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GRAFICA, IMPAGINAZIONE E STAMPA

Ancora srl
Via Benigno Crespi, 30
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DIRETTORE RESPONSABILE: P. ROVELLINI
REDAZIONE: C. ZIGLIANI

REDAZIONE

Chiara Zigliani
risg@mi.camcom.it

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Supercritical extraction from *Rosa canina* L. fruits: fatty acids composition and biological activities

Antonella Ibb^a
Antonella Rosa^b
Antonella Fais^c
Benedetta Era^c
Silvia Porcedda^a
Alessandra Piras^a

^a Department of Chemical and Geological Sciences, University of Cagliari, Cittadella Universitaria, SP 8, Monserrato-Sestu km 0.700, Monserrato, CA, Italy

^b Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria SP 8, Monserrato-Sestu km 0.700, Monserrato, CA, Italy

^c Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria, SP 8, Monserrato-Sestu km 0.700, Monserrato, CA, Italy.

✉ CORRESPONDING AUTHOR:
Alessandra Piras
E-mail: apiras@unica.it
Phone: +39 0706754413

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The supercritical fluid extraction from pulp of *Rosa canina* L. fruits, using CO₂ as a solvent, is presented in this study. The extraction experiment was carried out at pressures of 300 bar and a temperature of 40°C, SFE[300:40]. The extract yield was 0.3% for the weight of the charge. The extraction and saponification processes produced a fraction mainly formed by free fatty acids, FA, determined by HPLC-DAD and GC-FID analyses. Pulp extract was characterised by a high level of linolenic acid, 18:3 n-3, (28.37% of total FA); linoleic acid, 18:2 n-6, (26.74%); palmitic acid, 16:0, (18.20%); and oleic acid, 18:1 n-9, (15.74%). Followed by low amounts of stearic acid, 18:0, palmitoleic acid, 16:1 n-7, lauric acid, 12:0, and myristic acid, 14:0.

The amounts of the main unsaturated fatty acids, UFA, in SFE[300:40], determined by HPLC analysis, were 121.43 ± 3.21 mg/g, 102.16 ± 2.84 mg/g, and 49.95 ± 2.75 mg/g of extract for 18:3 n-3, 18:2 n-6 and 18:1 n-9, respectively. Interestingly, the sample was characterised by a high proportion of polyunsaturated fatty acids, PUFA, and the ratio value of UFA to SFA, saturated fatty acids, was 2.8.

The quality of the SFE[300:40] extract, in terms of its chemical composition, was compared with that obtained using n-hexane in a Soxhlet apparatus, Sx. The sample obtained by solvent extraction showed a chemical profile similar to the one obtained by means of SFE but without the added benefit of not having unwanted traces of solvent.

The extracts were evaluated for antioxidant properties, polyphenol content, and inhibitory activity on the xanthine oxidase (XO) enzyme. The antioxidant properties were determined with ABTS assay. The results indicated that the SFE[300:40] extract had low antioxidant activity (EC₅₀ = 0.241 ± 0.022 mg/mL) and the Sx extract had no antioxidant activity. The total phenolics of SFE[300:40] extract was 17.7 mg GAE/g of weight. Both extracts showed a very low inhibition activity towards the XO enzyme.

Keywords: *Rosa canina* L.; Supercritical extraction; fatty acids; antioxidant activity; phenolic content.

1. INTRODUCTION

Rosa canina L. (rose hip) is a species of plant belonging to the Rosacea family, which includes about 5000 species. This plant is a shrub up to 3.5 m of height and widespread in northern Europe, Asia, the Middle East, and North America [1,2].

Besides vitamins, minerals, carotenoids, and polyphenols, the rose hip fruit is also a good source of lipid substances as essential fatty acids that humans cannot synthesize and must be taken through diet. Essential fatty acids are long-chain polyunsaturated fatty acids derived from linolenic, linoleic, and oleic acids. These chemicals regulate numerous body functions, including blood pressure, blood viscosity, immune, and inflammatory responses [3].

The oil content of rose hip fruits ranges from 5 to 18%. Composed of unsaturated fatty acids such as linoleic acid (36-55%), which is the most abun-

dant one, linolenic (17-27%), and oleic acid (15-22%) respectively [4]. According to the literature, there are few studies on the fatty acid content of rose hip pulp [3]. Data on the chemical composition and biological activity mainly concern seed extracts [5,6]. This work aimed to present a description of the fatty acid profile, the polyphenolic content, and the antioxidant and xanthine oxidase inhibitory activities of extracts derived from the pulp of rosehip fruits obtained using CO₂ in the supercritical state. Xanthine oxidase (XO, EC 1.1.3.22) is an important enzyme that catalyses the oxidation of hypoxanthine to xanthine and subsequently to uric acid. In both steps, molecular oxygen is reduced, forming superoxide anion, followed by the generation of hydrogen peroxide. The overactivity of XO has been associated with the development of gout [7].

Thus, the inhibition of xanthine oxidase can reduce both circulating uric acid levels and the production of reactive oxygen species (ROS) [8]. In this context, the research is directed towards the discovery of extracts with potential beneficial properties, as well as antioxidant, anti-XO, and rich in phenolic compounds, polyphenols and flavonoids.

Supercritical fluid extraction (SFE) is an important alternative to conventional methods. It offers many favourable features over traditional techniques since it uses a clean, inexpensive, non-flammable, and non-toxic solvent. The efficiency of SFE and the bioactive components' extractability are ascribed to many factors such as temperature, pressure, and flow rate [9,10]. In contrast to the organic solvent extraction, SFE works at low temperatures and short process times, thus reducing the thermal damage and degradation of oxygen-sensitive compounds. CO₂ is commonly used as a solvent for SFE, because is non-toxic, inert, non-flammable, odourless, and cheaper [11].

2. EXPERIMENTAL PART

2.1. PLANT MATERIAL

Rosa canina L. fruits were supplied by Minardi (Bagnacavallo-Ravenna, Italy). Before use, the pulp was separated from the seeds and ground using a Malavasi mill (Bologna, Italy).

2.2. SUPERCRITICAL FLUID EXTRACTION

Supercritical CO₂ extraction (SFE-CO₂) was performed in a laboratory apparatus equipped with a 320 cm³ extraction vessel, as reported by Piras et al. 2017 [12]. Extraction was carried out in a semi-batch mode: batch charging of vegetable matter and continuous flow solvent, adopting an experimental arrangement that leaves out the first separator. About 300 g of *R. canina* pulp was charged in each run. Operative conditions were 300 bar and 40°C in the extraction section and 20 bar and 15°C in the separator. The extract obtained was stored at -20°C for chemical and biological assays.

2.3. SOLVENT EXTRACTION IN SOXHLET APPARATUS

Approximately 20 g of material was weighed in a cellulose extraction thimble, which was inserted into the cylindrical part of the apparatus. 60 mL of *n*-hexane was heated to reflux. After 6 h of extraction at a temperature above the solvent boiling point, the solvent was removed from the extract solution using a rotary evaporator until the extract was dried before determining yield. The dry extract obtained was stored at -20°C for chemical and biological assays.

2.4. OIL SAPONIFICATION

The extracts (2 mg, in EtOH solution) obtained from *R. canina* pulp by SFE and Soxhlet extraction were subjected to mild saponification as previously reported [13]. Dried saponifiable fractions, dissolved in acetonitrile with 0.14% acetic acid (v/v), were analysed by high-performance liquid chromatography (HPLC). A portion of dried fatty acid (FA) after saponification was methylated with 3 N methanolic HCl (at room temperature) as reported [14], and FA methyl esters (FAME) were analysed by gas chromatography (GC).

2.5. ANALYSIS OF FATTY ACIDS

FAME were analysed on a gas chromatograph HP-6890 (Hewlett-Packard, Palo Alto, USA) with a flame ionisation detector (GC-FID) and equipped with a cyanopropyl methyl-polysiloxane HP-23 FAME column as reported [13]. FAME were identified with standard compounds and quantified as a percentage of the total amount of FA. After the extract saponification, the analyses of total unsaturated FA (UFA) were carried out with an Agilent Technologies 1100 HPLC system (Palo Alto, CA) equipped with a diode array detector (HPLC-DAD). UFA, detected at a wavelength of 200 nm, were eluted with CH₃CN/H₂O/CH₃COOH (75/25/0.12, v/v/v) as the mobile phase at a flow rate of 2.3 mL/min using an Agilent Technologies XDB-C18 Eclipse column. The chromatogram data were recorded and integrated through an Agilent OpenLAB Chromatography data system. UFA identification was performed using standard compounds and conventional UV spectra. Calibration curves of FA were constructed using standards and were found to be linear (correlation coefficients > 0.995) [13].

2.6. STATISTICAL ANALYSES

Statistical differences were evaluated using Graph Pad INSTAT software (GraphPad Software, San Diego, CA, USA). Student's unpaired t-test assessed comparison between groups with Welch's correction and one-way analysis of variance (One-way ANOVA), followed by the Bonferroni Multiple Comparisons Test. The values with $p < 0.05$ were considered significant.

2.7. DETERMINATION OF TOTAL POLYPHENOL CONTENT (TPC)

The Folin-Ciocalteu test was chosen to measure the TPC of *R. canina* extracts. This test was performed

by referring to the method previously reported [15]. About 5 μL of the extract were mixed with 50 μL of the Folin–Ciocalteu reagent in the test tube. The mixture was allowed to stand for 5 min at room temperature. The mixture was then added about 150 μL of sodium carbonate (Na_2CO_3) aqueous solution, and the test tube was shaken gently to mix them. The absorbance of the mixture was measured using the UV-Vis spectrophotometer at $\lambda = 750\text{ nm}$.

A calibration curve of standard reference was established using gallic acid (in a range of concentration from 0.01 to 0.1 mM) as standard references plotted. TPC was expressed as gallic acid equivalents, in milligrams per gram of dry weight (dw)

2.8. ANTIOXIDANT ACTIVITY

The method adopted [16] is based on the capacity of an antioxidant to scavenge the free radical $\text{ABTS}^{\bullet+}$. The $\text{ABTS}^{\bullet+}$ was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate (final concentration) in an aqueous solution, and the mixture was kept in the dark at room temperature for 24 h before use. Extracted samples (10 μL) were added, at different concentrations, to 990 μL of diluted $\text{ABTS}^{\bullet+}$ solution and mixed vigorously. After the reaction at room temperature for 1 min, the absorbance at $\lambda = 734\text{ nm}$ (A734) was measured. The decrease in A734 was calculated, and the results were expressed as the extract concentration necessary to give a 50% reduction in the original absorbance (half maximal effective concentration, EC_{50}).

2.9. XANTHINE OXIDASE INHIBITION ASSAY

Xanthine oxidase (XO) activity was determined spectrophotometrically by measuring the formation of uric acid from xanthine, according to the method previously reported [17].

The xanthine solution was prepared by initially dissolving xanthine in a minimal volume of NaOH, adjusting the pH to 7.5. The XO (from bovine milk) solution was prepared by diluting it to a final concentration of 0.5 U/ μL in cold 0.1 M phosphate buffer (pH 7.5). The reaction mixture contained: 435 μL of 0.1 M phosphate buffer, 10 μL of plant extract solution, 30 μL of xanthine solution, and 25 μL of XO. The change in absorbance was recorded at 295 nm for 3 min at room temperature. All assays were performed in triplicate. XO activity was expressed as percent inhibition of XO, calculated as $[1-(B/A)] \cdot 100$, where A is the change in absorbance of the assay without the plant extract, and B is the change in absorbance of the assay with the plant extract.

3. RESULTS WITH DISCUSSION

This work concerns the extraction using CO_2 in the supercritical state from the pulp of dried fruits of *R. canina* L. The extraction experiment was carried out at a pressure of 300 bar and a temperature of 40°C.

After 4 h of extraction, the extract yield amounted to 0.30% by weight of the charged material. The chemical composition of SFE[300:40] was compared to the one of sample Sx, obtained using *n*-hexane at the boiling temperature in a Soxhlet apparatus (yield = 0.86%).

3.1. FATTY ACID COMPOSITION

Quali-quantitative information on FA that composes the extracts from *R. canina* pulp was obtained by HPLC-DAD and GC-FID analyses. The extract obtained by SFE[300:40] showed a high level of linolenic acid, 18:3 *n*-3, (28.37%) of total FA; linoleic acid, 18:2 *n*-6, (26.74%); palmitic acid, 16:0, (18.20%); and oleic acid, 18:1 *n*-9, (15.72%). Followed by low amounts of stearic acid, 18:0, palmitoleic acid, 16:1 *n*-7, lauric acid, 12:0, and myristic acid, 14:0, Table I. The concentrations of the main unsaturated fatty acids, UFA, in the sample were (121.43 \pm 3.21) mg/g, (102.16 \pm 2.84) mg/g, and (49.95 \pm 2.75) mg/g of

Table I - Fatty acids composition (% of total FA)

Fatty acids	SFE[300:40], %	Sx, %
Lauric (12:0)	1.65 \pm 0.29 ^a	1.99 \pm 0.04 ^a
Myristic (14:0)	1.28 \pm 0.05 ^a	1.17 \pm 0.09 ^b
Palmitic (16:0)	18.20 \pm 0.11 ^a	14.15 \pm 0.16 ^b
Palmitoleic (16:1 <i>n</i> -7)	2.01 \pm 0.30 ^a	1.70 \pm 0.09 ^a
Stearic (18:0)	3.98 \pm 0.30 ^a	3.52 \pm 0.01 ^b
Oleic (18:1 <i>n</i> -9)	15.72 \pm 0.01 ^d	16.62 \pm 0.04 ^c
Linoleic (18:2 <i>n</i> -6)	26.74 \pm 0.23 ^d	32.66 \pm 0.21 ^c
Linolenic (18:3 <i>n</i> -3)	28.37 \pm 0.23 ^a	24.94 \pm 0.30 ^b
Arachidic (20:0)	0.65 \pm 0.06 ^b	0.54 \pm 0.01 ^b
Gondoic (20:1)	0.10 \pm 0.02 ^b	0.20 \pm 0.13 ^b
ΣSFA	25.77 \pm 0.47 ^a	21.38 \pm 0.03 ^b
ΣMUFA	17.83 \pm 0.29 ^b	18.52 \pm 0.26 ^a
ΣPUFA	55.10 \pm 0.46 ^d	57.59 \pm 0.51 ^c
ΣUFA	72.93 \pm 0.74 ^d	76.12 \pm 0.25 ^c

SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Extract analysis was performed in triplicate and all data are expressed as mean values \pm standard deviations (SD); (n = 3). Mean values in the same row having different letters are significantly different (P < 0.05; One-way ANOVA followed by the Bonferroni Multiple Comparisons Test).

Table II - Fatty acid composition (expressed as mg/g of oil)

Fatty acids	SFE[300:40], mg/g	Sx, mg/g
Oleic (18:1 <i>n</i> -9)	49.95 \pm 2.75 ^a	64.68 \pm 0.99 ^a
Linoleic (18:2 <i>n</i> -6)	102.16 \pm 2.84 ^b	141.89 \pm 4.12 ^b
Linolenic (18:3 <i>n</i> -3)	121.43 \pm 3.21 ^a	127.78 \pm 4.15 ^a

Extract analysis was performed in triplicate and all data are expressed as mean values \pm standard deviations (SD); (n = 3). Mean values in the same row having different letters are significantly different (P < 0.05).

extract for 18:3 *n*-3, 18:2 *n*-6, and 18:1 *n*-9, respectively, Table II.

The quality of the sample SFE[300:40], in terms of its chemical composition, was compared with Sx. The extract obtained by solvent extraction showed chemical profiles similar to the ones obtained using SFE-CO₂ (Tables I and II; Figures 1 and 2) but without the additional benefit of not having unwanted traces of solvent. Interestingly, SFE-CO₂ pulp oil was characterised by a high proportion of PUFA and UFA; UFA to SFA ratio value was 2.8 *versus* 3.6 in Sx.

Our results on the FA profiles of *R. canina* pulp extracts align with those previously reported.

In 1997, Illes et al. [18] conducted an extraction from the *R. canina* peel with supercritical CO₂ at 35°C and 250 bar, obtaining similar yield (0.37%) and similar fatty acid profile for linoleic (51.8%), linolenic (23.1%)

and oleic (17.8%) acids.

Ercisli, 2007 [3] reported that the major fatty acid in the hexanic extract was linolenic acid, followed by linoleic and palmitic acids (40.5, 16.4, and 16.0%, respectively). Linoleic (39.5-15.9%), linolenic (26.3-16.9%), oleic (14.4-11.8%), and palmitic (6.7%) acids were previously identified as the main components in the oil obtained by petroleum ether extraction, using a Soxhlet apparatus, of fruits of *R. canina* collected in Portugal by Barros et al. 2010, 2011 [19,20]. Also, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were found in similar proportions.

3.2. TOTAL PHENOL CONTENT

To evaluate the total phenolic content in the fruit extracts, the Folin-Ciocalteu method was used. *R. cani-*

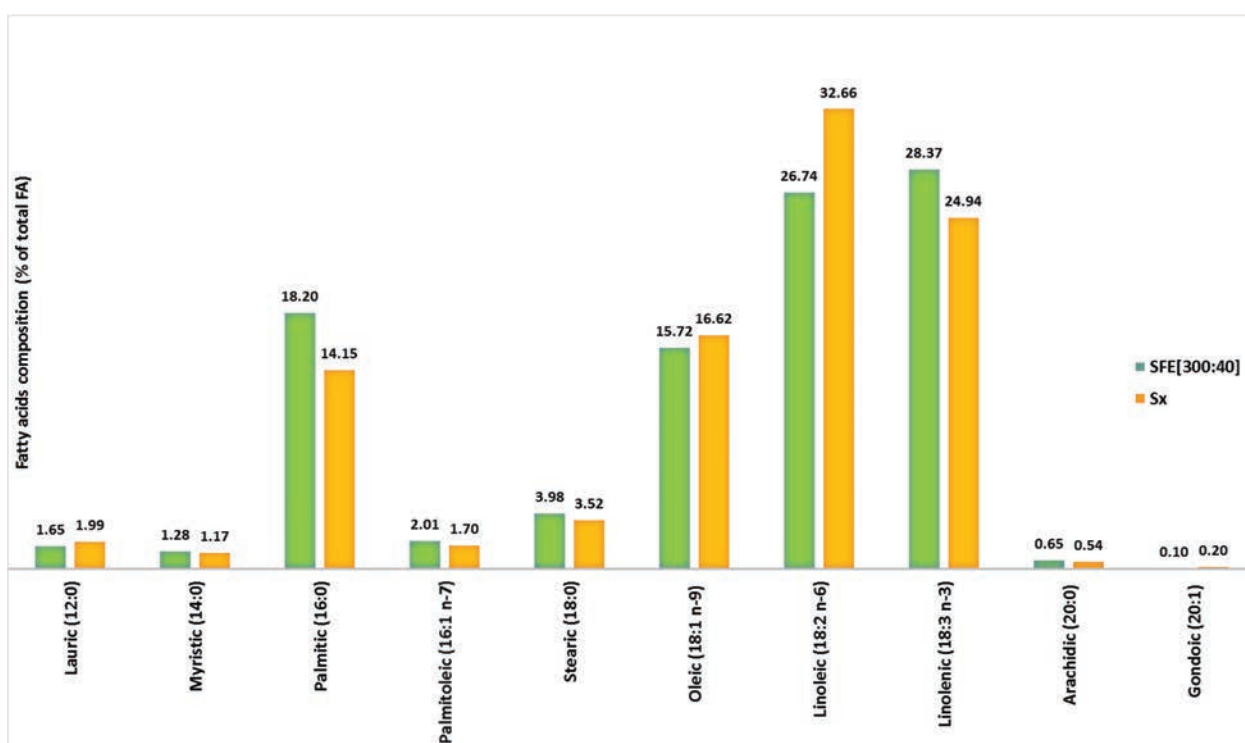


Figure 1 - Fatty acids abundance in *R. canina* extracts.

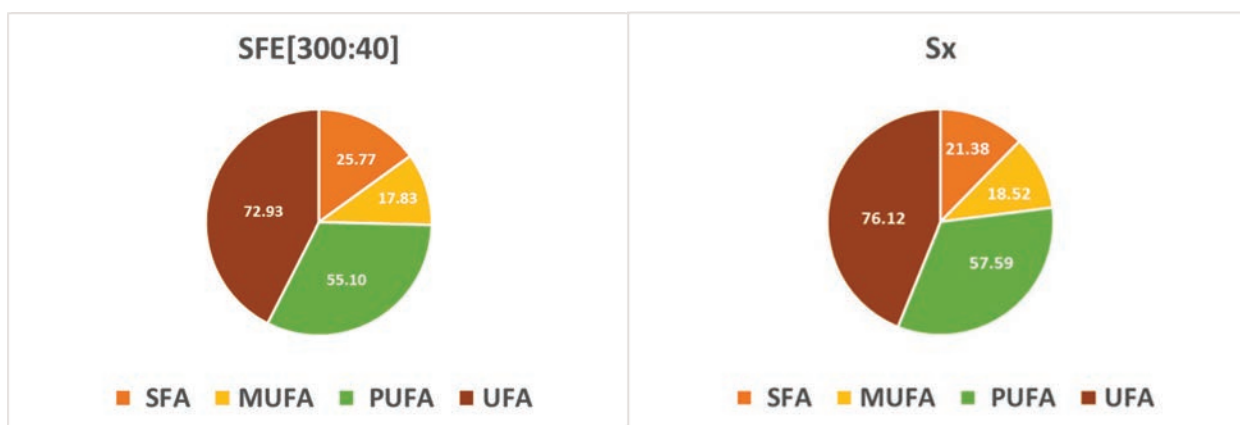


Figure 2 - Saturated and unsaturated fatty acids ratio in *R. canina* extracts.

na is a species known for its phenolic compound-rich composition [21]. Our research shows that the total phenolic content, expressed as the concentration of Gallic Acid Equivalents (GAE), in the SFE-CO₂ sample was 17.7 mg/g of extract and a concentration below the detection limit of the method for Sx (Table III). This value is greater than the value found in *R. canina* var. *transitoria* from Romania (5.751 ± 0.1464 mg GAE/g frozen pulp) [2], is lower than the value of 96 mg GAE/g of dry raw fruit from Turkey [3] and of 96.2 ± 4.35 mg GAE/g of extract from Serbia [22].

3.3. ANTIOXIDANT AND XANTHINE OXIDASE INHIBITORY ACTIVITIES

The antioxidant properties were determined by means of the ABTS assay performed in triplicate at different concentrations to estimate the EC₅₀ (the concentration of sample required to decrease ABTS radical cation concentration by 50% values). The results (see Table III) indicated that SFE[300:40] possessed antioxidant activity, EC₅₀ = (0.24 ± 0.02) mg/mL, and the Sx extract had no antioxidant activity. Both extracts showed a very low inhibition activity towards the XO enzyme. The inhibition percentages were 2.9% for SFE[300:40] and 6.0% for Sx extract.

In conclusion, the main benefit of the SFE method is that it produces a solvent-free, undiluted extract of natural substances applicable for medicinal purposes.

Variations in FAs profiles, polyphenol content, and rose hip pulp's antioxidant activity could result from numerous factors such as climatic, environmental, genetic, etc. No less important, these changes may occur due to the influence of water and enzymes. The aspects of fruit damage degree and humidity of studied raw materials and the drying method, storage conditions, and treatment in the technological process are of great significance for the degree of bioactive compounds.

This study showed the nutritional properties of *R. canina* pulp SFE extract due to their high content of essential fatty acids with health benefits, so qualify these extracts as a potential, environment-friendly, natural resource for food, nutraceutical, and pharmaceutical applications.

Table III - Total phenolic content, antioxidant and inhibitory activity of Xanthine oxidase (Ixo).

