ISSN 2611-9013 RISG on-line 102(1) 1-72 (2025)
Finito di stampare nel mese di aprile 2025

RISG

2025

VOL. 102

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

LA RIVISTA ITALIANA DELLE SOSTANZE GRASSE

N.1 - GENNAIO - MARZO 2025

La RIVISTA ITALIANA delle SOSTANZE GRASSE

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RISG
LA RIVISTA ITALIANA DELLE
SOSTANZE GRASSE

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

Indice

GRAFICA, IMPAGINAZIONE E STAMPA
Tipografia Caregnato
Via Trieste, 10
21040 Gerenzano

DIRETTORE RESPONSABILE: P. ROVELLINI
REDAZIONE: C. ZIGLIANI

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Autorizzazione del Tribunale di Milano 28.9.1948, N. 591

DIREZIONE E REDAZIONE
Via Giuseppe Colombo 79 – 20133 Milano

Sito web: www.innovhub-ssi.it

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

Impact Factor: 0.6
Subject Area: Organic Chemistry (Q4)

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

Effect of different pretreatments to process lyophilised avocado

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Prior to freeze-drying avocado pulp, four pulp treatments were evaluated. The pulps were first divided into two groups: untreated pulp and pulp treated with high pressure, HP, and then each group was treated with two different methods of particle reduction, compression, and friction, and they were subsequently freeze-dried. Physicochemical analyses were conducted on the different lyophilised powders, pH, acidity, wettability and water activity. The HP treatments lowered the pH and increased the acidity compared to fresh avocado pulp without any treatment. The opposite was true for the non-HP treatments. On the other hand, those treated by compression have a lower pH and higher acidity than those treated by friction. Each treatment presented different wettability times, with the shortest wetting time in the HP-free and compression, and this same batch presented the lowest water activity. The most effective treatment was compression without high pressure, as it demonstrated the least variability in pH and acidity compared to fresh avocado and it had the shortest wettability time and water activity. Likewise, gas chromatography was used to study the volatile compounds in each of the four treatments; these compounds are mostly the product of the degradation of fatty acids present in the lipid phase. The elimination or formation of compounds in relation to fresh avocado was observed for each treatment and varied between them. The fresh avocado oil contains ten main compounds, namely hexanal, 2-hexenal, caryophyllene, benzeneacetaldehyde, copaene, α-bergamotene, hexane-2,3-dimethyl, α-cubebene, hexane, and 3-carene, which make up 91% of the total. An optical microscopy analysis showed that all treatments caused a break in the cell wall of avocado cells, but compression resulted in less intensity.
Keywords: Persea americana, Hass, pretreatments, lyophilisation.

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Received: February 15, 2024
Accepted: July 12, 2024

1. INTRODUCTION

The avocado, *Persea americana*, is a fruit native to Mexico and Central America. Its Spanish name, 'aguacate' derives from the Aztec word "ahuacatl" which means testicle-shaped fruit. Today, this fruit is cultivated worldwide in tropical and subtropical regions, and it has several varieties, and the most popular is the Hass. Mexico is the world's leading exporter of avocados, representing approximately 45% of global exports, and Colombia and Peru follow as the next largest exporters [2,12,20]. Avocado is a rich source of essential nutrients including protein, lipids, minerals, vitamins, fibre, carotenoids, phytosterols, phytostanols, seven-carbon sugars, resveratrol, and phenolic compounds. These nutrients are crucial for maintaining good health [8,25].
The fruit contains several phytochemicals with antioxidant, anti-inflammatory, antitumor, and antimicrobial properties [14].
Most avocados are marketed and consumed in their fresh form on the domestic and export markets, but its short ripening time and susceptibility to oxidation

are the main problem for producers. Freeze-drying is the optimal dehydration process for preserving the shelf-life and sensory and nutritional characteristics of avocado [3]. Avocado is known for its high lipid content, ranging from 10-30% in fresh fruit, depending on the variety and seasonality, and, overall, the oil contains 71% monounsaturated fatty acids, 13% polyunsaturated, and 16% saturated fatty acids. Currently, reducing the consumption of saturated fats and increasing the intake of polyunsaturated fats is important in mitigating the risk of coronary heart disease. Preliminary studies suggest that avocados may aid in weight management and promote healthy aging [5,21,4].

