preparation. This may be due to the complex and intensive processing steps required for rice bran oil production. Rice bran, in particular the raw material, is particularly prone to rapid lipid oxidation and enzymatic degradation, necessitating immediate stabilization, often involving thermal treatment or the addition of processing aids, which can be potential sources of contamination.
Moreover, solvent extraction is commonly used to maximize oil yield, increasing the risk of co-extracting mineral oil residues from equipment or solvents themselves. In general, attention must be focused on searching for solvents that are free from MOAH contaminants for use in plants.
The refining process, although effective in reducing some contaminants, may not fully eliminate aromatic hydrocarbons, particularly if they are embedded in the bran-derived matrix or introduced during high-temperature treatments.
Additional contamination may occur during storage or bulk transport if proper handling practices are not rigorously applied.
In maize seed oil and olive oils, the results were broadly comparable, though HPLC-FLUO tended to slightly underestimate MOAH compared to LC-GC-FID. Extra virgin olive oils, despite their lower expected MOAH content, showed a more pronounced discrepancy, which may be attributed to the presence of natural polyphenols that interfere with fluorescence detection. Overall, while LC-GC-FID remains the reference method for accurate quantification of MOAH, HPLC-FLUO offers a reliable and efficient alternative for pre-screening purposes, especially in high-throughput settings.
The results obtained using the RP-HPLC-FLUO technique were also found to be well-aligned with expected or reference values from the organising bodies, confirming the method's robustness and accuracy under external validation conditions (Table XII).
This alignment not only supports the method's reliability across different oil matrices but also emphasizes its reproducibility and suitability for use in collaborative settings. Despite being a semi-quantitative approach, HPLC-FLUO consistently delivered MOAH values within acceptable ranges, reinforcing its role as an effective pre-screening tool compliant with current analytical performance requirements.
Such findings provide additional confidence in the method's application for routine quality control and early risk assessment, particularly when rapid throughput and operational simplicity are essential.

4. CONCLUSIONS

The comparison between RP-HPLC-FLUO and the reference LC-GC-FID method across a wide range of vegetable oil matrices confirms the validity of the fluorometric approach as a reliable and accessible screening tool for MOAH detection. The good correlation observed between the two techniques - both in routine samples and in interlaboratory proficiency test materials - highlights the robustness, reproducibility, and practical applicability of the HPLC-FLUO method, particularly in matrices such as sunflower, grapeseed, and rice oils.
While LC-GC-FID remains the gold standard for definitive quantification, RP-HPLC-FLUO offers significant advantages in terms of simplicity, speed, and cost-effectiveness, making it ideal for high-throughput environments or for preliminary risk assessment where rapid decision-making is critical. Its ability to detect a broad range of MOAH compounds, combined with minimal sample preparation and the use of standard laboratory instrumentation, further supports its integration into monitoring programs, especially in industrial and regulatory contexts where early detection and screening are essential.
Looking ahead, future developments may focus on refining the method's selectivity to reduce matrix interferences and on expanding its application to more complex food and environmental matrices.
Overall, this study supports the continued adoption of RP-HPLC-FLUO as a valuable component of a tiered analytical strategy for MOAH surveillance.

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[3] JRC Technical Reports 2023 Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials-2nd Edition in the frame of Commission Reccomendation (EU) 2017/84 S. Bratinova, P. Robouch, E. Hoekstra.

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STAZIONI SPERIMENTALI
PER L'INDUSTRIA

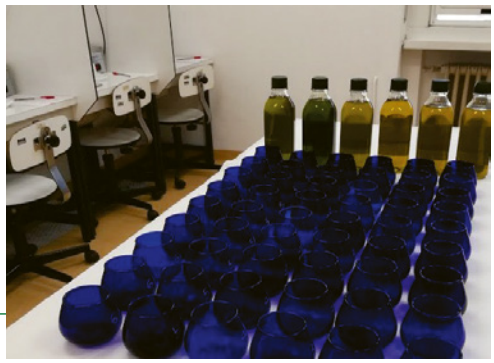
innovazione e ricerca

Virgin Olive Oil Organoleptic Assessment

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 fruitness 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 organoleptic 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
Team Chemistry, Technology and Food Safety

ANNUNCI DI RICERCA PARTNER

per progetti di ricerca e trasferimento tecnologico

Enterprise Europe Network (EEN)

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Anno 2026
(aggiornato al 30 giugno)