Extract	Total phenol (mg GAE/g)	ABTS EC ₅₀ (mg/mL)	Ixo (%)
SFE[300:40]	17.7 ± 1.5	0.24 ± 0.02	2.9 ± 2.6
Sx	N.C.	N.A.	6 ± 4
Trolox ^a		0.0013 ± 0.0004*	

N.C.: no classified; N.A.: no activity; Extract analysis was performed in triplicate and all data are expressed as mean values ± standard deviations (SD); (n = 3). GAE = gallic acid equivalent; ^a positive control; *A. Rosa et al. 2017

Disclosure statement

We declare the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Genus *Prangos* (Apiaceae): A systematic review on essential oils composition and biological activities

Abubakar Siddiq Salihu^{1,2}
Wan Mohd Nuzul Hakimi Wan
Salleh¹ ✉
Nurul Syafiqah Rezali³

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

²Department of Pure and Industrial
Chemistry
Faculty of Natural and Applied
Sciences
Umaru Musa Yar'adua
University
Katsina, Nigeria

³Chemical Sciences Programme
School of Distance Education
Universiti Sains Malaysia
Gelugor, Penang, Malaysia

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

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Essential oils are recognised for their exceptional medicinal value and are considered among the most attractive and potent plant-derived products. Accordingly, growing evidence of the health benefits of these natural essences has prompted researchers to further investigate essential oils and their components, which have been showing promising prospects so far. The objective of this study was to review the essential oils of the genus *Prangos* and their biological activities. The data were collected from scientific electronic databases including SciFinder, Scopus, Elsevier, Pubmed, Google Scholar, and Web of Science. A revision of the genus *Prangos* in Western Asia and in the East of the Mediterranean Sea claimed that there are about 45 species available. The genus *Prangos* distributed from Portugal to Tibet mainly consists of 45 species. *Prangos* species possess great importance as spices, and they are largely used as medicinal plants in Asia, especially in Iran, Turkey, and Iraq. It has been shown to possess gastrointestinal symptoms, as well as aphrodisiac, coagulant, carminative and tonic properties. A total of twenty-one *Prangos* species have been reported for their essential oils and biological activities. Monoterpenes were identified as the major components for several *Prangos* species with an abundance of α -pinene, β -pinene, (*Z*)- β -ocimene, δ -3-carene, and sabinene. The essential oils also presented remarkable biological activities such as antimicrobial, antioxidant, allelopathic, insecticidal, larvicidal, antiproliferative, anticholinesterases, antityrosinase, antidiabetic, repellent, antiobesity, wound healing, and fumigant toxicity effects. This is the first attempt to compile essential oils composition and their biological activities as well as the medicinal uses of the genus *Prangos*. In the future, several scientific investigations are required to understand the mechanism of the action of essential oils and their bioactive components.

Keywords: Apiaceae, *Prangos*, essential oil, composition, α -pinene, antimicrobial

1. INTRODUCTION

The Apiaceae family (also known as Umbelliferae) consists of nearly 3800 species, classified into four subfamilies and 29 tribes. According to the Plants of the World Online (POWO) database, there are 446 genera assigned to this flowering-plant type family [1]. The name originally derived from a celery-like plant of the genus *Apium* which has a wide distribution in Europe and Asia especially Turkey, as well as Africa, South America, and Australia. In Turkey, there are approximately 47 genera reported and 9 are endemic taxa plants [2].

Several plants of this family are well-known harvested crops including leaf and root vegetables such as carrots and celery, as well as herbs and spices such as parsley, anise, and caraway. Meanwhile, some members of the family have been used as an indigenous medicine over generations mainly in tropical and subtropical regions [3].

Prangos (Figure 1) is one of the genera in the Apiaceae family that is mainly



Figure 1 – Some species of the genus *Prangos*

found in western Asia and east of the Mediterranean Sea. It is reported that the majority of these perennial hemicryptophytes are clustered around Turkey, Iran, and Iraq [4]. About 45 species of *Prangos* were recorded and have an economic and medicinal impact. Generally, several *Prangos* species have been consumed in folk medicine such as *P. meliocarpoides* as a wound healing agent and treatment of gastrointestinal abnormalities [5-7]. Meanwhile, *P. ferulacea* and *P. heyniae* were used as a natural aphrodisiac. The most highlighted species of this genus are *P. ferulacea* and *P. pabularia* due to their extensive use by natives [8-10].

Phytochemical studies on the non-volatile metabolites of the genus *Prangos* reported the presence of coumarins, which includes prenylated coumarin (osthole) [11], aglycone coumarin (oxypeucedanin and isoarnottinin 4'-glucoside) [12], furanocoumarin (psoralen) [13] and other coumarin derivatives [10]. Due to this coumarins content, the genus *Prangos* shows various pharmacological properties such as natural anti-HIV agent [14], antifungal [15], antibacterial, antioxidant, and cytotoxic effects [16].

Essential oils have been used for thousands of years in countless cultures for their incredible health-promoting and medicinal properties. This secondary metabolite consists of versatile organic structures and complex mixtures of natural compounds including unsaturated hydrocarbons like terpenic compounds, aldehydes, ketones, esters, and phenols. The oils are mainly utilised for aromatherapy, insect repellents, and medical purposes and some are food-grade oils that are being used to flavour. Nowadays, many studies have revealed that the oils retain remarkably antimicrobial, antioxidant, and anti-inflammatory properties [17-19].

Hence, the review concerning the genus *Prangos* essential oils focusing on their medicinal uses, chemical compositions, and biological activities is necessary to clearly observe the potential of this genus, especially in the food and pharmaceutical industries. The facts pertaining to the *Prangos* essential oils and the biological activities of numerous species were searched thoroughly via online comprehensive discovery databases (SciFinder, Scopus, Elsevier, Pubmed, Google Scholar, and Web of Science) and the articles published in peer-reviewed journals were collected via a library search.

2. SEARCH STRATEGY

The protocol for performing this study was developed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) [20] (a) the first step was to exclude duplicate articles, (b) titles and abstracts were then read and the inclusion and exclusion criteria were applied and (c) all articles resulting from this stage were read in full, and the inclusion and exclusion criteria were applied again.

Figure 2 shows the flow diagram of the identification and selection of articles. Following this step, we reached the articles chosen for this study. This systematic review was conducted through searches using Scopus, PubMed, Science Direct, SciFinder, and Google Scholar. The keywords used were '*Prangos*', 'essential oil', and 'biological activity' articles over the period from the beginning of the database until December 2022.

The inclusion of articles considered the following criteria: (1) type of publication - original research articles, (2) only articles in English, (3) articles must present the chemical composition of *Prangos* essential oils, (4) articles must discuss the bioactivity of the essential oils.

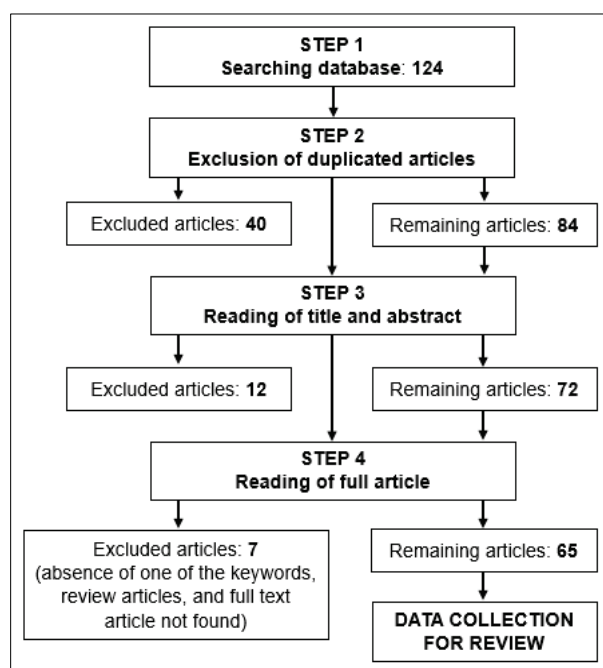


Figure 2 - PRISMA flow diagram of included studies

As the exclusion criteria, the following were used: (1) articles that did not present the search terms in the title and abstract; (2) review articles, (3) full-text articles not found, (4) articles without one of the keywords and (5) articles that did not present the composition of the essential oils.

3. MEDICINAL USES

Since a long time ago, traditional medicinal plants have been used for the treatment of different types of diseases around the world. It is proven that each part of the plants including roots, stems, flowers, seeds, and leaves is beneficial to humans. Recently, *Prangos* species have been used to treat multiple traditional therapies. Meanwhile, the aerial part of essential oils is the major contributor to the genus *Prangos* in terms of benefits of pharmacological effects. In Turkey, several *Prangos* species are used as sexual stimulants and to control external bleeding. Table I shows several *Prangos* species and their medicinal uses [21-33].

4. ESSENTIAL OILS COMPOSITION

The composition of essential oils contributes significantly to the determination of the pharmacological potential attributed to the plant species (indicated mainly by the major compounds) and is constantly being transformed, due to factors external to the biology of the plants (edaphic or environmental) and/or intrinsic to the biology of plants (physiological and genetic) [19].

The essential oils from *Prangos* species characterised by chemical diversity are shown in Table II [34-87]. The chemical composition for species of genus *Prangos* reported in the study consulted from 1996 to the present day. All the information collected was organised taking into account plant species, origin, part of the plant used, yield, identification of components, and main components. The essential oils from twenty-one species of *Prangos* were analysed according to the data reported in Table II. The important qualitative and quantitative differences in the chemical composition of the essential oils of genus *Prangos* can be estimated; the aerial parts are the most studied part of the plant, as well as fruits, inflorescences, leaves, roots, shoots, stems, and umbels. The most commonly used extraction processes were hydrodistillation. The components were characterised using mass spectrometry, retention indexes, and retention times. The amount of each component is given as a percentage of the total oil and, in general, 80-90% of the oil was identified. Analysis of the essential oils demonstrated the highest yield given by the roots oil of *P. denticulata* which gave 3.20% [79]. In addition, the highest components identified were from the fruit oil of *P. denticulata* (121 components) [79], followed by the aerial parts' oil of *P. pabularia* (86 components) [63]. Monoterpenes (hydrocarbons and oxygenated) were found and classified as the major components. α -Pinene was reported as the major component in 23 studies. Besides, 5 studies were also reported; β -pinene, (*Z*)- β -ocimene, and δ -3-carene as the most abundant components. α -Pinene was found its richness in the fruit's oil of *P.*

Table I - Medicinal uses of several *Prangos* species

Species	Plant Part	Plant Origin	Traditional uses	References
<i>P. pabularia</i>	Roots, Fruits, Leaves	Turkey, India	Traditionally used as laxative, antihypertensive, and carminative agents and are also recommended for the treatment of digestive disorders	[21]
<i>P. ferulacea</i>	Roots, Shoots, Leaves	Iran, Turkey	Treat gastrointestinal abnormalities, act as natural aphrodisiac, and increase body strength, anti-diabetic agent	[22]
<i>P. platychnaena</i>	Roots	Turkey	Treat wounds of cattle, stop external bleeding, prevent gum ailment and cavity formation	[23]
<i>P. uechtritzi</i>	Whole plant	Turkey	Treat haemorrhoids	[24]
	Roots	Turkey	Act as natural aphrodisiac	[25]
<i>P. haussknechtii</i>	Aerial parts	Iraq	Has carminative, diuretic, and act as natural sedative	[26]
<i>P. asperula</i>	Aerial parts	Lebanon	Reduce blood pressure, treat skin disease, digestive disorder and haemorrhoids	[27]
<i>P. heyniae</i>	Roots	Turkey	Natural aphrodisiac	[28]
<i>P. meliocarpoides</i>	Fruits	Turkey	Treat external bleeding, digestive disorder, wounds, scars and mucosal disease	[5]
	Roots	Turkey	Natural aphrodisiac	
<i>P. uloptera</i>	Aerial parts	Iran	Treat leukoplakia, digestive disorders and wounds	[29]
<i>P. tschimganica</i>	Aerial parts	Uzbekistan	Treat leukoplakia	[30]
<i>P. gaubae</i>	Fruits	Iran	Natural nutritional agent	[31]
<i>P. acaulis</i>	Aerial parts	Iran	Used as sedative and anti-infective agent, as well as pain relief and tooth whitener	[32]
<i>P. peucedanifolia</i>	Aerial parts	Iran	Treating kidney disorders, bladder inflammation, and hemorrhoids	[33]

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

Species	Locality	Part	Yield (%)	Identification (No. %)	Major components	References
<i>P. ferulacea</i>	Italy	Flowers	0.62	15, 96.58	(Z)- β -Ocimene (44.44%), sabinene (20.1%), γ -terpinene (8.09%), 3-thujene (5.79%), and α -pinene (4.28%)	[34]
		Leaves	0.28	19, 96.22	(Z)- β -Ocimene (61.91%), sabinene (10.11%), caryophyllene (7.65%), and (E)- β -ocimene (4.62%)	
		Aerial parts	0.62	31, 89.10	(E)- β -ocimene (43.1%), (Z)- β -ocimene (15.8%), and α -pinene (5.6%)	[35]
	Iran	Aerial parts	1.80	22, 99.87	β -Pinene (27.01%), δ -3-carene (24.78%), α -pinene (18.34%), and β -caryophyllene (17.69%)	[36]
			0.17-0.29	34, 99.90	(E)-Caryophyllene (48.21%), α -humulene (10.28%), spathulenol (9.36%), linalool (3.46%), and δ -3-carene (3.37%)	[37]
			0.20	27, 99.25	β -Pinene (43.1%), α -pinene (22.1%), and δ -3-carene (16.9%)	[38]
			1.90-2.10	38-47, 92.30-95.76	Terpinolene (56.3-38.1%), α -terpinene (2.25-1.6%), (E)-caryophyllene (4.7-3.6%), and bornyl acetate (2.9-1.8%)	[39]
			0.20	31, 98.00	α -Pinene (57.0%), 3-ethylidene-2-methyl-1-hexen-4-yne (5.3%), and β -pinene (4.5%)	[40]
			0.60	21, 96.23	β -Phellandrene (20.39%), α -terpinolene (15.26%), α -pinene (11.59%), δ -3-carene (11.06%), α -phellandrene (9.09%), and <i>trans</i> - β -ocimene (9.67%)	[41]
			1.60	10, NM	α -Pinene (36.6%) and β -pinene (31.1%)	[42]
		Leaves	1.17-1.23	32, 93.25-95.14	(E)- β -Ocimene (22.0-6-28.25%), limonene (12.19-15.18%), 2,3,6-trimethylbenzaldehyde (7.03-8.57%), and terpinolene (6.63-8.73%)	[43]
			0.54-1.55	22, 76.70-90.30	α -Pinene (4.8-16.4%), β -pinene (9.4-27.9%), and δ -3-carene (8.1-20.6%)	[44]
			0.43	39, 99.77	α -Pinene (36.82%), camphene (15.83%), limonene (10.52%), β -pinene (8.73%), and myrcene (5.88%)	[45]
			0.25	65, 96.20	β -Pinene (29.6%), α -pinene (19.8%), δ -3-carene (11.4%), and β -phellandrene (11.1%)	[46]
			0.90	10, 81.70	Linalool (36.7%), caryophyllene oxide (16.3%), and α -pinene (12.1%)	[47]
		Flowers	0.78	56, 99.42	α -Pinene (20.91%), bornyl acetate (13.80%), camphene (11.94%), limonene (8.57%), β -pinene (7.47%), and myrcene (6.03%)	[45]
			0.40	52, 91.50	β -Pinene (20.6%), α -pinene (8.8%), δ -3-carene (10.4%), and β -phellandrene (8.1%)	[46]
			1.10	17, 98.20	Linalool (19.0%), lavandulyl acetate (16.0%), 1,8-cineole (14.5%), α -pinene (12.4%), and geranyl isobutyrate (12.2%)	[47]
		Roots	1.20	14, 95.10	β -Phellandrene (32.1%), <i>m</i> -tolualdehyde (26.2%), and δ -3-carene (25.8%)	[48]
			0.20	53, 96.30	δ -3-Carene (22.5%), β -phellandrene (11.8%), α -pinene (8.6%), terpinolene (7.2%), <i>p</i> -cymene (6.3%), α -phellandrene (6.2%), and myrcene (4.5%)	[49]
		Stems	0.80	11, 43.30	1,8-Cineole (19.0%) and α -pinene (10.3%)	[47]
Fruits	0.80	14, 93.70	α -Pinene (63.1%), <i>cis</i> -ocimene (9.7%), and β -pinene (8.3%)	[50]		
Umbels	0.50	12, 94.70	α -Pinene (42.2%), <i>cis</i> -ocimene (36.3%), and myrcene (5.0%)			
Turkey	Aerial parts	NM	21, 98.86	2,3,6-Trimethyl benzaldehyde (66.59%), chrysanthemyl acetate (15.06%), β -ocimene (3.76%), and <i>p</i> -mentha-1,5-dien-8-ol (3.57%)	[51]	
	Fruits	0.36-0.98	23, 85.32-93.83	γ -Terpinene (30.22-33.27%) and α -pinene (16.71-12.83%)	[52]	
<i>P. asperula</i>	Lebanon	Fruits	0.52	22, 95.10	Sabinene (29.8%), β -phellandrene (19.2%), α -pinene (9.8%), <i>trans</i> -nerolidol (9.2%), and α -phellandrene (8.0%)	[53]
		Leaves	NM	42, 92.10	Sabinene (20.6%), β -phellandrene (19.0%), γ -terpinene (9.0%), and α -pinene (8.4%)	[54]
	Iran	Leaves	0.20	47, 98.80	2,3,6-Trimethyl benzaldehyde (18.4%), δ -3-carene (18.0%), and α -pinene (17.4%)	[55]

Species	Locality	Part	Yield (%)	Identification (No. %)	Major components	References
		Aerial parts	0.95	42, 99.62	δ-3-Carene (25.54%), α-terpinolene (14.76%) α-pinene (13.6%), limonene (12.94%), myrcene (8.1%), and β-pinene (5.4%)	[56]
<i>P. uloptera</i>	Iran	Aerial parts	0.85	12, 94.94	α-Pinene (25.20%), decanal (18.03%), β-caryophyllene (16.98%), limonene (7.15%), and caryophyllene oxide (6.25%)	[36]
			0.80	60, NM	δ-3-carene (26.3-32.1%), α-pinene (15.4-16.8%), and camphene (2.7-4.1%)	[57]
			0.41	60, NM	α-Pinene (14.26-15.37%), δ-3-carene (26.12-26.42%), β-myrcene (9.8-10.16%), p-cymene 88.21-8.60%), and β-phellandrene (7.62-8.16%)	[58]
			0.45	11, 90.96	Safrole (21.67%), α-pinene (20.09%), and spathulenol (13.66%)	[59]
			0.70	28, 89.10	β-Caryophyllene (27.1%), caryophyllene oxide (15.9%), and α-pinene (12.4%)	[60]
	Umbels	0.40	10, 93.20	α-Pinene (31.7%), β-bourbonene (15.9%), α-curcumene (10.6%), spathulenol (9.0%), and m-cymene (5.5%)	[61]	
	Fruits	0.40	18, 83.00	α-Pinene (14.98%), β-bourbonene (7.81%), α-humulene (7.74%), germacrene B (7.23%), and n-tetracosane (6.65%)		
<i>P. pabularia</i>	Tajikistan	Roots	0.10	42, 97.30	5-Pentylcyclohexa-1,3-diene (44.6%), menthone (12.6%), 1-tridecyne (10.9%), and osthole (6.0%)	[62]
	Uzbekistan	Aerial parts	0.42	86, 93.40	cis-allo-Ocimene (17.6%), δ-3-carene (14.2%), limonene (7.6%), 2,4,6-trimethylbenzaldehyde (6.8%), and α-terpinolene (6.1%)	[63]
	Turkey	Aerial parts	NM	34, 91.30	α-Pinene (32.4%), δ-3-carene (12.4%), germacrene D (8.1%), limonene (6.4%), and bicyclogermacrene (6.2%)	[64]
	Iran	Leaves	0.20	23, 90.31	Spathulenol (16.0%), α-bisabolol (14.3%), and (Z)-4-methoxycinnamaldehyde (9.47%)	[65]
		Fruits	0.40	15, 70.01	α-Pinene (33.87%), spathulenol (9.32%), and α-santalene (7.05%)	
		Umbels	0.30	23, 71.31	α-Pinene (21.46%), α-santalene (6.36%), and p-methoxyacetophenone (5.39%)	
India	Shoots	0.30	31, 97.34	Durylaldehyde (62.16%), bicyclo[3.1.1]hept-2-en-4-ol (8.84%), and chrysanthenyl acetate (5.12%)	[81]	
<i>P. heyntiae</i>	Turkey	Aerial parts	0.10-12.2	41, 95.90	β-Bisabolonal (12.2%), caryophyllene oxide (7.9%), germacrene D (7.8%), elemol (7.4%) and α-humulene (6.7%)	[66]
			0.30-0.90	20-66, 96.30-97.40%	Germacrene D (10.3-12.1%), β-bisabolene (14.4%), kessane (26.9%), germacrene B (8.2%), elemol (3.4-46.9%), β-bisabolonal (1.4-70.7%), and β-bisabolol (8.4%)	[67]
		Fruits	1.40-2.70	34, 97.0-98.70	α-Pinene (6.8-12.8%), α-phellandrene (0.1-17.1%), and β-phellandrene (4.2-22.4%)	[68]
	0.30-0.90		61-79, 89.80-92.20	β-Bisabolonal (53.3-18.0%), β-bisabolol (14.6-2.3%), and β-bisabolene (12.1-10.1%)	[69]	
<i>P. platychlaena</i>	Iran	Aerial parts	0.04-2.85	35, 90.14-92.55	δ-3-Carene (9.25-43.17%), α-pinene (4.58-27.41%), β-pinene (3.72-25.55%), and β-phellandrene (4.02-17.88%)	[70]
		Leaves	0.27	36, 90.44	(E)-β-Ocimene (25.93%), bornyl acetate (24.58%), α-pinene (5.84%), sylvestrene (4.62%), and γ-terpinene (3.75%)	[71]
		Stems	0.04	38, 92.72	Bornyl acetate (25.49%), (E)-β-ocimene (22.94%), α-pinene (9.5%), p-cymene (6.48), γ-terpinene (4.13%), and sylvestrene (4.01%)	
		Flowers	1.02	43, 96.49	(E)-β-Ocimene (28.5%), bornyl acetate (24.18%), γ-terpinene (14.15%), p-cymene (6.48%), and α-pinene (4.16%)	
	Turkey	Fruits	0.40	15, 98.82	α-Pinene (69.75%), β-phellandrene (10.58%), δ-3-carene (3.39%), and p-cymene (3.38%)	[72]
<i>P. acaulis</i>	Iran	Aerial parts	NM	21-26, 89.10-98.74	α-Pinene (13.7-22.87%) and 3-ethylidene-2-methyl-1-hexen-4-yne (14.3-21.36%)	[73]
		Stems	0.18	11, 100	3-Ethylidene-2-methyl-1-hexen-4-yne (56.8%) and α-pinene (34.2%)	[74]
		Leaves	0.25	18, 99.74	α-Pinene (39.54%), 3-ethylidene-2-methyl-1-hexen-4-yne(37.94%), and α-terpinene (10.9%)	

Species	Locality	Part	Yield (%)	Identification (No. %)	Major components	References
		Flowers	0.38	22, 98.18	α -Pinene (25.04%), 3-ethylidene-2-methyl-1-hexen-4-yne (23.51%), α -terpinene (17.26%), and limonene (13.64%)	
<i>P. uechritzii</i>	Turkey	Aerial parts	0.10-24.60	30, 97.40	p-Cymene (24.6%), caryophyllene oxide (19.6%), 7-epi-1,2-dehydrosesquiceneole (12.6%), limonene (3.2%), α -bisabolol (3.2%)	[66]
		Fruits	2.10	18, 97.42	α -Pinene (40.82%), nonene (17.03%), β -phellandrene (11.14%), δ -3-carene (7.39%), and p-cymene (4.90%)	[72]
			0.76	32-109, 86.70-90.00	α -Pinene (11.23%), α -phellandrene (8.42%), β -phellandrene (8.26%), α -bisabolol (7.04%)	[75]
			0.70	38, 84.50	p-Cymene (10.9%), γ -terpinene (7.0%), β -phellandrene (7.8%), α -phellandrene (6.3%), and (Z)- β -ocimene (4.6%)	[76]
<i>P. latiloba</i>	Iran	Flowers	NM	28, 86.80	Limonene (18.3%), myrcene (10.4%), (E)- β -ocimene (7.8%), α -phellandrene (6.4%), and α -pinene (5.7%)	[77]
		Leaves	NM	23, 84.60	Limonene (17.4%), myrcene (9.4%), α -pinene (6.1%), α -phellandrene (5.4%), and (E)- β -ocimene (5.3%)	
		Stems	NM	29, 83.70	Limonene (13.5%), myrcene (8.6%), α -phellandrene (4.9%), germacrene D (4.5%), and γ -curcumene (4.3%)	
		Aerial parts	0.66	27, 87.00	α -Pinene (25.1%), limonene (16.1%), and myrcene (9.5%), elemol (5.7%)	[78]
<i>P. denticulata</i>	Turkey	Fruits	trace	121, 95.20	Sabinene (26.1%) and p-cymene (19.7%)	[79]
		Roots	3.20	70, 88.10	δ -3-Carene (49.3%) and (Z)-3,5-nonadiene-7-ene (20.4%)	
<i>P. turcica</i>	Turkey	Fruits	0.37	72, 87.20	α -Humulene (11.0%), germacrene D (10.6%), naphthalene (8.5%), terpinolene (7.9%), and bornyl acetate (6.9%)	[80]
<i>P. meliocarpoides</i>	Turkey	Aerial parts	0.10-16.70	40, 99.50	Sabinene (16.7%), p-cymene (13.2%), bornyl acetate (11.8%), α -pinene (6.2%), and p-cymen-8-ol (6.1%)	[66]
<i>P. trifida</i>	Italy	Aerial parts	0.52	25, 91.30	cis- β -Ocimene (18.12%), α -phellandrene (12.14%), sylvestrene (11.32%), p-mentha-1,3,8-triene (9.56%), and α -pinene (8.85%)	[82]
<i>P. odontalgica</i>	Russia	Aerial parts	0.07	38, 88.40	γ -Elemene (9.84%), bisabolol(9.41%), transnerolidol (3.90%), and linalyl isobutyrate (3.41%)	[83]
<i>P. gaubae</i>	Iran	Aerial parts	0.40	41, 92.80	Germacrene D (26.7%), caryophyllene oxide (14.3%), (E)-caryophyllene (13.8%), and spathulenol (11.3%)	[84]
<i>P. uechritzii</i>	Turkey	Aerial parts	NM	NM	β -Pinene (28.79%), methyle linolenate (7.27%), α -terpineol (7.20%), spathulenol (5.60%), and humulene-1,2-epoxyde (4.20%)	[85]
<i>P. peucedanifolia</i>	Turkey	Aerial parts	NM	37, 89.50	α -Pinene (38.1%), bicyclogermacrene (11.3%), and δ -3-carene (9.2%)	[64]
<i>P. corymbosa</i>	Iran	Aerial parts	0.40	21, 97.40	β -Elemene (40.7%), kessane (10.7%), and caryophyllene oxide (10.5%)	[59]
<i>P. scabra</i>	Iran	Fruits	1.60	20, 92.30	β -Elemene (19.9%), β -farnesene (16.2%), epi-globulol (1.5%), γ -cadinene (10.0%), and β -caryophyllene (9.2%)	[61]
		Inflo-rescens	0.30	14, 80.10	epi-Globulol (21.1%), β -elemene (19.7%), caryophyllene oxide (9.0%), and α -cadinol (6.2%)	
<i>P. serpentinica</i>	Iran	Aerial parts	0.64	43, 92.20	β -Caryophyllene (26.4%), δ -3-carene (6.1%), linalool (5.7%), α -phellandrene (5.3%), p-cymene (5.2%), and camphene (5.1%)	[86]
<i>P. cheilanthifolia</i>	Iran	Aerial parts	1.10	17, 98.00	β -Myrcene (16.8%), camphor (16.6%), and trans-caryophyllene (16.1%)	[87]

platychlaena (69.75%) [72], and *P. ferulacea* (63.1%) [50], whereas β -pinene gave the highest percentage from the aerial parts oil of *P. ferulacea* (43.1%) [38]. Other monoterpenes identified in principal amounts were sabinene [53, 54, 66, 79], terpinolene [39], β -phellandrene [41], linalool [47], 1,8-cineole [47], γ -terpinene [52], bornyl acetate [71], p-cymene [66], limonene [77], and β -myrcene [87]. In another study, sesquiterpenes (hydrocarbons and

oxygenated) were also described as the major group components. Among them, (E)-caryophyllene was reported from the aerial parts' oil of *P. ferulacea* [37] and *P. uloptera* [60]. In addition, spathulenol [65], germacrene D [67], α -humulene [80], γ -elemene [83], β -elemene [59], and epi-globulol [61] were also found as the highest percentage in several *Prangos* essential oils. Safrole was the only phenylpropanoid identified in aerial parts' oil of *P. uloptera* [59].

5. BIOLOGICAL ACTIVITIES

The genus *Prangos* is well-known for its diverse biological activities. The essential oils are reported to have antimicrobial [34, 36, 41, 47, 48, 50, 53, 71, 72, 82, 88], antioxidant [34, 45, 66, 82, 84, 89], allelopathic [40, 65], insecticidal [51], larvicidal [67, 90], antiproliferative [54, 81, 89], anticholinesterase [66, 84, 89], antityrosinase [66, 84], antidiabetic [62, 66, 84], repellent [91], antiobesity [84], wound healing [48], and fumigant toxicity [92] properties. All reported biological activities from *Prangos* essential oils are summarised in Table III.

Out of twelve tested profiles, *Prangos* species showed the highest antimicrobial, antioxidant and insecticidal capabilities. Some *Prangos* species were investigated for their antimicrobial and antioxidant activities. Preparations of essential oils of *Prangos* species have been widely researched for their activities against gram-positive and gram-negative bacteria, as well as some species of yeast-like fungi, and compared to the activity of standard drugs. Different antimicrobial activity assays with different antibiotic and antifungal controls were used, including disc diffusion and microdilution. According to this established profile, the *P. trifida* [82] and *P. platychlaena* [72] oils demonstrated good inhibitory potential against gram-positive (*B. subtilis*, *S. aureus*); *P. ferulacea* [88] and *P. uechtritzi* [72] oils against gram-negative bacteria (*E. coli*, *P. aeruginosa*), as well as *P. uloptera* [36] oil against fungi (*C. krusei*). The antimicrobial activity observed has been attributed to the presence of different bioactive components with an impact on the growth and metabolism of microorganisms.

In antioxidant activity, various assays have been performed on the *Prangos* essential oils such as DPPH, CUPRAC, ABTS, FRAP, MCA, PBD, and metal chelating. The flowers and leaves' oils of *P. ferulacea* [45] showed a strong activity in DPPH with IC₅₀ values 23.9 and 22.9 µl/mL, respectively. Besides, *P. heynei* oil showed a significant activity in ABTS (92.9 mg TE/g) and FRAP (61.2 mg TE/g) assays [66]. Meanwhile, *P. meliocarpoides* oil have a potential activity in CUPRAC (113.4 mg TE/g) and PBD (24.3 mmol TE/g) assays [66].

Interestingly, it was also verified that the *Prangos* essential oils have significant mosquitocidal and insecticidal properties. The *P. ferulacea* oils exhibited a strong protection against two mosquito species *Culex quinquefasciatus* and *Anopheles stephensi* [90], as well as egg stages of *Ephestia kuehniella* and *Trichogramma embryophagum* [51]. While *P. heynei* showed positive inhibitory properties against the first instar *Aedes aegypti* lice cycle [67]. Not limited to mosquito, *Prangos* species are also able to serve as a beetle repellent. For example, the *P. acaulis* has demonstrated resistance to three beetle species including the red flour beetle (*Tribolium castaneum*), rice weevil (*Sitophilus oryzae*) and cowpea seed beetle (*Callosobruchus maculatus*) [91]. The profiles of

these plant-derived insect repellents could be utilised for developing eco-friendly and safer alternatives to current synthetic repellents.

6. CONCLUSION AND FUTURE PERSPECTIVES

In this review, we summarise the information on the chemical composition and biological activities of the genus *Prangos* as well as its medicinal uses. The principal chemical components of *Prangos* essential oils were α-pinene, β-pinene, (Z)-β-ocimene, δ-3-carene, and sabinene. These predominant chemical components of *Prangos* essential oils can serve as a novel potential natural source, which can be used in the pharmaceutical and food industries. Previous studies revealed that *Prangos* essential oils can protect people from several diseases due to their potent biological activities including antimicrobial, antioxidant, allelopathic, insecticidal, larvicidal, antiproliferative, anticholinesterases, antityrosinase, antidiabetic, repellent, antiobesity, wound healing, and fumigant toxicity effects. Hence, future studies need to conduct systematic revisions using cell and animal models, as well as clinical and experimental investigations of *Prangos* essential oils. Besides, future research should look at the toxicity, bioavailability, and pharmacokinetics of *Prangos* essential oils to find the chemical components responsible for their activities and expand the existing medical application of the genus *Prangos*.

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

Bioactivities	Species	Description	References
Antimicrobial	<i>P. ferulacea</i>	The flower oil showed activity against <i>S. tiphymurium</i> , <i>B. cereus</i> , and <i>B. subtilis</i> while the leaves oil against <i>B. cereus</i> , and <i>B. subtilis</i> , with same MIC value 100 µg/mL	[34]
		The essential oil gave activity against <i>E. faecalis</i> with MIC value 2.27 µg/mL	[88]
		The roots oil inhibited the growth of <i>S. aureus</i> and <i>P. aeruginosa</i> each with MIC value of 20 µg/mL	[48]
		The essential oil shows activity against <i>E. coli</i> and <i>S. saprophyticus</i> with MIC values 3.27 and 8.19 µg/mL, respectively	[41]
		The leaves and flower oils showed activity against <i>S. aureus</i> with MIC value 0.5 µg/mL	[47]
		The essential oil exhibited modest activities against <i>E. coli</i> , <i>S. epidermidis</i> , <i>P. aeruginosa</i> and <i>C. kefyri</i> with inhibition zones of 9-12 mm	[50]
	<i>P. platychlaena</i>	The leaves, stems and flowers oil gave MIC value 1.16 (<i>P. aeruginosa</i>), 3.08 (<i>S. aureus</i>), and 0.86 (<i>S. aureus</i>) mg/mL, respectively	[71]
		The essential oil showed activity against <i>B. subtilis</i> with MIC value 36 mg/mL	[72]
	<i>P. asperula</i>	The essential oil showed activity against <i>Trichophyton rubrum</i> and <i>T. tonsurans</i> with MIC value 64 µg/mL	[53]
	<i>P. uechtritzi</i>	The essential oil showed activity against <i>E. coli</i> with MIC value 9 mg/mL	[72]
<i>P. trifida</i>	The essential oil showed activity against <i>B. subtilis</i> with MIC value 0.12 mg/mL	[82]	
<i>P. uloptera</i>	The essential oil was able to inhibit <i>C. krusei</i> with MIC value 0.039 µL/mL	[36]	
Antioxidant	<i>P. ferulacea</i>	The essential oil gave IC ₅₀ values 100 (flower oil) and 500 µg/mL (leaves oil) in ABTS assay	[34]
		The essential oil gave IC ₅₀ values 726.5 µg/mL (DPPH), 89.5 µg/mL (ABTS), and 52.5 µg/mL (FRAP)	[89]
		The flowers and leaves oil gave IC ₅₀ values 23.9 and 22.9 µL/mL, respectively in DPPH	[45]
	<i>P. heyniae</i>	The essential oil gave 0.43 mg TE/g (DPPH), 92.9 mg TE/g (ABTS), 103.1 mg TE/g (CUPRAC), 61.2 mg TE/g (FRAP), 30.0 mg TE/g (MCA), and 20.3 mmol TE/g (PBD)	[66]
	<i>P. meliocarpoides</i>	The essential oil gave 1.0 mg TE/g (DPPH), 24.1 mg TE/g (ABTS), 113.4 mg TE/g (CUPRAC), 47.9 mg TE/g (FRAP), 28.6 mg TE/g (MCA), and 24.3 mmol TE/g (PBD)	
	<i>P. uechtritzi</i>	The essential oil gave 1.7 mg TE/g (DPPH), 58.1 mg TE/g (ABTS), 109.1 mg TE/g (CUPRAC), 56.4 mg TE/g (FRAP), 30.9 mg TE/g (MCA), and 15.6 mmol TE/g (PBD)	
	<i>P. trifida</i>	The essential oil gave IC ₅₀ values 0.8 mg/mL (ABTS) and 0.11 mg/mL (H ₂ O ₂)	[82]
	<i>P. gaubae</i>	The essential oil gave 2.02 mmol TE/g (ABTS), 0.47 mmol TE/g (CUPRAC), 0.37 mmol TE/g (FRAP), and 37.8 mg EDTAEs/g (Metal chelating)	[84]
Allelopathic	<i>P. pabularia</i>	The essential oil exhibited high activity with IC ₅₀ values 0.11, 0.14 and 0.12 mg/mL for inhibition of the seed germination, shoot and root elongation, respectively	[65]
	<i>P. ferulacea</i>	The essential oil stunted the root growth of lettuce with an IC ₅₀ value 244.19 mg/mL	[40]
Insecticidal	<i>P. ferulacea</i>	The LC ₅₀ and LC ₉₉ values of the essential oil against the egg stages of <i>E. kuehniella</i> and <i>T. embryophagum</i> were 320.3-486.8 µL/L air and 2.1-5.6 µL/L air, respectively	[51]
Larvicidal	<i>P. ferulacea</i>	The LC ₅₀ of essential oil against <i>Cx. quinquefasciatus</i> and <i>An. stephensi</i> were respectively 1.95 and 24.20 ppm for the fruits, 2.75 and 19.60 ppm for leaves, and 2.60 and 21.07 ppm for stems	[90]
	<i>P. heyniae</i>	The essential oil showed good activity at 125 and 62.5 ppm against 1 st instar <i>A. aegypti</i>	[67]
Antiproliferative	<i>P. pabularia</i>	The essential oil by MTT assay against human lung adenocarcinoma epithelial (A549) cells revealed that the activity of 56.12%	[81]
	<i>P. ferulacea</i>	The essential oil exhibited a moderate activity on MDAMB 231 cell line (IC ₅₀ value 22.41 mg/mL), HCT116 (IC ₅₀ value 30.35 mg/mL) and A375 (IC ₅₀ value 25.08 mg/mL)	[89]
	<i>P. asperula</i>	The essential oil exerted activity with IC ₅₀ value 139.17 µg/mL on the renal adenocarcinoma cell line	[54]
Anticholinesterases	<i>P. heyniae</i>	The essential oil gave 9.85 mg GALAE/g against BChE	[66]
	<i>P. ferulacea</i>	The essential oil gave IC ₅₀ value 86.1 µg/mL against AChE	[89]
	<i>P. gaubae</i>	The essential oil gave 2.97 mg GEs/g (AChE) and 3.30 mg GEs/g (BChE)	[84]
Tyrosinase	<i>P. heyniae</i>	The essential oil gave 53.9 mg KAE/g	[66]
	<i>P. meliocarpoides</i>	The essential oil gave 69.5 mg KAE/g	
	<i>P. uechtritzi</i>	The essential oil gave mg 46.3 KAE/g	

Bioactivities	Species	Description	References
	<i>P. gaubae</i>	The essential oil gave 29.2 mg KAEs/g	[84]
Antidiabetic	<i>P. gaubae</i>	The essential oil gave 1.35 mmol AEs/g (α -amylase) and 38.8 mmol AEs/g (α -glucosidase)	[84]
	<i>P. heyniae</i>	The essential oil gave 0.09 mmol ACAE/g (α -amylase)	[66]
	<i>P. meliocarpoides</i>	The essential oil gave 0.41 mmol ACAE/g (α -amylase)	
	<i>P. uechtritzi</i>	The essential oil gave 0.61 mmol ACAE/g (α -amylase)	
	<i>P. pabularia</i>	The essential oil showed PTP-1B enzymatic inhibition with IC ₅₀ value 0.06 μ g/mL	[62]
Repellent	<i>P. acaulis</i>	The repellency at 2.0 μ l/mL was 63.6, 83.6 and 71.6% against <i>T. castaneum</i> , <i>S. oryzae</i> and <i>C. maculatus</i> , respectively	[91]
Antiobesity	<i>P. gaubae</i>	The essential oil gave 1.59 mmol OEs/g (Lipase)	[84]
Wound healing	<i>P. ferulacea</i>	The essential oil significantly enhanced the migration rate of L929 cells by 87.05% at conc. 4.0 μ g/mL	[48]
Toxicity	<i>P. ferulacea</i>	The essential oil showed the fumigant toxicity against <i>Sitophilus oryzae</i> (34.4% mortality, 72 h)	[92]

TE: Trolox equivalent; MCA: Metal chelating ability; PBD: Phosphomolybdenum assay; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; GALAE: Galantamine equivalent; GEs: galanthamine equivalents; AEs: acarbose equivalents; KAEs: kojic acid equivalents; OEs: orlistat equivalents; AChE: acetylcholinesterase; BChE: butyrylcholinesterase

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Contacts: pierangela.rovellini@mi.camcom.it | +39 02 8515.3571



Lipid contents and fatty acid compositions of some soybean varieties and lines

Kagan Kokten¹ ✉

Meliha Feryal Sarikaya¹

Muhammed Tatar¹

Ilker Yuce¹

Pinar Cubukcu²

Celile Aylin Oluk²

Tolga Karakoy³

The seeds of some soybean genotypes were obtained from the Directorate of Eastern Mediterranean Agricultural Research Institute in Adana/Türkiye were grown in 2022 on the Sivas Science and Technology University were investigated for their lipid contents and fatty acid compositions. The lipid contents of the soybean seeds were found to be between 13.1-20.9%. The seed lipids of different soybean genotypes contained linoleic, oleic, palmitic and linolenic acids as their major components. The seed lipids of the soybean lines contain more linoleic acid than the varieties, as the major unsaturated fatty acids, whereas in the seed lipids of Samsoy and Soyanam varieties and all lines except Line 12 contain palmitic acid as the main component of saturated fatty acids. In the study on soybean genotypes, palmitic acid was found in the major saturated fatty acids, instead linoleic oleic, and linolenic acids were found in major unsaturated fatty acids.

Keywords: Soybean, lipid, fatty acid, variety and line

1. INTRODUCTION

Soybean (*Glycine max* L.), belonging to the *Fabaceae* family, is an annual warm climate plant. Soybean, originating from East Asia, is one of the most cultivated oilseed crops in the world. It is grown as an essential dietary component due to its high grain protein (25.5-58.9%) and lipid (12.0-23.0%) content [1]. The total amount of soybean production in the world is approximately 371.7 million tons, while the countries with the highest production are Brazil, USA, Argentina and China, respectively, Turkey ranks 31st with a production of 182 thousand tons [2].

Many industrial products such as soy sauce, soy milk, soy flour, lecithin and animal feed are obtained from soybeans, especially lipid [3]. After palm lipid, soybean lipid is used the most in the world to meet the vegetable lipid need. The amount of 1/3 of the vegetable lipid used for cooking is produced from soybeans [4]. Lipid extracted from soybeans is widely used as a component of frying lipid, margarine and salad dressing, and in the manufacture of inks, paints and cosmetics [5].

Fatty acids are the predominant components of soybean lipid. Fatty acids consist of saturated fatty acids (palmitic acid and stearic acid) and unsaturated fatty acids (oleic acid, linoleic acid and linolenic acid) [6]. Soybean lipid contains approximately 9.96, 3.93, 22.09, 53.46 and 9.05%, palmitic, stearic, oleic, linoleic and linolenic acids, respectively. The amount and relative ratios of each fatty acid are important factors as they affect the flavour, stability and nutritional value of the lipid [7]. Therefore, different fatty acid compositions are desired depending on the end uses of soybean lipid [8].

Many studies have been conducted on the lipid and fatty acid contents of the seeds of plants belonging to the *Fabaceae* family in Turkey [9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21]. The aim of this study was to determine the lipids and fatty acid compositions of the seeds of some soybean genotypes.

¹ Department of Plant Production and Technologies
Faculty of Agricultural Sciences and Technology
Sivas University of Science and Technology
Sivas, Türkiye

² Eastern Mediterranean Agricultural Research Institute
Republic of Türkiye Ministry of Agriculture and Forestry
Adana, Türkiye

³ Department of Plant Protection
Faculty of Agricultural Sciences and Technology
Sivas University of Science and Technology
Sivas, Türkiye

✉ CORRESPONDING AUTHOR:

Kagan Kokten

Department of Plant Production and Technologies,
Faculty of Agricultural Sciences and Technology,

Sivas University of Science and Technology,
Sivas, Turkey.

Fax: +90 4262160030

Tel: +90 5379352592

E-mail: kahafe1974@yahoo.com

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2. MATERIALS AND METHODS

2.1. MATERIALS

The seeds of *Glycine max* were used in this study. The seeds of soybean genotypes obtained from the Directorate of Eastern Mediterranean Agricultural Research Institute in Adana/Türkiye were sown on 28 April 2022 and harvested on 29 October 2022 on the land of Agricultural Research and Development Centre, Sivas Science and Technology University.

2.2. METHODS

2.2.1. Oil Extraction and Preparation of Fatty Acid Methyl Esters (FAME)

Impurities were removed from the seeds of some cowpea genotypes, and the clean seeds were ground into powder using a ball mill. Lipids were extracted with hexane/isopropanol (3:2) [22]. The lipid extracts were centrifuged at 1 g for 10 min and filtered; then the solvent was removed on a rotary evaporator at 50°C. Lipid extraction is used to separate seed oil from lean tissue and measure the energy contained in the seed oil. Each sample was performed in three replications.

2.2.2. Capillary GLC

Fatty acid methylesters were prepared via the methylation of total lipids [23]. Methylesters were separated by gas chromatography in Agilent GC 7890A gas chromatograph equipped with a flame ionisation detector (FID) and a fused silica capillary column Agilent J&W GC Columns (100 m × 0.25 mm id, 0.25 µm film thickness, Part Number 112-88A7). The carrier gas flow (H₂) was 1.2, 30 mL min⁻¹ N₂ and a minimum of 300 mL min⁻¹ synthetic air (H₂). The operation parameters were as follows: the detector temperature was 260°C, the injection port temperature was 250°C and the column temperature was 175°C, programmed to increase at 5°C min⁻¹ to reach 230°C and to hold at this temperature for 21 min. for a running time of approximately 55 min. The sample splitting rate was 1:50. The samples (1 µL) were injected in triplicate. Peak areas were determined using Agilent Chem Station B04.03. To identify the fatty acids retention times were compared with those of standard methyl esters (Supelco 37 Component FAME Mix).

3. RESULTS AND DISCUSSION

In this study, the lipid contents and fatty acid compositions of 9 lines and 5 varieties of soybean [*Glycine max* (L.) Merr.] were detected and the results are shown in Table I. The lipid contents in seeds of 14 genotypes of soybean ranged from 13.1 to 20.9% (Table I). The highest lipid content was found in the Arisoy variety, while the lowest lipid content was found in Line 12. The lipid content was higher in 8

genotypes (Arisoy, Traksoy, Line 4, Line 7, Line 8, Line 9, Line 10 and Line 11) as compared to the other genotypes. It has been reported that the oil content of soybean seeds exposed to drought stress varies between 17.6 and 25.4% [24], and the oil contents of soybean seeds that are applied to different agricultural management systems vary between 21.65 and 22.01% [1], and some agricultural practices applied to soybeans in Central South USA had soybean oil contents between 20.1-24.5% [25]. On the other hand, it has been reported that the oil content of some soybean lines and varieties varies between 17.1-21.0% [26] and 19.9-21.7% [27], while the seed oil content of 94 soybean varieties is reported to vary between 12.2-23.6% [28].