A Spanish company specializing in the production of bioactive natural extracts from olive by-products for antibiotic-free animal feed
Ref. BOES20240723009
The Spanish company, which specializes in producing bioactive natural extracts from olive trees, applies protocols for various animal species—poultry, pigs, ruminants, and fish—that enable the elimination of antibiotics.
The company is seeking a Nordic partner (Denmark, Finland, Iceland, Norway, Sweden) interested in establishing a cooperation agreement based on distribution services or a commercial agency agreement.
Deadline: 29 July 2026

Ukrainian enterprise offers deoiled sunflower lecithin powder
Ref. BOUA20251021015
Ukrainian producer offers pure deoiled sunflower lecitin powder (food additive E-322) with the content of phospholipids min.97%. Product of food and dietary grade is available. In food industry lecitin is used as an emulsifier and antioxidant. Lecitin as a diet additive is used in food rations of people as an additional source of phospholipids. Producer is looking for long-term sales partners under distribution services agreement. Manufacturing under private label for retail chains is possible
Deadline: 30 October 2026

A Ukrainian producer of equipment for extraction and processing of vegetable oils
Ref. BOUA20251104004
The Ukrainian manufacturing company specializes in the manufacture of equipment for the oil-and-fat industry. The company seeks for foreign partners (preferably from the EU) to establish long-term partnerships and implement its export potential. Cooperation is possible under the subcontracting and manufacturing agreement.
Deadline: 4 November 2026

An Albanian producer of premium CO_2 botanical extracts seeks expansion into EU and global markets
Ref. BOAL20260202011
The Company combines advanced CO_2 extraction technology, microencapsulation, and natural formulation expertise to deliver pure, efficient, and sustainable botanical ingredients tailored for premium international markets.
The Company seeks to establish long-term commercial partnerships, distribution agreements, and strategic collaborations with companies looking for reliable, innovative, and traceable natural ingredients
Deadline: 2 February 2027

Turkish Manufacturer of Olive Processing Machinery based on Balıkesir Seeking International Partners
Ref. BOTR20260205062
A Turkey-based manufacturer specialized in olive processing machinery is seeking international partners for commercial cooperation, distribution, and technical collaboration within the olive oil and agri-food machinery sector.
Deadline: 5 February
For further information and to get in contact with the owners of the profiles, please send an e-mail to simpler@mi.camcom.it specifying the project reference code of interest.

Portuguese company of organic essential oil and vegetable oil offers raw materials
Ref. BOPT20260218014
A PT company with production sites in Angola and Portugal specialized in the supply of organic essential and vegetable oils, high-quality raw materials tailored to the cosmetics, personal care, perfumery, detergents and pharmaceutical applications is seeking for B2B partners to establish long-term commercial partnerships, appointing agents or distributors, as well as supplier agreement. Leveraging a strategic warehouse in PT, it ensures efficient distribution within the European market.
Deadline: 18 February 2027

Fermentation-derived bioactive lipid ingredients from renewable side-streams for cosmetic formulations
Ref. BOEE20260313005
ÄIO is an Estonian biotechnology company developing sustainable, fermentation-derived lipids from renewable sidestreams for skincare and body care applications. Its ingredients include RedOil, a carotenoid-rich oil positioned for hydration and skin renewal benefits, and ZymaLipid Complex, a purified fatty-acid blend supporting emollience, barrier-feel, and formulation stability.
Deadline: 13 March 2027

High-phenolic dietary and health supplements from Greece offered for commercial partnerships
Ref. BOGR20260519002
This small family-owned company specialises in producing extra virgin olive oil with exceptionally high polyphenol content, certified under EU Health Claim Regulation 432/2012. The product targets health-conscious consumers with specific dietary requirements. The client is interested in concluding trade agreements with experienced importer-distributors and commercial agents worldwide, with special focus on India, Japan, Singapore, and UK.
Deadline: 19 May 2027

Industrial partners to jointly develop innovative plant-based dairy alternatives based on a novel structured lipid ingredient technology are sought for an EIC Transition project
Ref. RDRIT20260422020
An innovative spin-off company has developed a patent-pending technology that transforms vegetable oils into self-structuring oleogels, creating clean-label food fats without additives. The company is looking for industrial partners from the food sector.
Deadline: 25 May 2027