The seeds of some soybean genotypes contain palmitic and stearic acids as the major component of fatty acids among the saturated acids. The major unsaturated fatty acids found in the seeds of some soybean genotypes were oleic, linoleic and linolenic acids. Palmitoleic, myristic, palmitoleic, margaric, margoleic, arachidic, gadoleic, behenic, erucic and docosadienoic acids were shown to be lower than 1%. Palmitic and stearic acid contents ranged from 6.69 to 10.54% and from 2.51 to 4.99%, respectively. While the lowest palmitic and stearic acid contents were found in Line 12, the highest palmitic acid content was found in Line 8 and the highest stearic acid content was found in Soyanam variety (Table I). It has been reported that palmitic and stearic acids vary between 11.0-12.2% and 3.4-4.6%, respectively, in soybean seeds exposed to drought stress [24], between 10.07-12.29% and 3.62-6.10%, respectively, in soybeans applied to different agricultural management systems [1], and between 8.2-17.2% and 2.7-5.2%, respectively, in various soybean germplasms from around the world and grown in China [29]. On the other hand, palmitic and stearic acids were reported to vary between 10.45-12.71% and 3.99-5.79%, respectively, [26], and between 11.28-11.97% and 3.62-4.45% [27] in some soybean lines and cultivars, and reported to vary between 3.14-16.56% and 2.14-4.74%, respectively, in 94 soybean cultivars [28].

Myristic, margaric, arachidic, behenic and lignoceric acids from saturated fatty acids were detected in small amounts in seeds of all soybean genotypes. While the lowest myristic, margaric, arachidic and behenic acid contents were found in line 12, the lowest lignoceric acid content was found in line 10. On the other hand, the highest margaric and arachidic acid contents were determined in the Soyanam variety, the highest myristic acid in Traksoy, the highest behenic acid in Atem-7 variety and the highest in Arisoy variety. Palmitoleic, margoleic and gadoleic acids from unsaturated fatty acids were detected in all soybean genotypes and in small amounts; erucic and docosadienoic acids were either not detected or detected in very small amounts.

Table I - Lipids and fatty acid composition of the seeds of some soybean varieties and lines

Genotypes	Lipid	C14:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2
	(%)								
Arisoy	20.9	0.05	9.81	0.06	0.08	0.06	3.98	24.18	51.31
Traksoy	20.3	0.08	9.86	0.06	0.08	0.04	4.11	24.07	50.94
Samsoy	18.9	0.05	10.30	0.10	0.09	0.06	4.30	24.32	50.25
Soyanam	18.8	0.05	10.47	0.07	0.11	0.06	4.99	24.81	49.38
Ataem-7	19.3	0.05	9.95	0.10	0.08	0.06	3.97	23.26	51.49
Line 4	20.4	0.06	10.20	0.10	0.08	0.06	3.94	23.10	52.09
Line 5	19.2	0.06	10.27	0.06	0.09	0.06	3.90	21.02	53.45
Line 6	18.8	0.06	10.25	0.07	0.10	0.06	3.83	21.60	53.52
Line 7	20.0	0.06	10.37	0.07	0.09	0.05	3.73	21.41	53.62
Line 8	20.4	0.07	10.54	0.07	0.09	0.05	3.77	21.18	53.50
Line 9	20.4	0.06	10.29	0.08	0.09	0.04	4.10	21.88	53.32
Line 10	20.3	0.06	10.19	0.08	0.09	0.05	3.93	21.96	53.29
Line 11	20.4	0.06	10.23	0.07	0.08	0.06	3.95	21.50	53.47
Line 12	13.1	0.04	6.69	0.05	0.05	0.03	2.51	14.97	68.85
SD	0.93	0.02	0.30	0.03	0.01	0.01	0.45	1.89	2.17
Genotypes	C18:3	C20:0	C20:1	C22:0	C22:1	C22:2	C24:0	SFA	USFA
	(%)								
Arisoy	8.42	0.34	0.39	0.06	0.06	0.05	1.16	15.48	84.52
Traksoy	8.68	0.35	0.48	0.12	0.21	0.03	0.89	15.48	84.52
Samsoy	9.15	0.39	0.44	0.09	0.21	0.05	0.21	15.43	84.57
Soyanam	8.86	0.43	0.46	0.09	< 0.01	0.04	0.19	16.33	83.67
Ataem-7	8.75	0.39	0.46	0.35	0.03	0.02	1.05	15.83	84.17
Line 4	9.14	0.34	0.45	0.05	0.20	0.02	0.19	14.85	85.15
Line 5	10.20	0.32	0.36	0.05	< 0.01	< 0.01	0.17	14.86	85.14
Line 6	9.60	0.32	0.36	0.04	< 0.01	0.03	0.17	14.77	85.23
Line 7	9.68	0.31	0.37	0.08	< 0.01	< 0.01	0.17	14.81	85.19
Line 8	9.80	0.31	0.37	0.05	< 0.01	0.02	0.17	15.00	85.00
Line 9	9.13	0.34	0.21	0.04	0.19	0.05	0.16	15.09	84.91
Line 10	9.49	0.31	0.19	0.04	0.13	0.05	0.13	14.75	85.25
Line 11	9.67	0.31	0.32	0.07	< 0.01	0.07	0.14	14.84	85.16
Line 12	6.13	0.20	0.22	0.02	< 0.01	0.07	0.17	9.68	90.31
SD	0.05	0.87	0.14	0.15	0.19	0.69	0.05	0.71	2.06

C14:0 Myristic acid; C16:0 Palmitic acid; C16:1 Palmitoleic acid, C17:0: Margaric acid, C17:1: Heptadecenoic acid, C18:0: Stearic acid, C18:1 Oleic acid; C18:2 Linoleic acid; C18:3 Linolenic acid; C20:0 Arachidic acid; C20:1: Eicosenoic acid, C22:0: Behenic acid, C22:1: Erucic acid, C22:2: Docosadienoic acid, C24:0: Lignoceric acid; SFA: Saturated fatty acid; USFA: Unsaturated fatty acid; SD: standard deviations

Linoleic, oleic and linolenic acids were identified as the main USFA components, and these acids constituted the majority of seed lipids. Oleic acid ranged from 14.97 to 24.81%. Soyanam, (24.81%), Samsoy (24.32%), Arisoy (24.18%) and Traksoy (24.07%) had the highest oleic acid contents. Linoleic acid was the predominant component of seed oils of all studied genotypes. Linoleic acid contents ranged from 49.38 to 68.85%, whereas linolenic acid contents ranged from 6.13 to 10.20%. The highest linoleic and linolenic acids contents were found in Line 12 and Line 5 genotypes, respectively, while the lowest linoleic and linolenic acid contents were found in Soyanam and Line 12 genotypes, respectively.

While oleic, linoleic and linolenic acids in soybean seeds were found to be 23.1-29.6%, 48.5-53.8% and 5.1-7.4%, respectively, [24], 18.7-28.2%, 48.2-57.2% and 5.4-9.7%, respectively, [1] in studies conducted in the USA, they were determined as 13.5-31.9%, 45.6-63.9% and 3.4-12.8%, respectively, in a study conducted in China [29], as 13.4-60.5%, 24.7-64.0% and 2.2-12.9%, respectively, in a study

conducted in Brazil [28], and as 21.7-27.6%, 49.2-54.2% and 5.2-6.8%, respectively, [26], as 22.7-27.9%, 50.0-55.3% and 5.4-6.6%, respectively, [27] in studies conducted in Türkiye.

Unsaturated fatty acid (USFA) contents of studied genotypes were between 83.67 and 90.31% (Table I). Saturated fatty acid (SFA) contents of the studied genotypes were between 9.68 and 16.33%. Soyanam variety had the highest level of SFA; it was respectively followed by Ataem-7 (15.83%), Arisoy (15.48), Traksoy (15.48%) and Samsoy (15.43%) varieties. It has been reported that the saturated and unsaturated fatty acids of some soybean lines and cultivars vary between 15.0-17.5% and 81.5%-85.3%, respectively [26].

4. CONCLUSION

In this study on soybean genotypes, the fatty acid content was composed of 15 different fatty acids. The carbon numbers of these fatty acids range from 14 to 24. The major fatty acids were linoleic (C18:2),

oleic (C18:1), palmitic (16:0) and linolenic (C18:3), respectively. Because of these high values, the seeds can be evaluated as a good source for food, pharmaceutical and other industrial uses. In addition, when compared to these values in the literature, it is understood that soybean seeds are richer in total fat than the other legume seeds. In conclusion, this study highlights the potential of soybean seeds due to their high total fat content.

Conflict of interest

The authors declare that they have no conflict of interest.

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Per informazioni:

Liliana Folegatti
liliana.folegatti@mi.camcom.it

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



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

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

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

according to the intensity of the defects and of the fruitiness perceived, as determined by a group of tasters selected, trained and monitored as a panel, using statistical techniques for data processing.

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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 Forestry Policies 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.



Virgin Olive Oil Organoleptic Assessment



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

Fatty acid profile in mice is modified by *Silybum marianum* oil supplementation in butter

Raja Chaaba^{1,2} ✉

Amel Nakbi^{1,3}

Hanan Jrah⁴

Zahra Amri¹

Sonia Hammami¹

Mohamed Hammami¹

Sounira Mehri¹

¹ University of Monastir

Faculty of medicine

Nutrition-Functional Food & Health-Lab

LR12ES05

Tunisia

² Higher School of Health Sciences and

Techniques

Sousse, Tunisia

³ Superior institute of applied sciences

and technology

Mahdia, Tunisia

⁴ Faculty of pharmacy

Monastir, Tunisia

✉ CORRESPONDING AUTHOR:

Raja CHAABA

Lab-NAFS "Nutrition-Functional Food &

Health"

Faculty of Medicine

Avicene street 5019

Monastir, Tunisia

Tel: 0021698676111

Email: rchaaba@yahoo.fr

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Butter, a dairy product, is rich in saturated fatty acids (FA). Its consumption was thought to be associated with cardiovascular disease (CVD). FA profile is associated to CVD and is influenced by *Silybum marianum* (SM). This study aims to investigate the effect of butter and SM oil supplementation in butter on the FA profile in mice red blood cells (RBC) and liver. Three groups are included: group feeding on a normal diet, group feeding on a normal diet plus 10 % butter and group feeding on a normal diet plus butter supplemented with SM oil (1%). In red blood cells, butter consumption is associated with increased total saturated FA (SFA) and decreased total unsaturated FA (UFA) mainly monounsaturated FA (MUFA). The supplementation of SM oil decreased further trans FA. However, for the liver, butter consumption did not affect the total SFA and UFA but SM oil supplementation is associated with a significant decrease in SFA and increase in UFA. The conclusion was that SM oil supplementation is in favour of a protective FA profile in mice.

Keywords: Butter, desaturase, fatty acids, mice, silybum marianum oil

INTRODUCTION

Numerous studies have examined the association between dietary fatty acids (FA) and the development of diseases, particularly cardiovascular diseases [1]. It is widely recognised that total saturated fatty acids (SFA) are linked to cardiovascular disease. Numerous investigations, however, contradicted this theory by demonstrating a neutral relationship between the total SFA and coronary heart disease [2]. According to other studies that focused on particular saturated fatty acids, increased long-chain SFA (LCSFA: C12 to 18) in the diet has been linked to an increased risk of cardiovascular disease [3]. Butter has a significant amount of these saturated fatty acids, yet there has been controversy over its link to cardiovascular disease and all-cause mortality. Butter was associated with myocardial infarction [4] and inversely associated with the risk of major adverse coronary events (cardiovascular disease, stroke...) [5]. However, it was inversely linked to diabetes [6]. Moreover, a blood FA profile has been linked to the risk of developing multiple diseases including diabetes [7], a cardiovascular disease [8], and breast cancer [9]. In fact, FA in blood and enzymes implicated in FA metabolism could be considered as a disease predictor [10].

The investigations available are not many, though, examining the connection between blood and dietary FAs. The majority of research focuses on the relationship between dietary FA and blood lipids specifically triglycerides, cholesterol (HDL and LDL) rather than blood FA [11]. It will be of great interest to investigate if dietary FA's impact on blood FA could explain the link between disease and diet. However, the blood concentration and nature of fatty acids depend on FA food intake, intestinal FA absorption, and FA metabolism. Any disturbance in those three pathways impact the blood fatty acid profile. As an example of factors that may influence those pathways, we

can include i/ FA supplemented diet [12] ii/ Alcohol extract of nutmeg downregulated the expression of fatty acid synthase [13] iii/ Sake lees extract ameliorates the hepatic lipid accumulation via suppressing fatty acid-induced intracellular lipid accumulation[14] iiiii/ Water extract of *Curcuma longa* L. suppressed the expression levels of fatty acid transport proteins[15] iiiiii/ Pomegranate seed oil and bitter melon extract affect the fatty acid composition and metabolism in the hepatic tissue in rats [16] iiiiii/ Grape skin extracts affect the Stearoyl-CoA Desaturase-1 expression in Caco 2 cells [17]. In fact, the fatty acid profile is influenced by oil and extract added to food.

Silybum marianum (SM) or milk thistle is common in the central region of Tunisia. It has been known to be used as a medicinal plant because it contains silymarin [18]. Silymarin is composed of a group of polyphenolic flavonoids with excellent hepatoprotective activities and hypocholesterolemic, neuroprotective, skin-protective and chemoprotective activities [19]. These biological activities are attributed to the antioxidant properties of silymarin [20]. Additionally, SM oil has been shown to attenuate hepatic steatosis and oxidative stress in high fat diet-fed mice [21]. *Silybum marianum* has a strong potential for usage in a variety of different disciplines besides medicine, including: human and animal nutrition, the cosmetic industry, phytoremediation, and bioenergy products [22].

The objective of our study is to investigate the relationship between butter consumption and the fatty acid profile in mice red blood cells (RBC) and liver, and then to see if the S.M oil supplementation to butter affect this relationship.

MATERIALS AND METHODS

BIOLOGIC MATERIALS

Silybum marianum (SM) oil (provided by huillerie Ben Selma) was extracted by cold pressure from seeds. The plant was collected from the central area of Tunisia.

Cow butter was purchased from the local market.

EXPERIMENTAL PLAN

Male Swiss mice, weighing between thirty and thirty-five grams were provided by the Society of Pharmaceutical Industries of Tunisia (SIPHAT). To discard the effect of sex hormones on fatty acid metabolism, male mice were used. Before starting the experiment, the mice were acclimatised for two weeks. Polyethylene home cages were used with sawdust covering the floor. They were maintained under controlled conditions: 12/12 h light/dark cycle, Temperature close to 22°C (21-23) with water and food (standard commercial pellet chow) offered ad libitum. The mice were housed in accordance with the EEC 609/86 Directives regulating the welfare of experimental animals. The experimental protocol was approved by the Ethics Committee for Research in Life Science and

Health of the Higher Institute of Biotechnology of Monastir (CERSVS/ISBM011/2024) in compliance with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) [23].

Three groups of five mice each were created: Group 1 (control group): following a normal diet, Group 2 (Butter group): feeding a normal diet enriched with 10% commercial butter, Group 3 (SMB group): feeding a normal diet enriched with 10% commercial butter supplemented with SM oil (1% and stored at 4°C).

The experience lasted one month. The animals were ultimately sacrificed. The red blood cells were obtained after manipulating blood and liver samples were collected and stored at -80°C for analysis.

ANALYTICAL METHODS

According to the analytical techniques outlined in the European Union Regulations EEC 2568/91 and EEC 1429/92, the peroxide value (PV), free fatty acid content (% of oleic acid), and UV absorption characteristics at 232 nm (K232) and 270 nm (K270) were measured [24].

To determine the fatty acid composition of SM oil, the Fatty acids were converted into fatty acid methyl esters (FAMES) prepared according to the Regulation EEC/2568/91, EEC/1429/92 of the European Union Commission and the International Olive Council [24,25]. However for butter, red blood cells and liver, fatty acids were extracted according to the Folch method, and then methylated by BF₃ in methanol [26]. The chromatographic separation was provided by a gas chromatography using model 5890 series II instrument (Hewlett-Packard Ca Palo Alto, Calif. USA) equipped with a flame ionisation detector and a fused silica capillary column HP - INNOWAX (30 m length × 0.25 mm and 0.25 µm of film thickness). The temperature was programmed to increase from 180 to 240°C. Nitrogen (1 ml/min) was used as gas carrier. The results were expressed as a relative area percent of the total FAMES.

Estimated fatty acid desaturase and elongase activities are determined by the product/precursor ratio: D9D = C16:1w7/C16:0; D6D = C18:3w6/C18:2w6; D5D = C20:5w3/C20:4w3; D4D = C22:6w3/C22:5w3; Elongase = C18:0/C16:0.

The phenolic compounds were estimated colorimetrically at 765 nm [27]. The result was expressed as mg of gallic acid equivalents/ g of oil (mg GAE/g). 5 g of oil are mixed with 5 ml of the methanol/tween solution (80%/20%), the mixture is stirred for one minute using an ultra turrax before being centrifuged for 12 min at 2850 rpm, and finally, we recover the upper phase. The extraction is repeated 3 times with the residue and the methanolic fraction is left at -20°C overnight. To 200 µl of extract, 800 µl of distilled water and 5 ml of folin reagent are added. The mixture is incubated for one minute in the dark and then 4 ml of sodium carbonate (7.5%) is added. After vortexing, the mixture is left in the dark for 2 hours and then the absor-

bance is read at 765 nm. Under the same conditions, a standard range is prepared with gallic acid.

Total flavonoid content was evaluated by colorimetric method at 510 nm [28]. 1 ml of the methanolic extract is mixed with 4 ml distilled water and 0.3 ml NaNO₂ (5%). After 5 min, 0.3 ml of AlCl₃ (10%) is added and following a 6 min rest, 2 ml NaOH (1M) is added. The volume is adjusted to 10 ml by 2.4 ml of distilled water. After using a vortex to homogenise the mixture, the absorbance at 500 nm is measured. The result was expressed as mg of catechin equivalent / g of oil).

Oxidative stability measures the resistance of the oil against oxidation. Measurement of the induction period was determined by using a Rancimat apparatus, model 734 (Metrohm, Herisau, Switzerland). The temperature was 120°C and the air flow was 20 l/h [29]. Antioxidant Activity (DPPH Radical Scavenging Assay) was evaluated according to the method described by Blois et al.[30].

Anti-pancreatic lipase activity was determined as described by Gooda Sahib et al. [31].

Calcium and zinc concentrations were measured by atomic absorption spectroscopy "Spectrum SP-AA 4000" (NF V 05-113, 1972).

STATISTICAL ANALYSIS

All experiments were carried out in triplicate. Results were expressed as means ± standard deviations. The comparison was performed using the student-t test and ANOVA. Data were analysed with SPSS (version 12.00 for Window, SPSS Inc., Chicago, IL 2003). Differences were deemed significant at P<0.05.

RESULTS

SM OIL CHARACTERISATION

Table I provides a summary of the extracted SM oil's characteristics. Total phenol and total flavonoid concentrations are important in SM oil. In Parallel, the antioxidant capacity (DPPH, reducing power) is high. The SM oil has a high content of UFA (81.93%), with PUFA being the most common (53.61%).

BUTTER CHARACTERISATION

Butter has low water content and is very rich in lipids. Butter has a dry matter content of 86.15 ± 1.05 percent (Table II). The percentage of lipid content are 76.25 ± 3.18%. Almost 69% of fatty acids are saturated (Table II). Palmitic acid is the most common SFA (29.71%). Unsaturated fatty acids (29.96%) are represented mainly by monounsaturated fatty acids (oleic acid (25.12%). The concentrations of calcium and zinc are respectively 10.9 and 0.782 mg/100 g of butter.

FATTY ACIDS IN MICE

RBC Fatty acid variation between all groups is summarised in Table III. Saturated fatty acids increased in butter and SMB groups compared to the control group (p < 0.001). Inversely, UFA is lower in the SMB and butter groups than in the control group (p < 0.001). From UFA, MUFA decreased significantly in the butter group. Compared to the control group, trans FA dropped in the butter group and dropped more in the SMB group (p < 0.001). The difference between butter and the SMB groups is significant (p

Table I - Characteristics and fatty acid composition of *Silybum marianum* seed oil

Characteristics			
Acidity [%]	0.733 ± 0.028	Total phenol (mg GAE/g)	5.1 ± 0.85
PV [MeqO2/Kg]	5.33 ± 0.001	Total flavonoid (mg CE/g)	3.92 ± 0.03
K232	1.908 ± 0.004	DPPH (%)	64
K270	0.169 ± 0.005	Reducing power	0.306
OSI: oxidative stability Index [hour]	4.14	Lipase inhibition (%)	75.45
Fatty acid (%)			
Palmitic acid C16:0	13.37		
Palmitoleic acid C16:1	0.157		
Margaric acid C17:0	0.093		
Margaroleic acid C17:1	0.139		
Stearic acid C18:0	1.492		
Oleic acid C18:1	28.029		
Linoleic acid C18:2	52.954		
Linolenic acid C18:3	0.656		
Arachidic acid C20:0	2.203		
SFA	17.164		
UFA	81.935		
MUFA	28.324		
PUFA	53.611		

Total phenol (mg GAE/g)

Total flavonoid (mg of catechin equivalent /g of oil)

DPPH free radical scavenging activity

SFA: saturated fatty acids, UFA: unsaturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Table II - Physicochemical properties, composition and fatty acids of commercial butter

Physicochemical properties		Composition	
Peroxide value	4.66 ± 0.11	Lipid content [%]	76.25 ± 3.18
TBARS	0.76 ± 0.08	Calcium [mg/100g]	10.9 ± 1.36
Dry matter [%]	86.15 ± 1.05	Zinc [mg/100g]	0.782 ± 0.58
Fatty acids [% of total FA]			
C4 :0	4.59 ± 0.83	C18:1 n11 (trans)	0.034 ± 0.02
C6 :0	4.31 ± 0.53	C18:1 n-9 (cis)	23.88 ± 2.47
C8 :0	3.58 ± 1.11	C18:1 n-7(cis)	1.046 ± 0.26
C10 :0	2.11 ± 0.16	C18:2 (t9,t12)	0.113 ± 0.14
C12 :0	2.78 ± 0.29	C18:2 (c9,t12)	0.017 ± 0.01
C13 :0	0.232 ± 0.06	C18:2 (t9,c12)	0.072 ± 0.05
C14 :0	9.737 ± 0.59	C18:2 n-6 (c9,c12)	0.123 ± 0.10
C14 :1 n-9	0.058 ± 0.02	C18:2 (c9,t11)	0.019 ± 0.02
C15 :0	0.142 ± 0.07	C18:2 (t10,c12)	0.011 ± 0.01
C16 :0	29.71 ± 0.77	C18:3 n-3 (cis)	0.944 ± 0.26
C16 :1 n-9	0.017 ± 0.02	C18:3 n-6	0.028 ± 0.01
C16:1 n-7 (cis)	2.235 ± 0.15	C20 :0	0.157 ± 0.13
C17:0	0.838 ± 0.13	C20 :1	0.671 ± 0.09
C17 :1	0.592 ± 0.13	C22 :0	0.090 ± 0.03
C18 :0	11.08 ± 0.88	C21 :0	0.526 ± 0.07
C18:1 n-9 (trans)	0.013 ± 0.001	C24 :0	0.119 ± 0.04

TBARS (Thiobarbituric acid reactive substances)

= 0.024). n-3 FA decreased in the SMB group compared to the butter group ($p < 0.001$). However, there is no noticeable difference from the control group. Liver fatty acid variation between all groups is summarised in Table IV. Total SFA and UFA did not differ between the control and butter groups.

In the SMB group SFA decreased and UFA with PUFA increased significantly compared to the control and butter groups. Also, n-3 FA increased in the SMB group compared to the control group (mainly DHA). Whereas trans FA did not differ between all groups.

There are no significant variations in D6D, D5D, and elongase across the three groups regarding desaturase and elongase indexes in RBC (figure 1). In contrast to the butter group, the SMB group has a lower D9D index. The D4D index was higher in the butter group compared to the control group with no difference in the SMB group.

In the liver, D4D and D5D indexes increased in the butter and SMB groups compared to the control group. There was no difference in the elongase index between the butter and SMB groups; instead, it declined in the butter group and then increased in the SMB group. There was no significant difference in the D9D and D6D indexes between all groups (figure 2).

DISCUSSION

The SM oil has a significant antiradical activity and oxidative stability. As far as we know, few studies focused on phenolic compounds and antiradical activity of SM seed oil produced under cold pressure [32] [33]. Our findings confirmed the high total phenolic

content of the Tunisian variety reported by Meddeb et al. [32]. In fact, the method used in our and in the last-mentioned study to measure polyphenols is not the optimal one. Other investigations, using HPLC technique, have found lower concentrations of phenolic compounds [33]. In contrast to numerous previous researches, ours has a better oxidative stability index [32].

Results on fatty acid composition from multiple studies are nearly identical [32] [34] [35]. The major fatty acid was Linoleic acid C18:2 (59.95%), followed by oleic acid (20.029%). The amount of those fatty acids varies throughout the research. Environmental variables or the use of various extraction techniques could account for this discrepancy [35]. Because of its fatty acid composition, SM oil has a significant impact on human health, particularly in terms of preventing cancer and cardiovascular diseases [36]. Our results, on FA in butter, are consistent with those of numerous earlier studies in which butter is high in saturated fat (almost 60%) [37].

In this work, we investigated how mice's fatty acid profiles, as well as desaturase and elongase indices, were affected by butter and butter supplemented with SM oil. We demonstrated that the butter group had higher SFA in RBC. The rise in butyric acid, capric acid, lauric acid, myristic acid, and palmitic acid is the primary cause of the increased SFA. Butter has those FAs in it. Thus, diet intake may account for their increase. Moreover, the butter and SMB groups showed an increase in butyric acid, one of the short-chain FAs (SCFA). Butyric acid, or SCFA, can be synthesised in the intestine by the "gut microbiota" or

consumed by food [38]. We can propose that adding SM oil to butter stimulates the gut microbiota to produce SCFA. Because SM oil contains a high concentration of phenolic compounds, a previous study showed that phenolic compounds have a beneficial effect on gut microbiota [39].

FA with more than 12 molecules of carbon increased in the butter group but decreased in the SMB group. Moreover, FA which decreased in the butter group, decreased more in the SMB group. This could be explained by the fact that the SM oil inhibits pancreatic lipase (shown in this study) and therefore some of the

Table III - Fatty acid composition of red blood cells in different groups of rats; control group (normal diet), butter group (diet supplemented with butter), SMB group (diet supplemented with butter and SM oil)

Fatty acids	control	Butter group	SMB group	pANOVA
C4:0	0.058 ± 0.012	0.741 ± 0.324 ^P	***2.315 ± 0.115***	0.00
C6:0	0.020 ± 0.001	0.400 ± 0.541	*1.439 ± 0.428*	0.005
C8:0	0.024 ± 0.003	0.594 ± 0.719	1.211 ± 1.104	0.215
C10:0	0.022 ± 0.001	0.149 ± 0.077 ^P	**1.659 ± 0.486**	0.000
C12:0	0.022 ± 0.001	1.073 ± 0.284 ^{P/P}	***1.491 ± 0.249	0.000
C13:0	0.064 ± 0.057	0.318 ± 0.052 ^{P/P}	**0.239 ± 0.040	0.000
C14:0	0.696 ± 0.109	2.248 ± 0.459 ^{P/P}	0.594 ± 0.100***	0.000
C14:1n-9	1.369 ± 0.012	1.097 ± 0.138 ^P	1.141 ± 0.227	0.133
C15:0	1.356 ± 0.167	0.153 ± 0.060 ^{P/P/P}	1.083 ± 0.333**	0.000
C16:0	26.180 ± 2.214	33.345 ± 2.262 ^P	25.135 ± 1.735**	0.001
C16:1 n-9	3.402 ± 0.294	0.527 ± 0.225 ^{P/P/P}	*1.785 ± 0.897*	0.001
C16:1 n-7 cis	1.401 ± 0.136	2.238 ± 0.278 ^{P/P}	1.242 ± 0.117**	0.000
C17:0	5.885 ± 1.052	0.875 ± 0.149 ^{P/P/P}	5.132 ± 1.158***	0.000
C18:0 acide stéarique	10.729 ± 1.086	11.397 ± 0.312	10.394 ± 0.931	0.335
C18:1 n-9 cis	27.118 ± 0.657	23.916 ± 0.737 ^{P/P}	27.181 ± 5.155	0.325
C18:1n-7cis	1.079 ± 0.160	0.718 ± 0.279	0.956 ± 0.124	0.112
C18:1 n-9 trans	0.624 ± 0.160	0.261 ± 0.060 ^{P/P}	0.521 ± 0.081**	0.004
C18:2 t9c12	1.193 ± 1.621	1.754 ± 0.507	0.079 ± 0.038**	0.068
C18:2 c9t12	1.802 ± 1.791	0.097 ± 0.031	0.437 ± 0.183*	0.090
C18:2 c9c12	12.561 ± 1.410	13.762 ± 0.786	13.007 ± 3.742	0.808
C18:3 n-3 cis	0.502 ± 0.082	0.934 ± 0.255 ^P	0.356 ± 0.113**	0.004
C18:2 t10c12	0.351 ± 0.091	0.511 ± 0.068 ^P	0.297 ± 0.040**	0.005
C18:2 t9 t11	0.150 ± 0.090	0.173 ± 0.016	0.073 ± 0.057*	0.096
C18:2 c11t13	0.495 ± 0.108	0.614 ± 0.118	0.353 ± 0.88*	0.024
C18:3 n-6	0.493 ± 0.107	0.488 ± 0.231	0.389 ± 0.127	0.653
C20:0	0.112 ± 0.049	0.080 ± 0.021	0.063 ± 0.019	0.173
C20:1 n-9	0.141 ± 0.066	0.076 ± 0.047	0.080 ± 0.023	0.191
C21:0	0.149 ± 0.064	0.204 ± 0.044	0.081 ± 0.017**	0.013
C22:0	0.527 ± 0.062	0.590 ± 0.082	*0.363 ± 0.097*	0.014
C20:3 n-3	0.102 ± 0.097	0.035 ± 0.033	0.035 ± 0.030	0.275
C20:4 n-6	0.112 ± 0.050	0.058 ± 0.013	0.072 ± 0.024	0.127
C20:4 n-3	0.239 ± 0.237	0.073 ± 0.035	0.111 ± 0.076	0.279
C23:0	0.131 ± 0.053	0.078 ± 0.040	0.127 ± 0.046	0.272
C20:5	0.147 ± 0.023	0.094 ± 0.046	*0.093 ± 0.029	0.140
C24:0	0.216 ± 0.065	0.136 ± 0.045	0.141 ± 0.022	0.096
C22:5 n-3	0.152 ± 0.096	0.021 ± 0.014 ^P	0.146 ± 0.101*	0.092
C22:6 n-3	0.177 ± 0.129	0.137 ± 0.086	0.081 ± 0.065	0.424
C24:1	0.223 ± 0.087	0.022 ± 0.008 ^{P/P}	*0.082 ± 0.045*	0.003
SFA	46.193 ± 1.855	52.388 ± 0.641 ^{P/P}	**51.473 ± 0.901	0.000
UFA	53.804 ± 1.854	47.613 ± 0.643 ^{P/P}	**48.525 ± 0.901	0.000
MUFA	35.360 ± 0.333	28.858 ± 0.549 ^{P/P/P}	32.991 ± 4.423	0.036
PUFA	18.444 ± 2.185	18.755 ± 0.785	15.534 ± 3.668	0.213
n-6 FA	16.020 ± 2.338	16.183 ± 0.586	13.893 ± 3.736	0.434
n-3 FA	1.667 ± 0.497	1.689 ± 0.112	1.120 ± 0.123***	0.031
Trans FA	4.587 ± 0.365	3.411 ± 0.772	*1.761 ± 0.203**	0.013

Significant difference ^Pbutter/control ♦SBM/control *butter/SMB groups

Table IV - Fatty acid composition of liver in different groups of rats; group1: control (normal diet), group 2: (diet supplemented with butter), group 3: (diet supplemented with butter and SM oil)

Fatty acids	Control	Butter group	SMB group	pANOVA
C4:0	0.058 ± 0.029	1.627 ± 0.336 ^{BP}	**0.205 ± 0.041 ^{***}	0.00
C6:0	0.061 ± 0.028	0.806 ± 0.351 ^P	0.067 ± 0.025 ^{**}	0.001
C8:0	0.021 ± 0.009	1.211 ± 1.528	0.073 ± 0.121	0.158
C10:0	0.100 ± 0.074	1.263 ± 1.138	0.137 ± 0.043 [*]	0.057
C12:0	0.207 ± 0.328	1.953 ± 0.628 ^{BP}	*0.804 ± 0.210 ^{**}	0.001
C13:0	0.618 ± 0.427	0.111 ± 0.024	*0.121 ± 0.063	0.017
C14:0	0.728 ± 0.192	0.787 ± 0.152	0.633 ± 0.174	0.432
C14:1 n-9	0.469 ± 0.189	0.376 ± 0.326	0.721 ± 0.366	0.299
C15:0	0.155 ± 0.121	0.067 ± 0.095	0.168 ± 0.087	0.325
C16:0	20.087 ± 0.434	20.042 ± 0.722	18.863 ± 1.398	0.201
C16 :1 n-9 trans	0.439 ± 0.036	0.429 ± 0.042	0.422 ± 0.033	0.819
C16 :1 n-7 cis	1.107 ± 0.010	1 .059 ± 0.060	1.146 ± 0.191	0.633
C17:0	0.581 ± 0.040	0.634 ± 0 .603	0.898 ± 0.771	0.737
C18:0	25.691 ± 0.262	23.775 ± 1.313	24.104 ± 1.867	0.248
C18 :1 n-9 cis	0.637 ± 0.172	0.809 ± 0.297	0.706 ± 0.285	0.703
C18:1 n-7 cis	7.624 ± 0.911	9.027 ± 2.856	7.715 ± 0.9634	0.508
C18:1 n-9 trans	1.229 ± 0.790	0.644 ± 0.246	0.860 ± 0.170	0.234
C18:2 t9c12	0.073 ± 0.003	0.076 ± 0.035	1.785 ± 2.456	0.256
C18:2 cis 9t12	0 .127 ± 0. 015	0.117 ± 0.021	2.193 ± 3.746	0.404
C18 :2 n-6 c9c12	1.208 ± 0.078	1.092 ± 0.175	1.505 ± 0.502	0.245
C18 :3 n-3 cis	6.596 ± 0.308	7.344 ± 2.627	7.390 ± 2.297	0.867
C18 :2 t10c12	0.108 ± 0.008	0.082 ± 0.028	0.112 ± 0. 021	0.163
C18 :2 t9 t11	0.297 ± 0.014	0.224 ± 0.152	0.321 ± 0.084	0.405
C18 :2c11t13	0.007 ± 0.003	0.005 ± 0.003	0.016 ± 0.022	0.506
C18:3 n-6	1.723 ± 0.024	1.344 ± 0.552	1.497 ± 0.368	0.496
C20:0	0.596 ± 0.036	0.490 ± 0.366	0.533 ± 0.103	0.828
C20:1 n-9	0.083 ± 0.011	0 .094 ± 0.066	0.091 ± 0.031	0.940
C21:0	0.242 ± 0.006	0. 285 ± 0.112	0. 240 ± 0.069	0.674
C22:0	0.336 ± 0.056	0 .663 ± 0.025 ^{BPBP}	**0.652 ± 0.085	0.000
C20:3 n-3	0.593 ± 0.060	0. 634 ± 0.036 ^P	0.629 ± 0.143	0.856
C20:4 n-6	10.775 ± 0.486	9.583 ± 0.538 ^{BP}	10.158 ± 0.688	0.079
C20:4 n-3	0.580 ± 0.018	0.207 ± 0.146	**0.178 ± 0.129	0.003
C23:0	0.715 ± 0.036	0.236 ± 0.142 ^{BP}	0.268 ± 0.117	0.001
C20:5	2.388 ± 0.050	1.684 ± 0.981	1.903 ± 0.861	0.537
C24:0	4.788 ± 0.173	0.563 ± 0.089 ^{BPBP}	0.726 ± 0.313	0.000
C22:5 n-3	8.253 ± 0.133	4.583 ± 1.893 ^P	*5.207 ± 1.403	0.020
C22:6 n-3	0.576 ± 0.331	6.008 ± 3.179 ^P	**6.854 ± 2.397	0.016
C24:1	0.109 ± 0.111	0.051 ± 0.056	0.084 ± 0.033	0.531 BioMed
SFA	54.990 ± 0.562	54.519 ± 1.540	*48.497 ± 2.888 ^{**}	0.003
UFA	45.008 ± 0.561	45.479 ± 1.540	*51.503 ± 2.890 ^{**}	0.003
MUFA	11.700 ± 0.624	12.492 ± 3.155	11.749 ± 1.900	0.859
PUFA	33.307 ± 0.455	32.987 ± 4.441	*39.754 ± 3.606 [*]	0.032
n-6 FA	14.014 ± 0.365	12.295 ± 1.002 ^P	17.252 ± 3.60	0.148
n-3 FA	16.600 ± 0.061	18.778 ± 2.614	*20.259 ± 2.041	0.098
Trans FA	1.847 ± 0.784	1.158 ± 0.183	5.292 ± 5.481	0.248

Significant difference ^Pbutter/control [◆]SBM/control ^{*}butter/SMB groups

FA will not cross the intestinal wall.

FA in red blood cells represents an imprint of FA in food [40]. Circulating FA could be considered as biomarkers of disease development [10]. Studies showed that butyrate can decrease the coronary artery disease risk [41]. Another study considers C22:0, C24:0, C26:0, C25 n-3 (EPA), and C20:4 n-6 (AA) as biomarkers of CAD and trans FA as CAD severity

predictor [42]. In this study, we showed that butter is linked to higher levels of butyric acid and lower levels of oleic acid. This finding proved the correlation between butter consumption and a preventative FA profile. On the other hand, SM oil supplementation is associated with decreased C22:0, C20:5, and trans FA. Therefore, adding SM oil to a diet helps improve the fatty acid profile, which helps avoid CAD.

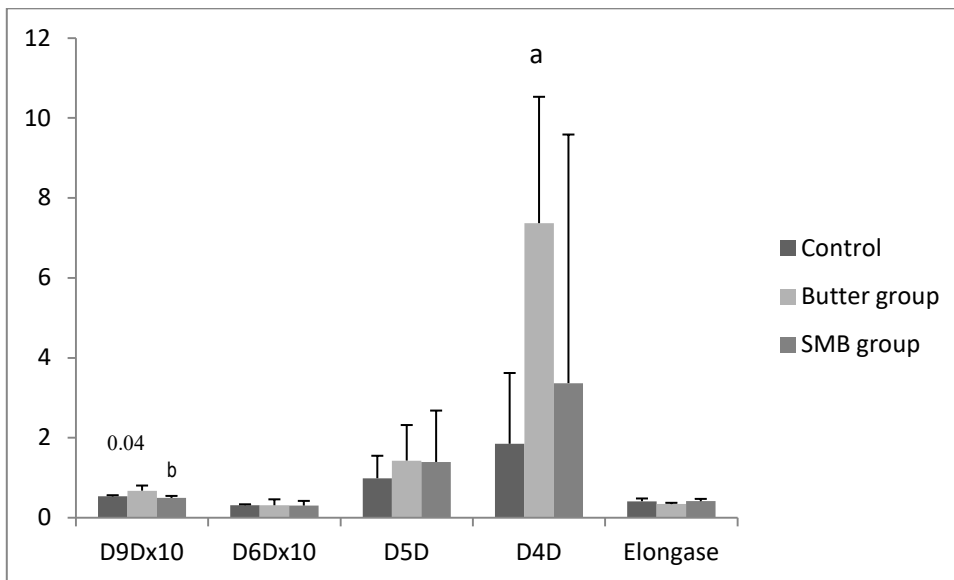


Figure 1 - Estimated desaturase and elongase activity variation in red blood cells of different groups of mice: control (normal diet), Butter (diet supplemented with butter), SMB (diet supplemented with butter and SM oil). D9D = C16:1n-7/C16:0; D6D = C18:3n-6/C18:2n-6; D5D = C20:5n-3/C20:4n-3; D4D = C22:6n-3/C22:5n-3; Elongase = C18:0/C16:0.

The values displayed above the histograms are the significant values of pANOVA: difference between three groups. ^asignificant difference with control; ^bsignificant difference with butter group

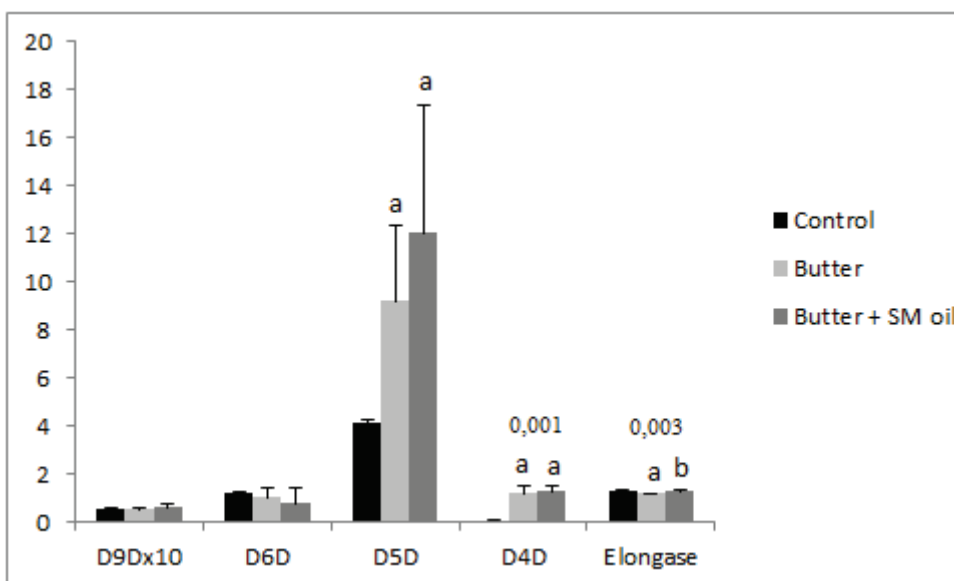


Figure 2 - Desaturase and elongase index variation in liver of different groups of mice: control (normal diet), Butter (diet supplemented with butter), SMB (diet supplemented with butter and SM oil). D9D = C16:1n-7/C16:0; D6D = C18:3n-6/C18:2n-6; D5D = C20:5n-3/C20:4n-3; D4D = C22:6n-3/C22:5n-3; Elongase = C18:0/C16:0.

The values displayed above the histograms are the significant values of pANOVA: difference between three groups. ^asignificant difference with control; ^bsignificant difference with butter group

In the liver, there are no differences in total SFA or UFA between the butter and control groups. However, many SFA increased in the butter group such as C4:0, C6:0, C12:0, and C22:0 and other SFA decreased such as C23:0 and C24:0. The change in fatty acid content in the liver may be the result of changes in dietary FA. The FAs that increased in the butter

group, are decreasing in the SMB group compared to the butter one. Thus, two hypotheses are plausible: i- SM oil masks the effect of butter consumption on fat profile. ii- SM oil decreases the content of FA which have raised under the effect of butter consumption. In summary, the impact of butter and SM oil on fatty acid metabolism may account for the differences in

fatty acid profiles observed in the control, butter, and SMB groups. Butter and SM oil supplementations are associated with increased D5D and D4D enzyme activities. Moreover, butter inhibits the elongase enzyme. However, SM oil activates it. This could be explained by the effect of butter and SM oil compounds (FA, zinc, phenolic compounds, flavonoids...). Previous studies showed that α -linolenic acid and anthocyanins affect desaturase activities [43]. Hydroxytyrosol and high fat diet influence desaturase expression and activity [44]. Silibinin, a phenolic compound of SMO, downregulates many lipogenic genes (FATP5, SREBP-1) and gluconeogenic genes [45]. We can suggest that SMO enhances the lipid (FA) composition of the liver by raising UFA, primarily ω 3, in addition to its lipogenic effect (ascribed to silibinin).

CONCLUSION

SM oil supplementation influences fatty acid profile in RBC and liver. It enhances the fatty acid profile to help prevent an illness. It would be very interesting to add SM oil to butter for good health. However, to further understand the impact of adding SM oil to the taste and stability of butter, additional research ought to be considered.

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Improving efficiency and sustainability with a new smart device for the olive oil chain

Marco Menegon, Valentina Giovenzana, Andrea Casson, Alessia Pampuri, Alessio Tugnolo, Roberto Beghi, Riccardo Guidetti

Department of Agricultural and Environmental Sciences
Production, Territory, Agroenergy
Università degli Studi di Milano, via Celoria 2, 20133 Milano, Italy

✉ CORRESPONDING AUTHOR:

Valentina Giovenzana
valentina.giovenzana@unimi.it, +39 025021974

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Presentato al centenario RISG

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

15 novembre 2023

The control of the fruit ripening phase in the olive oil production chain has always been an important aspect of managing the quality of the final product. Currently, laboratory analyses are applied to olives to assess their degree of ripeness. However, these chemical analyses are destructive, require qualified operators, and expensive equipment, and are often unsustainable due to chemical reagents. Optical technologies offer a promising alternative for estimating quality parameters. These methods are non-destructive, rapid, objective, and environmentally friendly. Unfortunately, commercially available spectrophotometers are mainly benchtop instruments, expensive, and they are difficult to use for field measurements.

Nowadays, technological innovation has led to the miniaturization of optical sensors and the development of portable devices with performance like benchtop instruments. SmartHAND (Smart Handheld Analyzer Non Destructive) is a low-cost optical prototype that operates in the visible (Vis) and near-infrared (NIR) spectra; this device consists of photodiodes, optical filters, and LEDs capable of analysing 12 different wavelengths. Using field-collected data, and applying different chemometric processing, it is possible to develop predictive models to estimate quality parameters such as water and oil content in olives.

Furthermore, the environmental impact of using vis/NIR optical technologies instead of conventional laboratory analyses (chemical analyses performed on olives and olive oil) has been evaluated using the internationally recognized Life Cycle Assessment (LCA) method (ISO 14040 and 14044 standards). The results demonstrate the advantages of innovative optical methods over traditional chemical approaches.

In conclusion, the adoption of portable optical technologies could revolutionize the monitoring of olive ripening, allowing for faster and more accurate assessments directly in the field.

Keywords: optical sensor, agrifood sector, life cycle assessment, quality, vis/NIR, spectroscopy

INTRODUCTION

In the olive oil sector monitoring the fruit ripening is a very important aspect to consider for obtaining a high-quality finished product. The evaluation of the degree of maturation is undergoing an evolution from a technological point of view. In the past (1975) a colorimetric index was developed to evaluate the degree of ripeness of olives: the Uceda and Farias ripeness index (Figure 1). The index does not

determine the degree of ripeness with scientific methodologies but it is based on the visual evaluation of the epicarp of the fruit and the farmer's experience. This method, still partially in use, is not completely objective, implying a high variability in the evaluation of the sample [5].

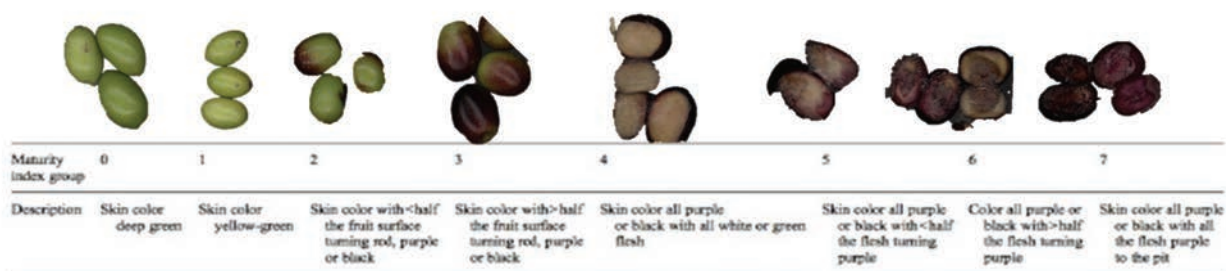


Figure 1 – Uceda and Frias index, developed in 1975 [5]

Nowadays, however, the analysis of drupe ripening through the use of chemical analyses is consolidated, as they are objective measures, but on the other hand not very sustainable in several respects. Chemical analyses require qualified staff to be carried out, require very expensive instrumentation, and sample preparation, are time-consuming, require the use of chemical reagents, and for this reason, could have a high impact on the environment [1]. A modern alternative to chemical analyses is represented by optical technologies, capable of estimating the desired parameters, after careful calibration of the instrument. Despite chemical analyses, optical analyses do not require the use of chemical reagents and are simpler to use, have a real-time response time (from a few milliseconds to a maximum of one minute), and in most cases they are non-destructive as no preparation of the sample is required, highlighting a potential low environmental impact [3].