Seeking expertise in supercritical CO_2 extraction to isolate bioactive compounds in propolis
Ref. TRFR20260520018
A French company specialising in bee products is looking for a partner with expertise in supercritical CO2 extraction to develop a process aimed at isolating the bioactive compounds in propolis - particularly polyphenols and flavonoids - while reducing contaminants such as pesticides and polycyclic aromatic hydrocarbons (PAHs). Commercial agreement with technical cooperation or R&D partnership is sought. The goal is to obtain purer propolis extracts to be used as as ingredients for nutraceutics.
Deadline: 26 May 2027

Seeking industrial confectionery manufacturing partner for thermostable premium chocolate technology
Ref. BRPL20260528006
Company developing trans-fat-free structured lipid systems for thermostable confectionery fats enabling chocolate-like sensory performance at up to 40°C. The technology preserves snap, creaminess and controlled melt for confectionery, coatings, bakery fillings and snacks.
Deadline: 28 May 2027

Seeking confectionery and bakery partners to co-develop heat-resistant premium chocolate products
Ref. BRPL20260528007
Develops next-generation structured lipid systems for confectionery and bakery applications, including a thermostable fat platform that maintains chocolate-like sensory quality up to 40°C. Solutions improve heat resistance, prevent bloom and deformation, and reduce cold-chain dependency. Seeking partners for co-development and commercialization in confectionery, bakery, snacks and nutrition bars.
Deadline: 28 May 2027

Seeking strategic investment and industrial partnerships for structured lipid technology in heat-resistant confectionery
Ref. BRPL20260528008
Food tech company developing patent-pending thermostable structured lipid systems for premium confectionery applications. Its trans-fat-free fat technology enables chocolate-like performance with heat stability up to 40°C, reducing reliance on cold chain and improving product durability and quality. The company is seeking strategic investors and industrial partners to support scale-up and global commercialization.
Deadline: 28 May 2027

FoodTech company offering natural fermentation-based culinary ingredients seeks partners in collective catering for commercial agreement with technical assistance
Ref. TOFR20260527003
A French FoodTech SME offers natural, fermentation-based plant stocks to enhance flavor while reducing additives and improving sustainability. The company seeks central kitchens and collective catering operators to integrate its customised products into large-scale food production through co-development of recipes and applications.
Deadline: 10 June 2027

Per ricevere ulteriori informazioni e per entrare in contatto con i soggetti titolari degli annunci si prega di inviare una mail a: ***simpler@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/partnering-opportunities**

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

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RECENSIONI DI LIBRI

RIPENSARE L'OLIVICOLTURA

VASO POLICONICO, BIODIVERSITÀ, HUMUS E TORROIR

A CURA DI:
GIORGIO PANNELLI, BARBARA ALFEI

I Edizione - 2026
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Pagine 480
https://www.tecnichenuove.com/libri/ripensare-l-olivicoltura

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Come può l'olivicoltura affrontare le sfide dei cambiamenti climatici, della sostenibilità economica e della crescente richiesta di qualità?
Questo volume propone una risposta concreta attraverso i principi dell'olivicoltura rigenerativa, un approccio che mira a migliorare nel tempo la fertilità del suolo, la biodiversità dell'agroecosistema e la capacità produttiva degli oliveti, creando sistemi agricoli più resilienti, sostenibili e redditizi.
In 480 pagine ricche di contenuti tecnici, esperienze di campo e approfondimenti scientifici, il lettore viene guidato lungo l'intera filiera produttiva dell'olivo, dalla progettazione dei nuovi impianti alla gestione degli oliveti tradizionali, dalla cura del terreno alla valorizzazione dell'olio extravergine di oliva.
Ampio spazio è dedicato al vaso policonico, considerato dagli autori il modello di riferimento per una gestione moderna, sostenibile ed economicamente efficiente dell'oliveto. Il volume approfondisce inoltre il ruolo strategico della biodiversità, dell'humus e della fertilità del suolo, elementi fondamentali per contrastare erosione, siccità e degrado ambientale.
Particolare attenzione viene riservata alla qualità dell'olio, al concetto di terroir, alla valorizzazione delle varietà autoctone e al legame tra oliveto, paesaggio e territorio. Completano l'opera gli approfondimenti sulla difesa dell'olivo, sulla progettazione dei nuovi oliveti e un prezioso catalogo varietale, utile per orientare le scelte tecniche e produttive.

INDICE:
Olivicoltura rigenerativa - Vaso policonico - Biodiversità - Nuovi oliveti - Humus - Difesa - Olio: obiettivo qualità - Terroir - Catalogo varietale.