Spectroscopy is an optical technique by which light radiation, characterized by a specific wavelength range, hits the matrix to be analysed, returning an optical fingerprint (spectrum) based on how much of this energy is absorbed, reflected, or transmitted by the sample itself. The characteristics of light radiation allow different information to be obtained from spectral acquisitions related to the nature of the molecules, since their bonds vibrate differently at certain wavelengths, making them recognisable. The optical range most used in the agri-food sector is the visible (vis, 350 nm – 700 nm) and near-infrared (NIR, 700 nm – 2500 nm). The information deriving from the spectra is subsequently processed using chemometrics, a statistical science that deals with the analysis of multivariate data to extract useful information relating to the characteristics of the analysed matrix [4].

At a commercial level, the spectrophotometers on the market are often large, expensive instruments, classified as "benchtop" and they can only be used in laboratories or large companies. Technological innovation, however, has made it possible to miniaturize optical sensors in an increasingly significant way, leading to the development of a new generation of devices, compact, portable, and with excellent performances comparable to benchtop instruments. The Department of Agricultural and Environmental Sciences of the University of Milan has developed and patented a new optical, smart, cost-effective, user-friendly, and environmentally sustainable prototype to support the olive supply chain, the SmartHAND [3].

EXPERIMENTAL PART

To develop a new smart device for analysing olive ripening and identifying the ideal harvest time, it was necessary to proceed through well-defined steps, namely (i) a feasibility study, which involved chemometrics analysis with multivariate statistical analysis, sampling at different ripening stages, optical acquisition, and corresponding reference measurement (ripening index), and model creation for ripening degree identification, (ii) selection of representative wavelengths for drupe ripening, and (iii) prototype development (SmartHAND) for analysing olive ripening directly in the field.

APPLICATION OF vis/NIR SPECTROSCOPY FOR MATURITY DEGREE ANALYSIS

For the feasibility study, a commercial portable instrument (Jaz, OceanOptics, The Netherlands) was used to acquire the optical spectra necessary for final device development. Jaz is a portable vis/NIR spectrophotometer that operates in a wavelength range between 400 and 1000 nm. It consists of a halogen lamp as a light source, an optical fiber cable that works in reflectance (i.e., acquiring light reflected from the sample), a measurement management module, and a microprocessor for spectrum acquisition, which are saved on a microSD memory card for data storage. The instrument acquires spectra in reflectance; therefore, the light radiation from the lamp is guided through the optical fiber to the sample. The light reflected from the sample, through the optical fiber reaches the spectrophotometer, recording for each wavelength the intensity signal of light reflected from the olive. The feasibility study used the Maturity Index (MI) as a reference [4].

SELECTION OF WAVELENGTHS

The selection of wavelengths characterizing olive ripening degree started from the Uceda and Frias 1975 index. The 8 classes of the index were reduced to 4, creating a new index called S.C.I. (Superficial Colorimetric Index). This method involves a visual evaluation of skin colour only, and was applied to simplify the MI procedure, classifying olives as green, less than 50% ripe, more than 50% ripe, and fully ripe [4].



Figure 2 – S.C.I. Superficial Colorimetric Index

The experimentation involved dividing the samples into the 4 S.C.I. classes (Figure 2) and acquiring optical spectra using the portable Jaz spectrophotometer. From the spectra obtained, chemometric techniques were used to select the most informative wavelengths for ripening degree estimation.

PROTOTYPE DEVELOPMENT

The idea of using a simplified instrument with a reduced number of wavelengths, and thus low cost, is to directly assess olive ripeness in the field by farmers, providing immediate, easily interpretable information on olive ripeness (Figure 3). This allows for standardizing harvesting time and consequently oil quality. The information gathered in the field is then sent to a service cloud for data management. Speed of response is one of the strengths of these technologies, along with no training costs, transportation costs, use of chemical reagents, and time savings, all of which classify these technologies as low-impact and entirely green.



Figure 3 – Representation of olive ripeness assessment directly in the field using a portable optical instrument

ENVIRONMENTAL IMPACT ASSESSMENT

Optical instrumentation can be considered a green technology, and therefore, the environmental impact of these optical analyses compared to conventional chemical analyses was evaluated using the Life Cycle Assessment (LCA) method, recognized internationally according to ISO 14040 and 14014 standards.

The LCA study took a "cradle-to-grave" approach (Figure 4), considering all inputs and outputs contributing to the environmental impact by measuring three key analytical parameters for olive ripening: moisture content, oil content, and phenols content [2].

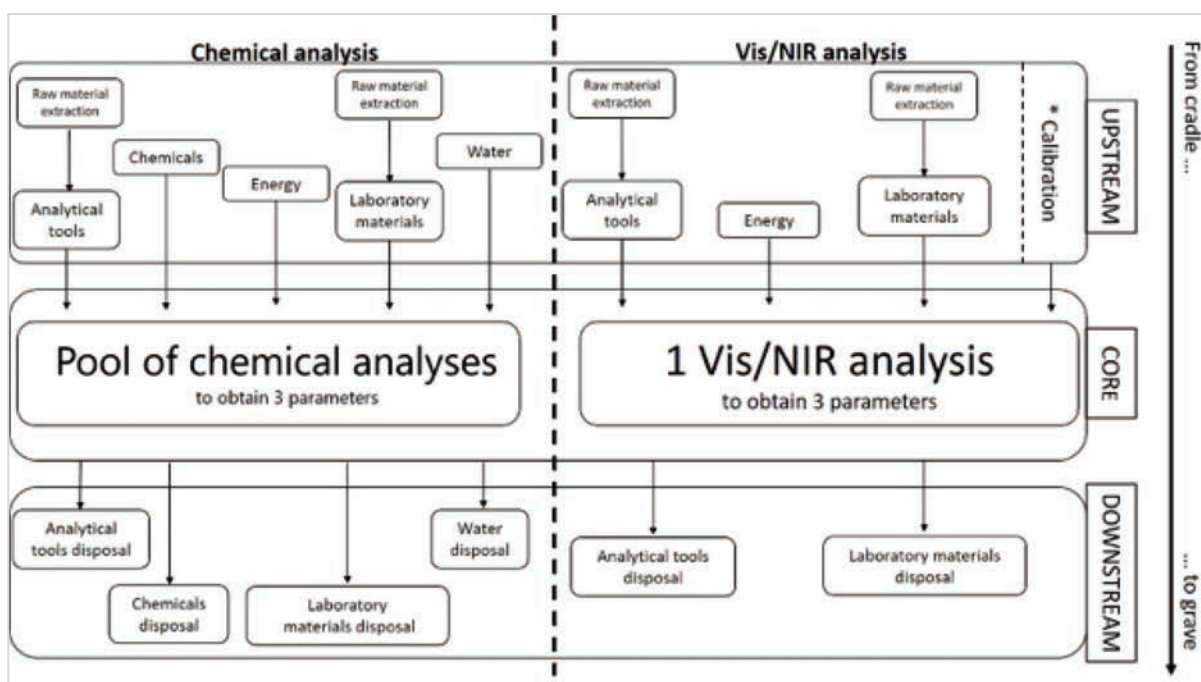


Figure 4 – Cradle-to-grave approach for assessing the environmental impact of chemical and optical analyses

As regards chemical analyses as input, the impact on the construction of the analytical instruments was considered, also considering their energy consumption and their average life (10-15 years), the chemical reagents used, the laboratory material useful for analysis and consumption of water; for all the components considered, the impact of their disposal was then assessed. Similarly, as regards optical analyses, the impacts of the creation of vis/NIR analytical instruments, their duration over time, their energy consumption, and the laboratory material necessary for their operation were considered, in addition to the evaluation also in this case of their disposal costs [1].

Using 16 indicators, the environmental impact relating to the two measurement methods (traditional and optical) used to measure the three reference parameters was assessed using LCA. The impact categories taken into consideration are reported in Table I.

Table I – Impact Categories

Impact category	Acronyms	Unit
Climate change	CC	kg CO ₂ eq
Ozone depletion	OD	kg CFC-11 eq
Human toxicity, non-cancer effects	HT-NC	CTUh
Human toxicity, cancer effects	HT-C	CTUh
Particulate matter	PM	kg PM _{2.5} eq
Ionizing radiation HH	IRHH	kBq U235 eq
Ionizing radiation E (interim)	IRE	CTUe
Photochemical ozone formation	POF	kg NMVOC eq
Acidification	ACID	molc H ⁺ eq
Terrestrial eutrophication	TEU	molc N eq
Freshwater eutrophication	FEU	kg P eq
Marine eutrophication	MEU	kg N eq
Freshwater ecotoxicity	FECO	CTUe
Land use	LU	kg C deficit
Water resource depletion	WRD	m ³ water eq
Mineral, fossil & ren resource depletion	RRD	kg Sb eq

RESULTS WITH DISCUSSION

Spectroscopic analysis using the full-range Jaz instrument yielded spectra highlighting differences based on harvest time and therefore olive ripening degree (Figure 5). Variations at specific wavelengths can be observed both in the visible and near-infrared regions.

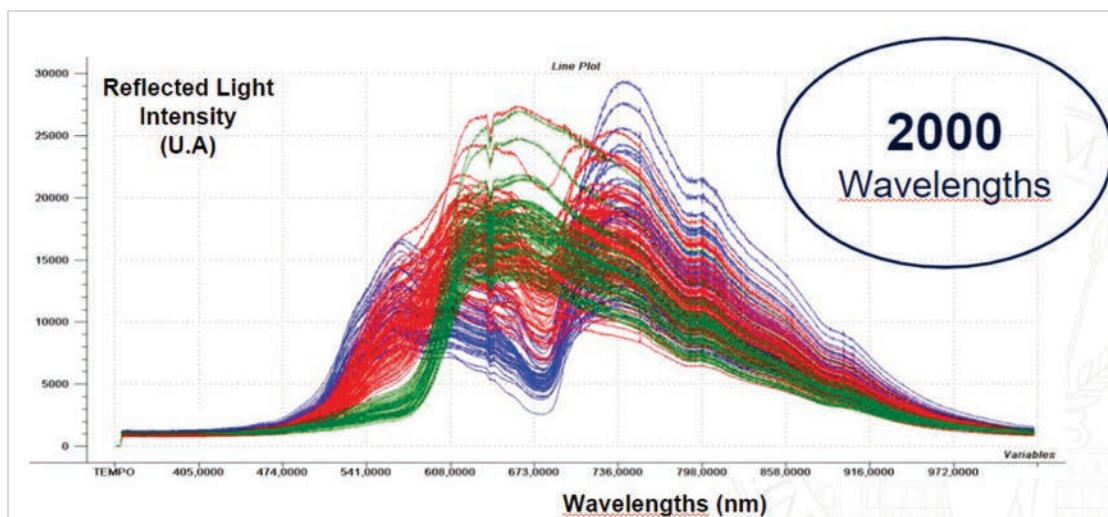


Figure 5 – Dataset of olive spectra acquired using the portable Jaz instrument, color-coded according to different ripening times

Data analysis through chemometric techniques involved applying appropriate preprocessing to eliminate instrumental and environmental noise, providing precise information on wavelength bands showing significant variability related to ripening degree. Three wavelength bands correlating best with a ripening degree were selected, all belonging to the visible region (Figure 6). Indeed, components expressing maximum variance during olive ripening are also responsible for the concurrent change in fruit epicarp colour.

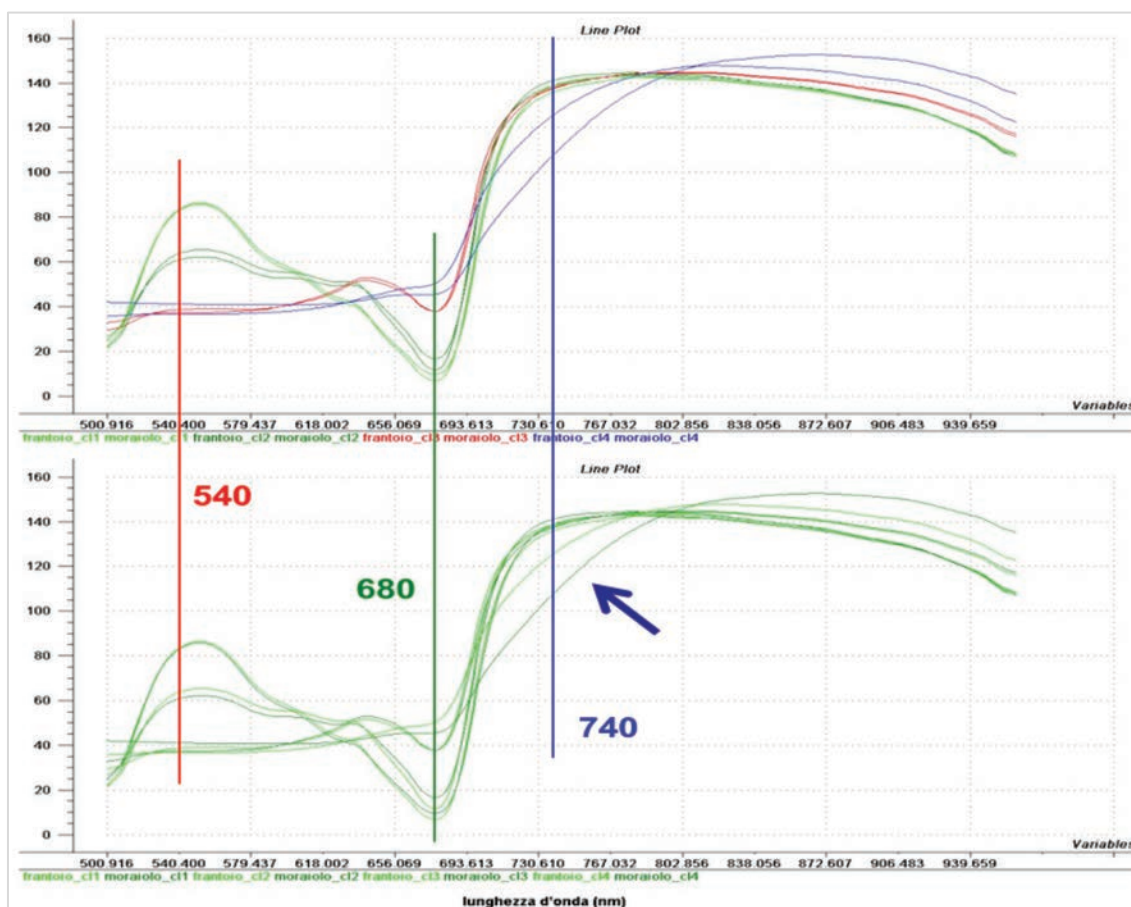


Figure 6 – Identification of the most informative wavelengths for measuring olive ripeness

Based on this data analysis, a new optical prototype was developed, focusing on acquiring optical data at specific wavelengths to simplify data collection and processing, providing preliminary information on olive ripening in a rapid and environmentally sustainable manner without the need for laboratory analysis. Specifically, three wavelengths were selected for measuring olive ripeness (Figure 6), and using 3D printed components, the prototype of the small new device, called 'SmarHAND' (Smart Handheld Analyzer Non-Destructive, Figure 7), was developed. The SmarHAND prototype has been patented in 2022 by Università degli Studi di Milano as "Portable device for analysing vegetable matrices on the field and related system and method" (patent n. WO 2022/172153).

SmarHAND is a low-cost portable instrument operating in the visible (Vis) and near-infrared (NIR) spectra, composed of photodiodes, optical filters, and a LED capable of analysing at 12 wavelengths (Vis sensor measures at 450, 500, 550, 570, 600, and 650 nm, while the NIR sensor measures at 610, 680, 730, 760, 810, and 860 nm). The device is still in the prototype phase TRL 5 approx. (technology demonstrated in a relevant environment) on a scale 1 to 9, where 9 is a fully qualified system ready for commercialization. The wavelengths present in SmarHAND are 12 (a miniaturized optical bench already available on the market was used for simplicity and reliability), but these include the 3 obtained from the specific selection as seen earlier. Sample illumination is provided by a wide-spectrum white LED.



Figure 7 – The SmarHAND prototype

SmarHAND returns as acquisition result a discontinuous spectrum composed of the set of wavelengths installed on the device (Figure 8). This spectrum is simplified compared to a full-range spectrum but retains useful information associated with epicarp colour evolution, and thus ripening.

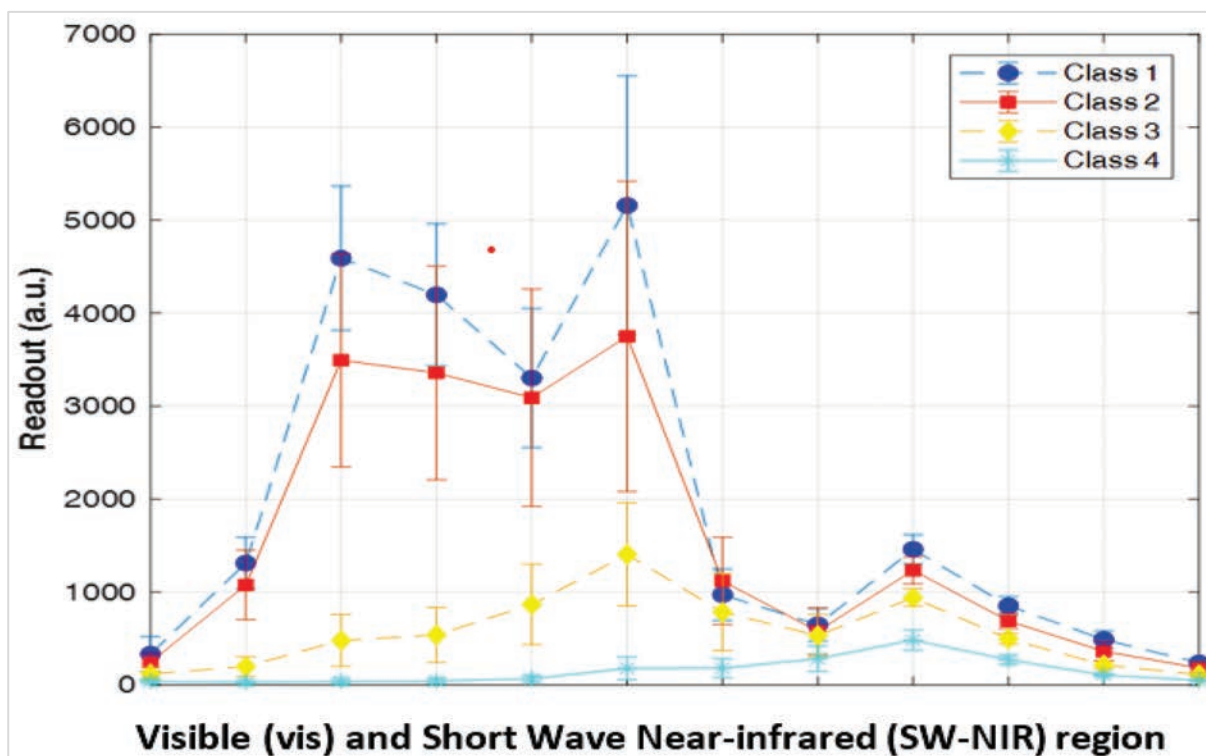


Figure 8 – Spectral dataset acquired using SmartHAND prototype at different harvest times. Each class represents a different time and ripeness degree.

LCA EVALUATION

Regarding the comparative LCA study on the impact of optical and traditional analysis instrumentation, extremely positive results emerged for optical instrumentation.

In Figure 8, it can be observed how traditional chemical analyses have a significantly higher impact compared to spectroscopic techniques. To achieve the same results, traditional methods require three different analyses, while optical instrumentation only necessitates a single spectral measurement.

Considering the analysis frequency relative to the total oil production, the impact of the analyses on the entire process of extra virgin olive oil production can also be estimated. Laboratory chemical analyses account for a maximum of 21% of the total impact when the analysis frequency involves analysing a sample for every 10 litres of oil, decreasing to 0.2% of the total impact for samplings conducted every 1000 litres of oil. Comparing the two analysis scenarios (laboratory chemical vs. optical analyses), it emerged that optical analysis has an average impact 36 times lower than traditional analysis on oil samples.

Furthermore, traditional laboratory and optical analyses were compared, even on whole olive sample analyses, resulting in a reduced impact of 15 times for optical compared to traditional methods.

By using predictive models capable of simultaneously estimating a larger number of parameters and/or increasing the number of samples analysed during the life cycle of optical instrumentation, the impact gap between the two methodologies becomes even wider [2].

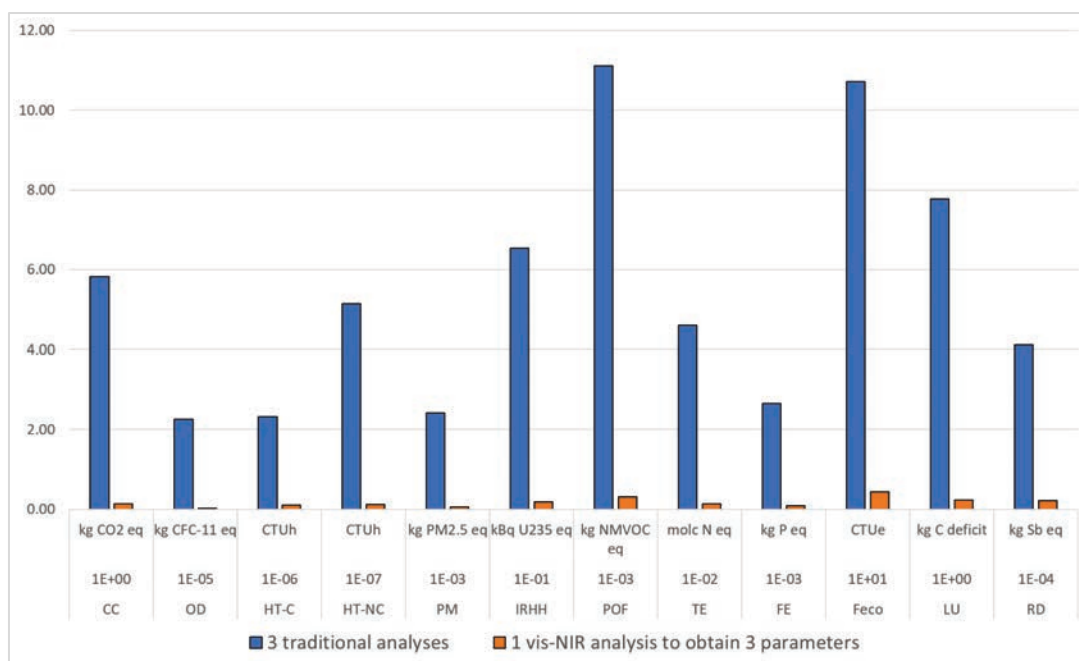


Figure 9 - Comparison of environmental impact between traditional chemical and optical analyses [2]

Overall, quantifying the environmental damage, the results have shown clear advantages for optical analysis, confirming that vis/NIR spectroscopy can be rightly considered a green analytical technology. Among the various techniques available, vis/NIR and NIR spectroscopy are valid tools for the monitoring of qualitative parameters and control in the olive oil sector. The optical instruments currently available on the market are mainly laboratory instruments with dimensions and costs that are not suitable for use in real pre- and post-harvest applications, especially for SMEs. To overcome recent years, research has focused on feasibility studies and simulations of simplified systems. This study has focused on the preliminary design, build, and test of a cost-effective, real-time measurement, device to support operators of the olive sector who want to use a sustainable approach and olive-growing 4.0.

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The evolution of analytical methods of fats and oils: the role of “La Rivista Italiana delle Sostanze Grasse”

Lanfranco Conte

Italian Society for Fats and Oils Researches (SISSG - Società Italiana per lo Studio delle Sostanze Grasse)

Formerly Full professor of Food Chemistry at University of Udine – Italy

lanfranco.conte@gmail.com

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Presentato al centenario RISG

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

15 novembre 2023

FOREWORD: AN HISTORICAL APPROACH

The assessment of purity of foods had been an hot topic through the whole human history, the higher was the value of a food, the higher were the possibilities that is underwent to tentative of frauds

In the beginning, to check for olive oil purity and quality, analysts were compelled to use some tests of the so-called “Chemistry of indexes” because they were within the Italian legislation.

Some examples are reported in Table I; in some cases, they were chromatic (not colorimetric) reactions e.g. the Kreiss reaction for rancidity, the Halphen reaction to assess the presence of Malvaceae oils (that are characterized by the presence of fatty acids with cyclopropane and cyclopropene ring), or the reaction of Villavecchia Fabris and later Isodoro Pavolini, used to look for the presence of sesame oil that in Italy at that eve was mandatory added to seed oils. A number of false positive results occurred

Table I - The “chemistry of indexes” parameters and related meaning

Index	Nowadays
Refractive index	Purity Unsaturation degree still used
Halphen reaction sterculic acid (Malvaceae oils)	Purity No more used, GC of FAMES and sterols
Villavecchia-Fabris and Isodoro Pavolini reactions (In Italy, for Sesame oil)	Purity No more used
Saponification Index	Purity Amount of fatty acids, no more used
Esters index	Purity Amount of TAGs, no more used
Iodine number	Purity Unsaturation degree still used
Thermosulphuric index	Purity Unsaturation degree no more used
Acidity index	Quality, still used
Peroxide value	Quality, still used

Many of the methods reported in the previous table were adopted by Italian Standards for Control of Fats and Related Substances (NGD), (Figure 1 reproduces the front cover of the second edition of the collection of methods) published in 1942, then updated in 1953 and following years; information about these methods were published on the newspaper “Oli Minerali, Grassi e Saponi, Colori e Vernici” (Mineral oils, Fats and Soaps, Paints and Varnishes), then named “Rivista Italiana delle Sostanze Grasse”.



Figure 1 - Front cover of the “Norme grassi e derivati” (NGD) method collection

This scientific journal is edited by “Stazione Sperimentale per le Industrie degli Oli e dei Grassi” that was founded by Stefano Fachini in 1919, even if it already existed since 1906, as “Laboratory school for industry of oils and fats”.

Stefano Fachini was a pioneer of a nowadays current methodology: in 1913 he founded the “Committee for alignment of analytical methods” and in 1924 the “Technical Governmental Committee for Mineral Oils, Fats and Soaps, Paints and Varnishes and Detergents”, in 1930 he founded the “International Committee for the Study of Fats and Oils” that in 1951 became the “Division of Fatty Substances” of IUPAC.

The needed works finalised to develop and standardise analytical methods were carried out by the “Technical Governmental Committee for Mineral oils, Fats and Soaps, Paints and Varnishes and Detergents”, appointed by the Italian Ministry of Industries (at that eve).

Every proposed analytical method underwent to a collective experimentation and once it was standardized, it was published on the “Rivista Italiana delle Sostanze Grasse” to undergo to a public evaluation; after a certain number of days, amendments (if any) were evaluated and eventually applied to reach the ultimate release of the method that became part of the above cited collection of official methods.

It's quite clear the key role of RISG in dissemination of information and facilitation of scientific debate.

Some examples of method development, updating and improvement:

1. FATTY ACID COMPOSITION

In 1965, the method for fatty acid composition by gas chromatographic analysis was published on RISG and adopted as Italian official one as Method NGD Ba II-15 (1965).[1]

A packed column 2 m length with polar stationary phase Polyethylene Glycol Succinate (PEGS) was adopted; separation was satisfactory, even if time of analysis were rather long, if compared to those nowadays request (about 46 minutes to obtain the elution of lignoceric acid). The text of the method clearly described many aspects of the analysis, also enclosing the instruction to calculate the separation index between some critical pairs of peaks, e.g, C16:0/C16:1, C18:0/C18:1, C18:3/C20:0.

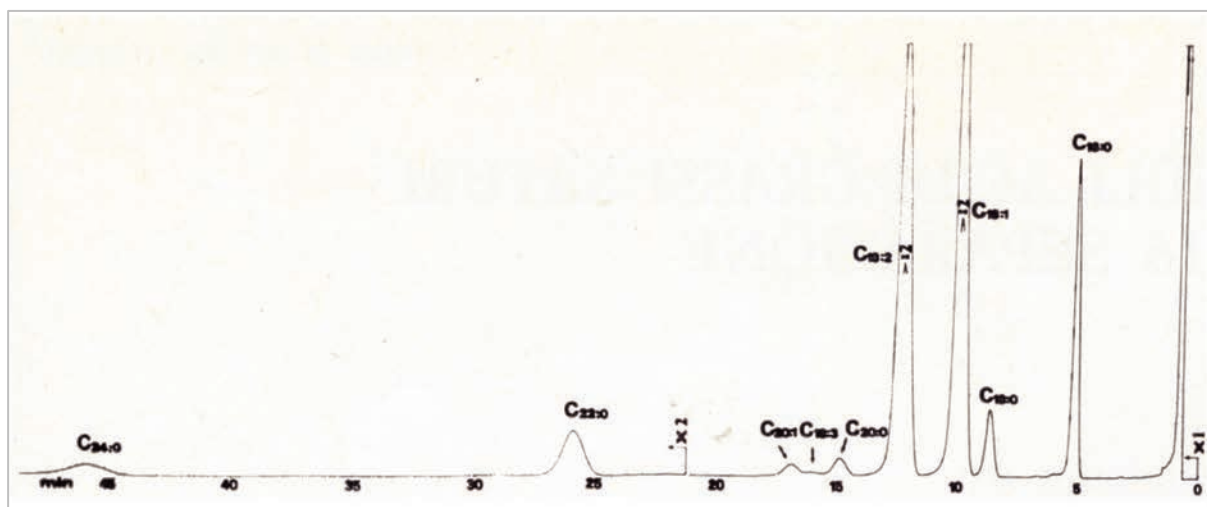


Figure 2 - Gas chromatographic trace of fatty acids methyl esters of a peanut oil, obtained by means of a packed column 2 m length, stainless steel, stationary phase polyethylene glycol succinate (PEGS) according to the NGD Ba II-15 (1965) method

Even if capillary gas chromatography was already published on RISG and adopted within NGD for the detection of elaidinic acid (Method NGD Ba IV, 39, 1968) [2] it became a widespread used technique only more than 30 years later, when two papers were published on RISG, dealing with the determination of fatty acids trans- isomers (Morchio et al, 1989 [3], Mariani et Al., 1991 [4]); these methods, too, were then adopted within NGD and then within EEC Reg. 3682/91 [5].

The formation of fatty acids isomers early highlighted the presence of position isomers of unsaturation and was published in 1959 by Montefredine and Laporta on "Oli Minerali, Grassi e Saponi" [6], then to bypass the detection of the admixture of olive refined oil with extra virgin ones, an illegal technique was developed by using maleic anhydride, however, unexpected trans- isomers also resulted by this reaction and for this reason their detection became very important.

2. STEROLS COMPOSITION

In order to improve the oxidative stability of edible oils, researchers looked at the reduction of unsaturation degree, with this aim, too, genetic improvement since 1967 obtained safflower and sunflower oils with high amount of oleic acid [7], quite similar to olive oil, at that time, this weakened the determination of fatty acids composition as a tool to assess the admixtures of olive oils with seed oils and led the attention to the analysis of the sterols fraction.

In 1971, the method for the determination of the sterols composition by gas chromatography with packed column was published and then, as usual, adopted within the NGD collection (NGB Ba-III-13)

[8]; the separation was rather poor, if compared to the one later obtained by several researchers, both in olive oils (Morchio and De Andreis, 1983 and 1984 [9,10]) and in other different oils (Lercker et al, 1981 [11], Giro and Marzo, 1987 [12]); these results, too, were published on RISG; a collaborative study lead to the standardization of the method on behalf of the Commissione Tecnica Governativa Italiana the method was published, too, on RISG in 1989 [13,14].

Figure 3 reports a comparison of the GLC trace obtained with a packed column and with a capillary one.

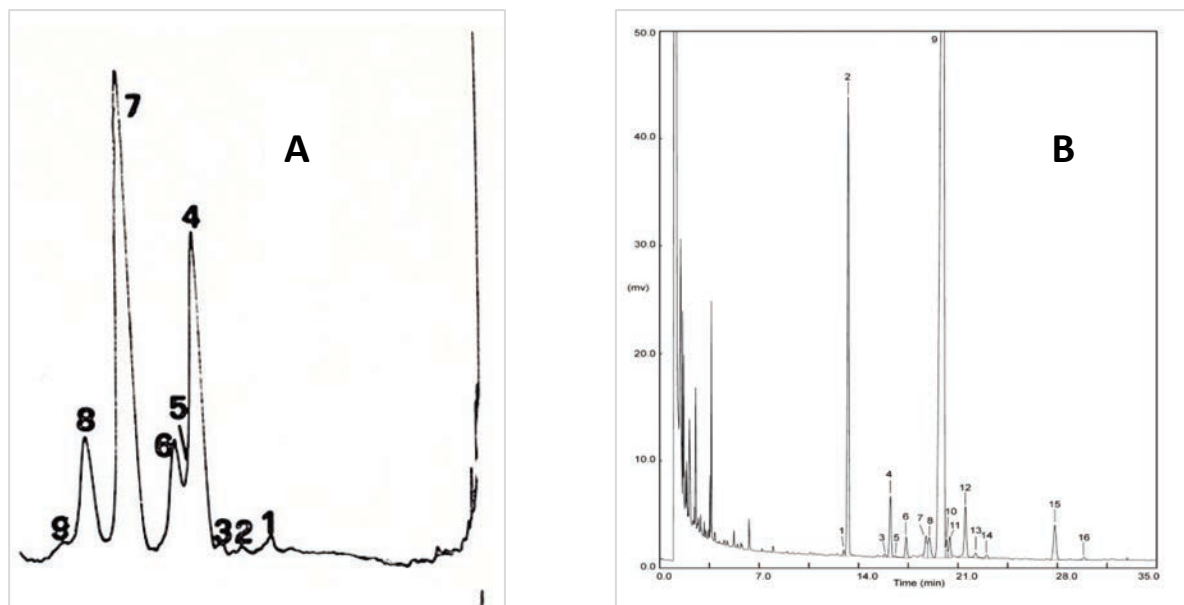


Figure 3 - Gas chromatographic analysis of sterol fraction of olive oil:

A with 3 m glass packed column (stationary phase SE30), according to the NGD Method NGD Ba III-13 1971 Method;

B with 30 m capillary column according to the NGD Method NGD C71 -1986

3. THE ASSESSMENT OF THE PRESENCE OF OTHER EXTRANEIOUS OIL

Knowledge of seeds oils composition was enough deep to avoid most of the illegal admixtures, at least for the years we are speaking about, so that the only other possible oil suitable to be mixed to virgin olive oils seemed could be olive pomace oil.

Early studies highlighted the presence of two diterpenic alcohols named erythrodiol and uvaol which presence was assessed within the analysis of sterols [15], however, several years after, this parameter, too, results not so reliable; starting from the observation that the lipid fraction of drupes skin also contain waxes that after saponification undergo to hydrolysis releasing aliphatic alcohols, the quantitative determination of these compound was proposed by Camera [16] in 1981, this study was modified and published on RISG later by Tiscornia et al [17] and adopted as NGD Method in 1981[18] and EEC in 1991 [19].

Then the presence of free aliphatic alcohols was however detected in selected virgin olive oils (South Apulia, Greece) [20], so that the determination of not hydrolysed waxes was proposed as NGD Method [21] and approved as EEC official method [22].

To get success in carrying out the gas chromatographic analysis of high molecular weight and high boiling point molecules the use of the cold on column injection port is mandatory, this is true for waxes, but also for glycerol esters with fatty acids: the first paper dealing with the direct GC analysis of the reaction mixture obtained by the lipolysis with pancreatic lipase (used to assess the presence of esterified oils) was published by Motta et al in 1983 [23] and later by Lercker et al [24].

This method, too, was then validated by the Italian technical Committee [25] and approved as IOC method [26] and as EEC official method [27].

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**Nota I^a: Impiego della spettrofotometria ultravioletta
per la caratterizzazione degli olii sopraffini vergini di oliva - I.**

MONTEFREDINE A. e LAPORTA L.
Laboratorio Chimico Provinciale di Pescara

Olii Minerali, Grassi e Saponi, Colori e Vernici 36 (2), 31-36 (1959)

Una nota di

Paolo Bondioli e Lanfranco Conte

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

Accademia dei Georgofili - Firenze

✉ paolo.bondioli1956@gmail.com

Continuando con la nostra escursione nella storia della chimica analitica delle sostanze grasse non possiamo non parlare di questo fondamentale lavoro, pubblicato in due diverse note nell'anno 1959. La prima parte, quella che andremo ad analizzare in dettaglio, descrive le conoscenze fondamentali che stanno alla base della analisi spettrofotometrica nell'ultravioletto per quanto riguarda le sostanze grasse. La seconda parte, pubblicata sul numero successivo della Rivista [36 (3), 63-72 (1959)] riporta i risultati delle analisi condotte su 206 campioni di olio di oliva provenienti da tutta Italia. Va qui evidenziato come, conformemente alla legislazione (italiana) vigente all'epoca, negli articoli si parli di olio sopraffino vergine, fino vergine e "mangiabile". Le analisi sono

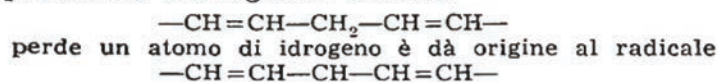
state realizzate nel 1958 e riguardavano i parametri: Acidità, Reazione di Kreis, Indice di Perossidi, Fluorescenza, Esame organolettico e Estinzione specifica nell'ultravioletto alle lunghezze d'onda di 232, 262, 264, 268, 270, 272, 274 e 280 nm. In tabella compaiono inoltre il rapporto R, inteso come rapporto tra le estinzioni specifiche misurate a 232 e 270 nm, nonché il ΔK , calcolato utilizzando le estinzioni specifiche misurate a 268, 262 e 274, come si fa tuttora. Interessante notare che gli Indici di Perossidi riportati nel lavoro assumono valori a prima vista inverosimili, compresi tra 70 e 500, misurati secondo il metodo NGD Ba-IV-20 (1957), che non differisce sostanzialmente dal metodo ISO attualmente in uso. La differenza risiede nell'espressione del risultato, qui riportato in mg di ossigeno attivo per kg di sostanza grassa e non in milliequivalenti. Tra i due risultati esiste un fattore 8, relativo al peso atomico dell'ossigeno, che fa in modo che i risultati in questo modo espressi siano otto volte superiori a quelli che avremmo calcolato secondo le modalità attuali.

L'introduzione dell'articolo ripercorre le conoscenze sino ad allora disponibili, per poi entrare nel dettaglio degli aspetti chimici della questione.

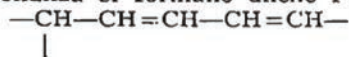
I sistemi di doppi legami coniugati possono originarsi nei grassi attraverso i fenomeni di ossidazione o con i trattamenti di raffinazione. Il problema, per quanto riguarda l'ossidazione, è stato studiato da Mitchell e Kraybill (2); secondo questi AA., il formarsi di sistemi con due o tre doppi legami coniugati è sempre preceduto da una ossidazione degli acidi grassi, alla quale fa seguito una perdita di acqua.

Limiteremo l'esame del complesso argomento ai casi che possono interessare l'olio d'oliva; possiamo presumere che negli olii si verifichino i seguenti fenomeni:

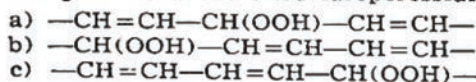
1) - Durante i primi stadi di ossidazione si formano idroperossidi; la loro formazione è spesso accompagnata da uno spostamento del doppio legame e conseguente formazione di un diene coniugato che determina un assorbimento a 232 m μ . La reazione può essere rappresentata dal seguente schema:



ma per risonanza si formano anche i radicali



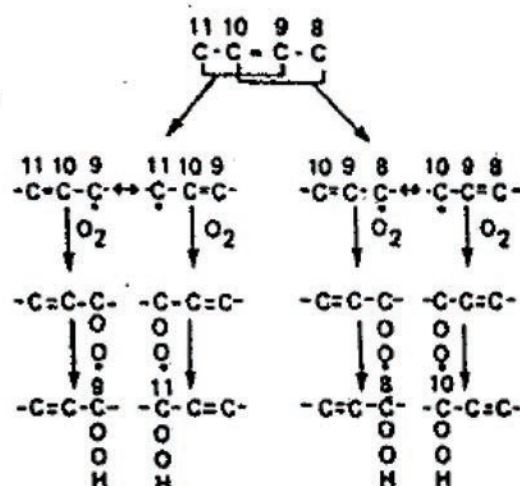
e quindi si possono avere i tre idroperossidi



in due dei quali si è formato un doppio legame coniugato.

2) - La decomposizione degli idroperossidi e la disidratazione degli ossiacidi etilenici danno origine ad acidi grassi dienici che presentano anche essi una banda di assorbimento a 232 m μ .

In modo piuttosto criptico al punto 2) viene affrontata la questione dei dieni coniugati che si formano a seguito della formazione dell'idroperossido a carico dell'acido oleico e successiva disidratazione, secondo la reazione:



Infatti l'estrazione di un atomo di idrogeno sull'atomo di carbonio 8 o 11 della molecola dell'acido oleico porta alla formazione di due diversi radicali allilici e l'attacco dell'ossigeno porta quindi, grazie alle forme di risonanza di tali radicali, alla formazione degli idroperossidi allilici in posizione 8, 9, 10 e 11 nella molecola.

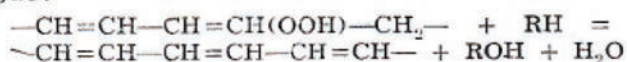
La disidratazione di questi composti porta in ogni caso alla formazione di dieni coniugati.

3) - Durante la conservazione degli olii, gli idroperossidi si trasformano in corpi chetonici, prodotti secondari di ossidazione, che assorbono da 260 a 270 $m\mu$. Questa ossidazione secondaria è favorita dall'acidità dell'olio. R. T. Holman, W. O. Lundberg, W. M. Lauer e G. O. Burr (3) trattarono l'argomento in numerosi lavori e stabilirono che nell'ossidazione dei grassi all'aria l'assorbimento ultravioletto aumenta fortemente, tanto che l'estinzione a 270 $m\mu$ è quasi proporzionale al numero di perossido; la curva dell'assorbimento dovuto a questi composti non presenta però spiccati punti di massimo. Sui rapporti tra lo stato di ossidazione di un grasso (rancidità) e l'assorbimento nell'ultravioletto ci intratterremo particolarmente in una nota successiva.

Gli Autori rimarcano un importante fatto relativo all'assorbimento UV per prodotti di degradazione dell'idroperossido, quali le aldeidi α , β insature qui identificati come corpi chetonici. Si sottolinea quindi che l'assorbimento UV nella regione trienica può essere pesantemente influenzata dalla presenza di questi prodotti secondari di ossidazione.

4) - Dalla distruzione dei perossidi esistenti negli olii, per azione di terre decoloranti a caldo, prendono origine acidi grassi trienici caratterizzati da una tripla banda principale con un massimo a 268 $m\mu$ e da una banda secondaria a 232 $m\mu$. La formazione di questi acidi grassi trienici fa diminuire l'indice di perossido dell'olio. La reazione può essere rappresentata come segue:

La reazione può essere rappresentata come segue:



5) - Ricordiamo infine che un'altra causa di formazione dei doppi legami coniugati è il trattamento con alcali che provoca uno spostamento dei doppi legami come è stato descritto per gli idrocarburi fin dal secolo scorso (4). Nei grassi questa isomerizzazione alcalina fu osservata per la prima volta da Dann e Moore (5) e, studiata poi da Moore (6), ha avuto una larga applicazione nella chimica analitica dei grassi per la determinazione quantitativa degli acidi polinsaturi.

Questa ultima considerazione assume un valore storico ma non ha rilevanza analitica al giorno d'oggi, per la disponibilità attuale di sistemi analitici cromatografici e spettroscopici, dotati di enorme potenzialità di separazione e di identificazione.

Non si ha inoltre evidenza del fatto che per trattamenti con alcali del tipo di quelli che vengono realizzati nel caso della raffinazione chimica degli oli si possano verificare sensibili incrementi degli assorbimenti nella regione dell'ultravioletto. Anche in bibliografia non è facile trovare lavori nei quali vengano discusse le caratteristiche UV di campioni di oli di oliva prelevati nelle tappe intermedie della raffinazione. L'unico articolo reperito (K. Essid et al., *Influence of the neutralization step on the thermal and oxidative stability of acid olive oil*, J. Oleo Sci. 58 (7), 339-346 (2009)) purtroppo è basato su due soli campioni analizzati, per i quali però viene registrato un incremento degli indici UV a seguito di neutralizzazione chimica.

Per i nostri scopi è interessante stabilire che il trattamento con terre decoloranti produce la coniugazione in seguito alla distruzione dei prodotti secondari di ossidazione, l'alcali invece sposta un doppio legame già esistente in una posizione coniugata. Il trattamento con alcali produce una banda a 232 $m\mu$ in seguito a coniugazione dienica, mentre il trattamento con terre decoloranti, produce bande trieniche con massimo di assorbimento a 268 $m\mu$.

(..omissis..)

D'accordo con Kaufmann, Thieme e Volbert (11), che si sono occupati soprattutto dell'assorbimento ultravioletto dello strutto di maiale, nelle curve di assorbimento dei grassi nell'ultravioletto sono da prendere in considerazione tre parametri distinti (fig. 1):

- a) L'assorbimento a 232 $m\mu$ dovuto agli acidi grassi dienici ed ai prodotti primari di ossidazione;
- b) L'assorbimento a 270 $m\mu$ dovuto ai prodotti secondari di ossidazione;
- c) Il picco nella zona di 268 $m\mu$ dovuto agli acidi grassi trienici.

Tra questi parametri si è cercato di stabilire una qualche relazione. Wolff J. P. (12) si è orientato verso il rapporto R tra l'assorbimento a 232 m μ e l'assorbimento a 270 m μ , basandosi sul concetto che nei prodotti naturali, con l'aumentare dell'indice di perossido, accanto agli idroperossidi che assorbono a 232 m μ , si formano composti chetonici, responsabili della svalutazione organolettica degli olii, che provocano un aumento dell'assorbimento a 270 m μ ; perciò il rapporto $R = \frac{K_{232}}{K_{270}}$ si abbassa con la formazione dei corpi chetonici e sarà tanto più basso quanto più l'olio sarà di qualità scadente.

Molto interessante è la proposta di collegare le estinzioni specifiche misurate a 232 e 270 nm e di utilizzarle come dato con significato diagnostico. Vedremo più avanti come questo rapporto sia proposto come indice per valutare la qualità degli oli di oliva vergini. Bisogna anche dire che questa proposta non ha trovato molta fortuna in quanto a nostra conoscenza non ha avuto seguito in nessun ambito normativo.

Nei successivi passi dell'articolo viene avanzata una delle prime proposte per il calcolo e l'interpretazione del valore di ΔK :

Kaufmann e coll. (11) hanno invece cercato di mettere in rilievo la formazione del picco che si presenta intorno a 268 m μ in presenza di acidi trienici: questi AA. fanno la media dell'assorbimento a 264 e 272 m μ e la sottraggono dal valore dell'assorbimento a 268 m μ ; evidentemente questa differenza, che moltiplicata per 100 viene indicata con T, sarà tanto maggiore quanto più netto è il picco che la curva presenta a 268 m μ . In definitiva $T = (K_{268} - \frac{K_{264} + K_{272}}{2}) \cdot 100$.

In buona sostanza si mette in pratica quanto sin qui discusso: i prodotti di ossidazione secondaria hanno assorbimenti nella zona delle lunghezze d'onda interessate, senza mostrare spiccati picchi di assorbimento, caratteristica invece dei trieni coniugati. Geometricamente il calcolo del ΔK ha quindi il significato di eliminare il background dovuto ai prodotti di ossidazione secondari, per isolare invece il segnale per il quale i trieni coniugati sono responsabili.