AUTORI:
Giorgio Pannelli è agronomo. Dal 1978 al 2011 ha svolto attività di ricerca presso il CREA-OFA di Spoleto in ambito esclusivamente olivicolo-oleario. Successivamente si è occupato anche di divulgazione e di formazione professionale certificata in olivicoltura. Dal 2004 cura rubriche tecniche sulla rivista “Olivo e Olio”. È attualmente Direttore scientifico di Scuola Potatura Olivo Giorgio Pannelli S.R.L. – Impresa sociale.

Barbara Alfei è agronomo e si occupa di olivicoltura sin dalla laurea, in collaborazione con Giorgio Pannelli. È responsabile del settore olivicoltura e capo Panel presso l’AMAP - Agenzia per l’innovazione nel settore agroalimentare e della pesca. È la “madrina” di Forbici d’oro e della Rassegna Nazionale degli oli monovarietali. Dal 2004 cura specifiche rubriche sulla rivista “Olivo e Olio”. Effettua docenze in corsi di olivicoltura, potatura a vaso policonico, assaggio olio e analisi sensoriale.

CONGRESSI

National Seminar on Palm Oil Milling, Refining, Environment and Quality (POMREQ 2026)

11-12 August | Sarawak, Malaysia

The Seminar is an established national seminar series supporting the continuous development of the Malaysian palm oil industry. The seminar will showcase recent advancements in milling technologies, including the practical applications of AI in palm oil mills to enhance operational efficiency and product

quality. Sessions will also address progress in refining technologies, renewable energy utilisation, biomass valorisation and sustainability practices. POMREQ 2026 brings together industry leaders and technology innovators to shape a smarter, more competitive and sustainable future for the palm oil processing sector.
Objectives:

- To share updates on technological improvements that enhance productivity and efficiency in palm oil milling and refining;
- To highlight recent research and innovations that improve product quality and environmental performance;
- To discuss key issues and challenges affecting the palm oil industry;
- To provide a platform for knowledge exchange, networking and collaborations among industry stakeholders.

Visit:
https://mpob.gov.my/2026/03/national-seminar-on-palm-oil-milling-refining-environment-and-quality/

ARA International Symposium 2026

15-17 September 2026 | Melbourne, Australia

The ARA International Symposium returns to Melbourne in 2026, bringing together the rendering and used cooking oil supply chains for three days of practical discussion, market insight and industry connection.
Hosted by the Australian Renderers Association, the 18th Symposium will focus on the issues shaping rendered products, tallow, used cooking oil and animal protein meals across domestic and international markets.
The program will connect science, policy and trade across plant operations, sustainability, market access, renewable fuels, global supply chains and the future direction of the sector.
Learn across the sector
Hear from regulators, researchers, market analysts, customers, technical specialists and industry partners across the three-day program.
Practical Value
From plant and export realities to market dynamics and regulation, gain practical insights relevant to rendering, agribusiness and the broader supply chain.
Connect with industry
Meet renderers, exporters, regulators, researchers, technology suppliers, customers and downstream users through networking events, Trade Hall conversations and program sessions.
The big picture
Explore how rendering is responding to change across sustainability, compliance, trade and renewable fuels, and what that means for agribusiness and global supply chains.
INDUSTRY DAY
Industry Day is a practical program for plant operators, managers and technical teams working directly across rendering operations. Sessions will focus on odour, wastewater, workforce capability, steam efficiency, compliance expectations and practical site-level issues affecting the sector.
SYMPOSIUM DAYS 1 AND 2
Across the following two days, speakers and panels will connect science, policy and trade across the issues shaping what's next for rendering. Sessions will cover low-carbon liquid fuels, certification, global protein and fats markets, emissions reporting, market access, international developments and the official launch of the ARA Sustainability Framework.
TRADE HALL & NETWORKING
The Trade Hall is the hub of the Symposium, bringing delegates, sponsors and exhibitors together across the three days. It will host meal breaks, sponsor activity, informal meetings, delegate conversations, the Welcome Reception and the Taste of Victoria networking event.
For more information: https://www.arasymposium.com.au/event/2026/summary

OFI International 2026

21-23 September | Amsterdam, The Netherlands

Bringing together the global oils and fats industry, this event unites four powerhouse conferences under one roof, delivering unmatched insight across markets, regulation, technology and innovation.
OFI Commercial/Technical Conference
This two-day conference presents updates on the key challenges currently facing the oils and fats industry including the EU Deforestation Regulation (EUDR), sustainability, traceability, biofuels/HVO/SAF, the global vegetable oil market and prices, trade agreements, food safety and innovations, and solutions in processing and refining.
Key takeaways for delegates:

- Understand the latest global vegetable oil market trends, pricing and trade flows
- Navigate EUDR, sustainability and traceability requirements with confidence
- Explore the impact and opportunities of biofuels (HVO, SAF) on the sector
- Discover innovations in processing, refining and food safety
- Gain practical strategies to adapt to evolving commercial and regulatory pressures

DGF 'Emerging and Persistent Contaminants in Oils and Fats – Challenges and Innovations'
This conference will spotlight one of the most pressing issues for the oils and fats industry: contaminant management and mitigation. As analytical methods become more sensitive and global regulations tighten, the list of substances under scrutiny continues to expand. Organised by the German Society

for Fat Science (DGF), leading experts from research, regulatory agencies and industry will gather to discuss both emerging contaminants, such as persistent organic pollutants (POPs) and process-related compounds, as well as ongoing challenges including mineral oil hydrocarbons (MOH), 3-MCPD, and glycidyl esters. Participants can expect insights into the latest scientific findings, risk assessment approaches, and innovative mitigation strategies that support compliance and consumer safety.

Key take-aways for delegates:

- Stay ahead of tightening global regulations and compliance requirements
- Understand risks around MOH, 3-MCPD, glycidyl esters and emerging contaminants (POPs)
- Learn the latest analytical methods and risk assessment approaches
- Discover innovative mitigation and contamination control strategies
- Gain insight into how regulation and consumer expectations are reshaping standards

Euro Fed Lipid Oleochemistry Conference; From Oils and Fats to Polymeric Materials: Innovations and Applications

This symposium brings together leading academic and industrial experts working at the forefront of bio-based polymers, surfactants, emulsions, and sustainable materials, who will present recent advances in the design, synthesis and functionalisation of renewable building blocks, with a particular emphasis on lipids and biobased amphiphiles as platforms for high-performance materials.

Key take-aways for delegates:

- Explore cutting-edge developments in bio-based polymers and sustainable materials
- Understand how lipids are used as building blocks for high-performance applications
- Discover innovations in surfactants, emulsions and functional materials
- Learn about advances in design, synthesis and material performance
- Gain insight into the future of bio-based and circular material solutions

Triact Oleochemicals Training: Technical and Market approach

This training session will give a global overview of vegetable oils and animal fats derivatives and broaden corporate scope and vision of oleochemicals. Dedicated to technical sales, product and application development, purchasers, business & product managers and team managers, the session will strengthen know-how and boost creativity around oleochemical products and their markets.

Key take-aways for delegates:

- Build a global understanding of oleochemical markets and value chains
- Explore applications and commercial opportunities for derivatives
- Strengthen expertise in technical sales and product development
- Identify new ways to drive innovation and business growth
- Expand your perspective on emerging uses and market dynamics

Visit the website for more information: https://www.ofimagazine.com/ofi-international-2026

3rd Berlin Symposium on Structured Lipid Phases

28-30 September | Berlin, Germany

In essence, a large group of established and early-career experts engaged in vivid discussions. Dealing with the different aspects of structured lipid phases the symposium is designed to allow for ample space for discussion. This is often sacrificed to the tight schedule at larger congresses with parallel sessions due to tight scheduling.

Furthermore, this is an attempt to stimulate dialogue between academia and industry, to relate global challenges and future ambitions to goals relevant to the community. An implication of this ambition is the willingness to share long-term ambitions (not strategy) and nagging questions (industry) as much as imperfect but inspiring work (academia).

The symposium is meant to be a stage for exchange, challenges, a stimulus for cooperation, while being an inspiration for the junior researchers.

The following subject areas should not be interpreted too tightly, but give guidance:

- fat crystallization
- non-TAG structuring/oleogelation
- analytical techniques and structure resolution
- modelling and data processing
- confectionary
- emulsions, delivery, and non-food applications

The set of invited talks, submitted contributions, either oral presentations or posters, are supposed to stimulate discussions such that new answers, questions and ideas result.