A margine di quanto riportato nell'articolo, giova ricordare che fino agli ultimi anni '90 anche le Norme Ufficiali (Reg. CEE 2568/91 e le Norme Commerciali del Consiglio Oleicolo Internazionale), nel caso in cui fosse rilevato un assorbimento 270 nm superiore al limite di specifica, era prevista la purificazione su allumina, in modo da trattenere in colonna i prodotti di ossidazione ed isolare il segnale dovuto ai trieni coniugati.

A seguire troviamo poi un paragrafo che riteniamo molto significativo e precursore dei tempi. In uno specifico riferimento alla qualità degli oli gli Autori affermano:

La conoscenza dei parametri K_{270} , R ed indice di perossidi permette, secondo Uzzan, di apprezzare in maniera razionale e scientifica la qualità di un olio: se un fabbricante vuol produrre olio d'oliva di alta qualità non deve mantenere bassa la sola acidità, ma è

necessario abbassare contemporaneamente anche le altre variabili.

L'A. auspica una nuova legislazione che completi quella esistente ed in base alla quale l'olio d'oliva extra, oltre ad essere un olio ottenuto per pressione a freddo delle olive, la cui acidità espressa in acido oleico non superi il 0,7%, sia anche un olio con assorbimento specifico a 270 m μ eguale, al massimo, a 0,14-0,15, con un rapporto R non superiore a 13 e con indice di perossido inferiore a 100. Per le altre qualità di olio i limiti entro i quali queste caratteristiche possono variare potrebbero essere i seguenti:

	Acidità	K ₂₇₀	R \leq	Indice di P.
Olio Extra . . .	0,7 %	0,14 - 0,15	13	100
Olio sopraffino .	1,0 %	0,16 - 0,17	13	120
Olio fino . . .	1,5 %	0,18 - 0,20	15	150
Olio mangiabile .	2,0 %	0,20 - 0,22	15	200

Come si vede questo articolo pone le basi per una classificazione degli oli di oliva edibili che troverà poi recepimento, con alcune modifiche, nella Legge 13 Novembre 1960, n. 1407.

Avviandosi verso la conclusione del lavoro gli Autori citano una affermazione di Wolff, che appare quanto mai attuale, anche dopo 65 anni:

Questo gruppo di lavori francesi, che sono dedicati soprattutto alla classifica di qualità degli olii, si concludono con un altro lavoro di Wolff (13), il quale enuncia con molta esattezza il concetto che ci ha spinto ad effettuare le nostre ricerche sugli olii d'oliva italiani: *«La distinzione tra prodotti vergini e raffinati non è suscettibile di un controllo soddisfacente se non si impone ai prodotti vergini un minimo di qualità. Non si può ritenere vergine un olio che per l'insieme dei prodotti di ossidazione che contiene ha tutti i caratteri di un prodotto raffinato, perchè mal preparato e mal conservato».*

La valutazione organolettica degli oli vergini di oliva è ancora di là da venire, anche se nella seconda nota degli stessi Autori una colonna della immensa tabella sulle caratteristiche di qualità di oltre 200 campioni di olio è dedicata la parametro "Giudizio", in verità non molto positivo in quanto vi abbondano termini quali rancido, acido, acido e rancido.

Consigliamo a tutti gli interessati di dedicare qualche tempo ad una attenta lettura dei lavori originali, in quanto rappresentano una importante testimonianza nella costruzione delle nostre conoscenze odierne e una importante trattazione teorica delle stesse.

Una copia dell'articolo originale può essere richiesta inviando una email a: risc@mi.camcom.it

La bibliografia originale degli articoli si trova alla fine della seconda parte e viene qui riportata di seguito.

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Antonio Montefredine

Laureato in Chimica nel 1926 presso l'Università di Roma, nel 1930 divenne direttore del Laboratorio Chimico del Laboratorio Provinciale di igiene e profilassi di Pescara; conseguì la laurea in Scienze Naturale nel 1939 e nel 1940 in Farmacia, fu libero docente di Industrie Agrarie (1942) e Bromatologia (1956) presso le Università di Milano, Bologna e Perugia, infine di Tecnica conserviera presso l'Università di Pescara. Nel 1963 fu eletto Presidente della Associazione Nazionale Chimici dei Laboratori Provinciali di Igiene, fu presidente della Società Italiana per lo Studio delle Sostanze Grasse, lavorò sulle sanse di olivo e sulla spettrofotometria UV e sulla gas cromatografia applicata agli oli d'oliva.

Presso la sede dell'edificio storico dei Laboratori Di Igiene e Profilassi di Pescara poi, a seguito delle riforme intervenute, del Presidio Multizonale di Igiene e prevenzione e, da ultimo, dell'Arta (Agenzia Regionale per la Tutela dell'Ambiente), esiste da oltre mezzo secolo la biblioteca scientifica intestata alla memoria di Antonio Montefredine, scienziato, bibliofilo e primo direttore di quei laboratori che sono stati a lungo di prestigio nazionale.

Non abbiamo purtroppo reperito notizie relative al secondo Autore, **L. Laporta**, che ha condiviso con il prof. Montefredine la responsabilità di questi importanti lavori. Se ci fosse un lettore in possesso di informazioni sarebbe un grande piacere pubblicare un'integrazione delle note biografiche in modo da rendere merito anche a lui per questi importanti lavori che non devono essere dimenticati.



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Anno 2024
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Progetto TRIT20230512014**Smart packaging solutions for food freshness monitoring: new partners are sought for tailored development and industrial scale-up**

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

Progetto RDRRO20230526011**A Romanian research institute is looking for partners for Eurostars calls**

The Romanian research institute is looking for international partners (SMEs, SME + research organization) involved in the production of food/food supplements or feed additives to form a consortium in the next Eurostars calls (September 2023, March 2024).

The main topics are the valorization of raw materials from natural renewable resources (plant peptide-rich protein hydrolysates) and identification and quantification of chemical compounds by conventional and modern methods; valorization of agri-food waste/by-products; classical and modern methods of extraction of active ingredients from yeasts and medicinal and aromatic plants with applications in the food and feed sectors.

Dead-line for EOIs: 25 May 2025

Progetto TRES20230526017**Spanish Natural ingredients company looks for new scientific evidence technologies to incorporate in its research and manufacturing processes. Collaboration under r&d , commercial with technical assistance or investment agreements is offered**

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

Dead-line for EOIs: 30 May 2025

Progetto BOFR20240223025**A French company offers a subcontract to produce white-label natural liquid food supplements for foreign partners**

A French company offers manufacturing and packaging products without heat treatment, preserva-

tives or additives in glass ampoules (5-15mL), bottles (20mL-1L) or sterile bags (10-1000L). The SME offers subcontracting agreement to companies in sectors of nutraceuticals, sport nutrition, health nutrition, nutricosmetics, animal nutrition or pet food.

23 Apr 2025

Progetto TODE20241004015**A German research institute specialized in nutrition food technology and bioeconomy offers opportunities for product and process development**

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

Dead-line for EOIs:16 Oct 2025

Progetto TRES20240612015**Spanish agricultural company is looking for new techniques for agricultural, ecological, self-made products through a technical cooperation or financial agreement**

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

Dead-line for EOIs:12 Jun 2025

Progetto BOUA20240529003**Production of dairy products and purchase of equipment in the dairy industry**

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

Dead-line for EOIs:03 Jun 2025

Progetto BOIT20240411011**An Italian organic olive oil producer is looking for distributors in specific European countries**

An Italian organic farm offers its organic extra virgin olive oil under distribution services agreements in specific European countries. The company carries out all the production process phases, from the

harvest to the packaging, processing local varieties of olives grown at their own plantings. It is a JAS (Japanese Agricultural Standard) and COR (Canada Organic) certified firm.
Dead-line for EOIs: 11 Apr 2025

Progetto TRCH20250123005

Swiss company seeks food-side-streams suppliers to turn waste into innovative biopolymers

A Swiss cleantech start-up specializing in elastic biobased and biodegradable materials is primarily seeking European suppliers of food side-streams or agricultural waste. These suppliers should either be interested in participating in the development of upcycling processes or be willing to sell their side-streams for integration into the start-up's innovative material portfolio. Commercial agreement or R&D cooperation agreement with the potential supplier is sought.

Dead-line for EOIs: 23 Jan 2026

Progetto TRFR20241007025

French company seeks solution for eliminating bacteria from high-quality vanilla (beans or powder)

A French SME specialises in importing vanilla and spices from Madagascar. It is looking for equipment to guarantee product free of microbiological load from high-quality vanilla (beans/powder) and spices (grains/powder) while preserving the organoleptic properties of the product. A commercial agreement with technical assistance is being sought with partner capable of providing food processing equipment dedicated to eliminating pathogenic microflora and with a sterile outlet for vacuum packaging

Dead-line for EOIs: 7 Oct 2025

Progetto BOCO20240620010

Social enterprise producing nut butters, cashew snacks and hardwood charcoal made by women survivors of war, using ethically sourced raw cashews seeks potential clients

Colombian company producing cashew butter, cashew snacks, and hardwood charcoal with the support of local farmers and war-affected women, seeks a partnership with a European buyer. They offer high-quality products for resale under their brand or other's brands, with flexible private label options. Their goal is continuous factory operation and mutual market growth. They propose a distribution agreement and joint marketing.

Dead-line for EOIs: 20 Jun 2025

Progetto TOES20240315006

Rapid analysis of Salmonella in food using the ELFA technique (enzyme linked fluorescent assay)

A Spanish research center offers a rapid analysis of Salmonella in food using enzyme linked fluores-

cent assay technique. The technology center is looking for commercial agreement with technical assistance with companies working in food field.
Dead-line for EOIs: 15 Mar 2025

Progetto BOCY20240617002

CY start up specializing in research and development of functional food and beverages seek partners in a form of an investment agreement.

A Cypriot research-phase startup using microbiology and modern tech to create sustainable, nutritious functional foods and beverages is seeking partners to help them move to commercial production under investment agreement.

Dead-line for EOIs: 28 Jun 2025

Progetto RDRTR20241204013

Transforming Resilient Grains into Nutritious Foods through Precision Fermentation

This project uses precision fermentation to transform drought-resistant and underutilized grains into consumable products, addressing food supply challenges and helping meet food supply demands.

Dead-line for EOIs: 4 Dec 2025

Progetto RDRES20230302015

Biotech SME with deep expertise on pro/postbiotics & microbiota study to regulate glucose metabolism in diabetes related conditions. Seeking complementary Eurostars3/other EU proposal partnership/consortium where their experience can provide added value.

The Spanish innovative SME is working on premium pro and postbiotics and microbiome science to prevent & regulate metabolic glucose metabolism (diabetes). The company is proprietary to a bacteria collection and microbiota and their efforts related on IP actions and science have a strong strategy in place. The company is seeking for partners to jointly apply to Eurostars3.

Dead-line for EOIs: 1 Mar 2025

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

Enterprise Europe Network (EEN)

È la rete nata nel 2008 per volontà della Commissione Europea con l'obiettivo di supportare l'innovazione, il trasferimento tecnologico e l'internazionalizzazione di piccole e medie imprese ed enti di ricerca.

Si avvale di oltre 600 organizzazioni presenti in 60 paesi e offre un sistema integrato di servizi gratuiti per aiutare le imprese a individuare nuovi partner commerciali, produttivi e tecnologici all'estero; per promuovere la partecipazione ai programmi Europei per la ricerca, come Horizon Europe, e per fornire gli strumenti utili per essere più competitivi sui mercati internazionali, migliorando la conoscenza dei mercati e della legislazione europea.

In Lombardia i servizi di Enterprise Europe Network sono garantiti da Simpler (Support Services to IMProve innovation and competitiveness of businesses in Lombardia and Emilia-Romagna), di cui Innovhub SSI è partner.

Come ti può aiutare la rete EEN?

Far crescere l'azienda e sostenere l'internazionalizzazione:

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

Sviluppare partneriati:

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

Implementare processi di innovazione e trasferimento tecnologico:

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

I servizi della rete EEN sono gratuiti.

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

Per maggiori informazioni contattare:

Federico Agostini

federico.agostini@mi.camcom.it



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innovazione e ricerca



..... CONGRESSI

Fats & Oils International Conference - Exhibition (FOIC)

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

The conference will focus on "Technological Innovations, Efficiency & Value Addition Following Principles of Green Chemistry."

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

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

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

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

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

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

NIOP Annual Convention

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

The NIOP – National Institute of Oilseed Production organizes the hub for industry-wide networking, offering a unique platform to connect with fellow professionals, exchange insights, and build valuable relationships. Attendees can look forward to engaging discussions with industry thought leaders, gaining invaluable perspectives on the latest trends and innovations. Beyond the enriching experience of knowledge sharing, the Convention also offers a vibrant social atmosphere, providing opportunities to unwind, relax, and simply enjoy the camaraderie of like-minded individuals. The 2025 Convention promises to be an exceptional event, setting the standard for industry-wide networking, featuring enlightening discussions with leading industry figures, and providing a captivating environment for socializing and relaxation. Attendees will not only bask in the wealth of professional opportunities but also immerse themselves in the breathtaking beauty of the California

landscape. It's a combination of business and leisure that ensures an unforgettable experience for all who attend.

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

2025 International Biomass Conference & Expo

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

The 18th annual International Biomass Conference & Expo unites industry professionals from all sectors of the world's interconnected biomass utilization industries—biobased power, thermal energy, fuels and chemicals.

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

International Biomass Conference & Expo is where future and existing producers of biobased power, fuels and thermal energy products go to network with waste generators and other industry suppliers and technology providers. It's where project developers converse with utility executives; where researchers and technology developers rub elbows with venture capitalists; and where Fortune 500 executives and influential policy makers sit side-by-side with American farmers and foresters.

International Biomass Conference & Expo is the largest, fastest-growing event of its kind. In 2024, this event is expected to draw nearly 900 attendees. In 2024, the event drew more than 900+ attendees. This growth is fueled by a world-class Expo and an acclaimed program.

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

Track 1: Pellets & Densified Biomass

Track 2: Biomass Power & Thermal

Track 3: Biogas & Renewable Natural Gas (RNG)

Track 4: Sustainable Aviation Fuel (SAF)

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

See the event page for the full Program and updates:

<https://ibce.bbconferences.com/ema/DisplayPage.aspx?pageld=Home>

Argus Agriculture & Feedstocks Conference

20-21 March 2025 | Buxelles, Belgium

Driving the future of the agriculture market and its role in providing solutions to the energy sector for decarbonisation. Agricultural markets are navigating significant challenges, from adverse weather and the Russia-Ukraine conflict to changing consumer preferences and evolving trade dynamics. Amid these disruptions, the global move towards energy transition is driving new investments and pushing agriculture to adapt. This shift is vital for addressing climate change while producing sustainable bioenergy and quality food.

The Argus Agriculture & Feedstocks Conference, a revolutionised evolution of the Argus Agritel Paris Grain Conference, brings together industry leaders to examine the production and market dynamics of grains and oilseeds. Building on a legacy of excellence, the event now offers a broader focus, covering agriculture's pivotal role in supporting the energy sector's transition and exploring innovative solutions to unlock global feedstock potential to meet growing demand.

Gain key insights, build strategic partnerships, and uncover innovative opportunities at the crossroads of agriculture and energy. Whether you work in agriculture, biofuels, or energy production, this event provides a platform to influence the future of sustainable practices and advance the global energy transition.

See: <https://www.argusmedia.com/en/events/conferences/agriculture-and-feedstocks-conference>

5th International Oil palm biomass conference 2025

14 - 15 April 2025 | Kuala Lumpur, Malaysia

As the world transitions into renewables, oil palm biomass has been flagged as one promising resource. Not just to generate biofuel and electricity but also to convert into higher value products which will find demand in the global economy striving to embrace sustainability. The fact that there is a fortune waiting is no longer a secret. Many studies have confirmed the presence of the untapped gold in oil palm biomass. Much work has gone into developing the right technology and business model to transform the oil palm biomass from a waste burden to a new climate-friendly resource that the world craves for. Countries around the world, including Malaysia, have already launched blueprints and roadmaps to tap on the vast wealth of the oil palm biomass.

The biggest suppliers are Indonesia and Malaysia. But the region is eagerly eyeing the diverse business potential that the oil palm biomass promises. This international oil palm biomass conference would provide a splendid platform to know and understand more about the immense business potential that the oil palm biomass offers.

This conference aims to bring together experts, industry leaders, researchers, and policymakers from around the world to discuss the latest advancements, innovations, and challenges in the field of oil palm biomass. It offers unparalleled opportunities for networking, education, and business development in the biomass industry. It's a great platform to learn about advancements, discover new technologies, and connect with global leaders and stakeholders.

Visit: <https://oilpalmbiomass.com/>

AOCS Annual Meeting & Expo

27-30 April 2025 | Portland, Oregon, USA

OCS is a community of scientists, technicians, nutritionists, researchers and other industry professionals advancing the science and technology of edible oils, fats, proteins, surfactants and related materials. AOCS is a leading international society with more than 2,000 members around the world.

The AOCS Annual Meeting & Expo is a premier international science and business forum on fats, oils, surfactants, proteins and related materials.

The annual meeting features a multidisciplinary technical program including 80+ sessions, special sessions including presentations on trending industry topics, networking events and receptions, and more. The technical program in 10 interest areas features more than 600 oral and poster presentations, plus additional break out sessions around important trending topics:

- Analytical
- Biotechnology
- Edible Applications Technology
- Health and Nutrition
- Industrial Oil Products
- Lipids Oxidation and Quality
- Phospholipids
- Processing
- Protein and Co-Products
- Surfactants and Detergents

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

15th ICIS World Surfactants Conference

7 - 8 May | Jersey City, USA

This is the premier event in the surfactants calendar, as the industry continues to play an increasingly important role in the global economy, from essential functions like health and hygiene to emerging, fast-growing applications in nanotechnology, 3D printing, precision agriculture and many

others.

Join leading consumer brands, industrial companies, manufacturers, feedstock providers and technology companies as we drive forward the future of surfactants.

Stay informed about the surfactants market. Explore a comprehensive program, featuring exclusive sessions on regional advances, market dynamics and industry innovations.

Meet the entire value chain. Our conference delivers a valuable and meaningful experience for everyone as we offer numerous opportunities for networking and collaboration, aiming to foster a dynamic and productive environment for all delegates. Key themes:

Accelerating Innovation. Examine how consumer trends and the adoption of sustainability principles are driving innovation in surfactant technologies. Learn how companies are developing cutting-edge solutions to meet evolving market demands while aligning with environmental goals.

Collaboration. Explore the impact of mergers and collaboration across the value chain in the surfactants industry. Delve into the role of biotechnology advancements and increased cross-industry investment in renewables as key drivers of sustainable growth and innovation.

Strategy. Discover how megatrends and evolving regulations at federal, state, and local levels impact success across the surfactants value chain.

See the program at:

<https://events.icis.com/website/8544/home/>

Argus Biofuels & Feedstocks Asia Conference

22 - 24 April | Singapore

Fuelling global collaboration and business: Asia's premier biofuels and feedstocks event.

The Conference serves as a vital platform to explore the latest developments in biofuel production and the diverse feedstocks that support this growing sector. The event offers a unique opportunity for participants to network, forge partnerships, and collaborate on solutions that can drive the biofuels industry towards greater sustainability and scalability in the coming years.

This is the flagship pan-Asian industry event which gathers 400+ senior representatives from 30+ countries across three days. Discuss key challenges and opportunities for the sector, including advancements in feedstock diversification, efficiency improvements in biofuel production, whilst exploring the commercialisation of new biofuel pathways, including bio-naphtha for sustainable chemical and plastic production, the opportunities for ethanol blending, as well as the growing demand for biofuels in the marine and aviation sectors.

See the conference website for more information: <https://www.argusmedia.com/en/events/conferences/biofuels-and-feedstocks-asia-conference>

IGC Grains Conference

10 - 11 June | London, UK

The aim of the conference is to bring to the International grain conference a wide range of topical issues, some within the industry but also to look at those outside the industry, such as geopolitics, that over the next few years will be major influencers within Agri-Business and policymaking.

Being part of a series of related industry events under the banner "London Grains Week", the International Grains Conference is a truly global platform for dialogue between policymakers and operators across the entire grains value chain. The event will be held over two full days, devoted to discussions surrounding the challenges, risks and opportunities in global trade, such as Trade finance, Biodiversity, ports connectivity and grains trade.

Trade opportunities will be explored in 2 regions: Africa and Middle-East. The event comprises a number of commodity-specific workshops, covering topical issues affecting markets for grains, rice, oilseeds, pulses and related sectors.

Check program updates at:

<https://www.igc.int/en/conference/confhome.aspx>

World Bio Markets

10 - 11 June | The Hague, Netherlands

World Bio Markets is a two-day, business development event for the global industrial biomanufacturing sector.

We facilitate commercial connections and generate deal flow between bio-developers and producers, global brands and buyers, community enablers, investors and financiers and suppliers.

Our unique 'meetings first' format makes it easy for companies to meet new customers and partners, grow new business development pipelines, generate sales, secure investment and scale at a faster rate than without us.

World Bio Markets takes a unique "meetings first" approach, prioritizing highly targeted, pre-arranged 1-2-1 commercial meetings that are key to accelerating growth in the sector.

By removing the element of luck from traditional networking, this format offers the most cost-effective and time-efficient way for you to connect with the new customers and partners needed to scale your businesses.

World Bio Markets is the industrial biomanufacturing conference dedicated to driving the commercialisation of the industry; where pre-arranged 1-2-1 commercial meetings are the focus; that attracts consumer-facing global brands;

with a truly international audience.

Companies attend World Bio Markets to meet potential new customers and partners; grow their new business development pipeline; build relationships with global brands; find investment; position themselves as bioeconomy pioneers and thought leaders.

See more at: <https://www.worldbiomarkets.com/>

Oleofuels 2025

11t - 12th June | Barcelona, Spain

It is the 16th edition for professionals and experts in the field of oleofuels, providing a unique platform for networking and knowledge exchange.

In this two-day conference, industry leaders, manufacturers, researchers, policymakers, and market experts will come together to discuss the latest advancements, challenges, and innovations in the field of oleofuels. The event will feature informative presentations, interactive panel discussions, and engaging networking sessions.

ACI's Oleofuels 2025 conference offers a valuable opportunity to gain insights into the current market trends, learn about the most recent technological developments, and explore potential collaborations within the industry. It will provide participants with an in-depth understanding of the global oleofuels market, its future prospects, and the regulatory framework shaping the industry.

By attending this conference, you will have the chance to meet and connect with over 300 professionals from various sectors related to oleofuels.

The event represents an ideal platform for expanding your professional network and fostering new

business relationships.

Call for papers is open. See updates at <https://www.wplgroup.com/aci/event/oleofuels/>

16th ISSFAL Congress

29 June - 2 July 2 | Québec City, Canada

The event will bring together leading experts, researchers, and industry professionals to share groundbreaking insights on lipid and fatty acid science. The aim is to create an environment where the scientific and industry communities can connect and build lasting relationships. Along with engaging sessions, you'll have the chance to network with peers and enjoy the rich cultural heritage of one of North America's most historic cities.

The ISSFAL 2025 abstract submission portal is OPEN! Don't miss your chance to showcase your work at the premier global event in lipid science and nutrition.

Some of Conference Topics:

Lipidomics and Precision Nutrition in Cardio-metabolic Disease Prevention

The Role of Fatty Acids in Retinopathy of Prematurity

Application of Lipids for Oral Delivery of Small Molecular Drugs and Peptides

Blue Transformation of Food Systems for Sustainable Lipid Production

The Role of VLC PUFA in Retinal Health and Disease

The Potential of Lipidomics for Population Health Research

For more information and updates visit:

<https://www.issfalcongress.com/>



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Chiara Zigliani
riscg@mi.camcom.it



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

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Sede Legale: Via Meravigli 9/b, 20123 Milano

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REA: MI -1798570 - Socio Unico: Camera di Commercio di Milano, Monza-Brianza, Lodi

Via Giuseppe Colombo 79 - 21033 MILANO

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