Check updates:
https://veranstaltungen.gdch.de/microsite/index.cfm?l=11917&sp_id=2

GLOBOIL India 2025

29 September-1 October | Mumbai, India

Where the global edible oils industry convenes and moves forward

The vegetable oils trade does not move forward in isolation. It moves when producers, refiners, traders, and policy shapers meet in the same room. When forward positions are taken with information that's hours old, not weeks old. When the next

phase of the cycle gets defined out loud, in front of the desks that will act on it.
GLOBOIL India is the platform where that happens - at scale.
Across three days, the full value chain gathers in Mumbai: from palm estates in Indonesia and Malaysia, soy operators in Brazil and Argentina, sunflower in the Black Sea, to the Indian refining and retail capacity that now consumes the largest share of global imports.
This is not a trade show. It is where forward cover gets priced, where sponsorship deals become distribution agreements, where producer-consumer alignment gets built in corridors - not committees.
GLOBOIL India launched in 1997 as Asia's first dedicated international edible oils conference. From 60 delegates in its inaugural year to over 2,000 today. From a single-day gathering to a 3-day convening. From a regional event to a platform that draws delegations from 60+ countries.
See the program at: https://globoilindia.com/programme

5th ICIS Pan American Oleochemicals Conference

1-2 October | Miami, United States

The event spotlighting the Pan American oleochemicals industry returns to Miami!
The 5th ICIS Pan American Oleochemicals Conference brings the industry together at a critical time. Soft demand, rising competition, and structural advantages for petrochemical alternatives are forcing producers and buyers to rethink strategy as supply continues to outpace consumption.
From fatty acids to downstream derivatives, margin pressure is reshaping trade flows and sourcing decisions. Elevated lauric oil prices are challenging natural alcohol economics, discouraging imports, and accelerating feedstock substitution. Against a backdrop of regulatory change, trade uncertainty, and evolving sustainability demands, clear insights have never been more important.
Join the event or exclusive ICIS data and market intelligence to uncover growth opportunities, track changing FMCG demand, and support smarter business and strategic decisions.
Oleochemicals Business Essentials Training
Build sharper oleochemical market confidence. Whether you're upskilling yourself or aligning your team, this course helps to assess pricing signals, manage risk and understand supply and demand - helping to protect margins and improve sourcing outcomes.
It's is a one-day, in-person program designed to give a clear, structured understanding of the market, covering production, pricing, applications, and end-use demand.
Key Themes
- Policy, trade, and market access risks

EUDR, US trade policy shifts, and the wider regulatory landscape are increasingly shaping costs, customer access, and supply chain decisions. Gain insight into how companies across the market are adjusting compliance strategies and protecting market access in real time.
- Feedstock security

Rising feedstock risk across the Americas is creating new challenges, particularly as future biofuels demand begins to influence availability and competition. Explore practical perspectives on the strategies being used to manage volatility and build more resilient sourcing.
- Sustainability and innovation

Evolving FMCG and consumer goods expectations on sustainability and carbon performance are reshaping buyer requirements. Discover which alternative feedstocks, sustainable technologies, and sourcing models are supporting stronger long-term market positioning.
- Rethinking competitiveness

Shifting dynamics in the oleochemicals market are emerging as petrochemical manufacturers leverage structural advantages and FMCG sourcing priorities evolve. Examine how portfolio diversification and clearer value propositions can reinforce competitiveness in a tightening market.
Discover more on: https://events.icis.com/website/13907/

6th International Symposium on Lipid Oxidation and Antioxidants

12-14 October | Wageningen, The Netherlands

Lipid oxidation and its control through antioxidant strategies have been studied for decades. Yet, these topics remain high relevant in today's world, striving for greater sustainability, higher nutritional quality, cleaner labels, and minimal processing. The symposium will highlight cutting-edge research on lipid oxidation mechanisms, analytical methods, antioxidant strategies, and nutritional implications. It will also offer exceptional networking opportunities with world-leading scientists, R&D specialists, and industry partners for which lipid oxidation is a key challenge.
The symposium will be hosted by Wageningen University, internationally recognized as a global center of excellence in food, agriculture, and life sciences. Wageningen is the home to world-renowned research institutions and a leading innovation ecosystem including numerous industrial R&D centers located directly on campus. The university mission "to explore the potential of nature to improve the quality of life" aligns closely with the scientific challenges surrounding lipid oxidation in food and health applications.
More information:
https://veranstaltungen.gdch.de/microsite/index.cfm?l=11952&sp_id=2

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