Pureed avocados quickly lose their nutritional and sensory quality due to oxidative enzymes, such as polyphenoloxidase and lipoxygenase. These enzymes attack phenolic-like compounds and affect fatty acids and carotenoids, resulting in rancid flavours and odours. In addition to the hydrolysis of ester bonds in triglycerides, lipases, also exhibit hydrolytic enzymatic activity. This results in the production of free fatty acids and volatile compounds, which can accelerate autooxidation reactions due to their susceptibility to free radical attack [13,19].

Avocado pulp can be directly consumed or further processed to create guacamole, avocado oil, puree, sauce and other commercial avocado products. Additionally, avocado contains considerable amounts of phytochemicals, especially phenolic acids and flavonoids, which provide antioxidative capabilities to be utilised in food and pharmaceutical industries [7].

Particle reduction in food processing is a unit operation that aims to decrease the average size of solids or liquids by applying various forces. In the case of solids, this process is also known as comminution and includes grinding, crushing, impacting, abrasion, or cutting [23]. On the other hand, high-pressure technology can be used to activate or inactivate enzymes. Low process conditions can increase enzyme activity and stabilise enzymes, while extreme conditions such as pressure, temperature, and time can induce denaturation. In molecular terms, high pressure breaks noncovalent bonds, such as ionic and hydrophobic bonds, and has little effect on covalent bonds. Changes in the secondary, tertiary, and quaternary structures affect large biomolecules like proteins and polysaccharides, but small molecules such as colour, flavour, and vitamins are usually unaffected [16].

The objective of this study was to investigate alternative methods for obtaining lyophilised avocado pulp. Two particle size reduction methods, compression and friction, were evaluated under two process conditions: one with high pressure treatment and one without treatment prior to lyophilisation.

2. MATERIALS AND METHODS

Hass variety avocado pulp from Michoacan, Mexico, was used and divided into two groups: untreated pulp and pulp treated with high pressure (HP), and each group was then subjected to two different methods of particle reduction, compression, and friction, before being freeze-dried. The study looked at four different treatments: T1A, which used avocado pulp without high pressure and friction; T1B, pulp without high pressure and compression; T2A, pulp with high pressure and friction; and T2B, pulp with high pressure and compression.

2.1. RAW MATERIAL

To ensure consistency of fresh Hass avocados, a single lot was used for all treatments. The avocados were subjected to accelerated ripening at 25°C ± 2°C and relative humidity (RH) of 85 ± 10% for 4 days until they reached optimum maturity with a texture of 13.5 ± 2.0 Newton (N). Maturation was carried out in a climatic chamber with temperature and relative humidity control (Thermo Scientific™-3949). The avocados were then washed, sanitised, and cut. The pericarp was manually extracted using a knife.

The avocados were selected based on a destructive test that evaluated their texture using a Brookfield® model CT3 texturometer and CT3 Texture Analyser software. The TA44 Cylinder 4 mm D tip was used with a penetration distance of 3 mm, a displacement speed of 3 mm/s, and a net activation load of 6 N.

2.2. HIGH PRESSURES

The high hydrostatic pressure process was carried out using a Hyperbaric® Wave 55 model equipment under the conditions of 550 MPa at 20°C for 120 s.

2.3. SIZE REDUCTION

Two different methods, friction and compression, were used to reduce the particle size of the pulp after treatment with and without high pressure. The resulting avocado paste was then freeze-dried. The friction process was conducted using an Oster® brand immersion blender model M2609-13 at minimum speed, while the compression process was conducted using a Metaltex® brand potato press with a mesh size of 1 cm. Figure 1 displays images of both equipment.

2.4. LYOPHILIZATION

The samples were flash frozen at -60°C for 15 min using a Revco® ULT1386-9-A36 freezer. Freeze-drying was performed using industrial equipment owned by SioSi Alimentos Company, Mexico. Identical process conditions were used for all batches. The process conditions are not disclosed due to confidentiality.

3. PHYSICOCHEMICAL ANALYSIS

The freeze-dried products underwent analysis for pH, acidity, water activity, and wettability. All analyses were performed in triplicate.

pH. An Oharus® model a-AB23PH pH meter was utilised. A dilution of 1:10 was used, lyophilised powder and distilled water.

Acidity. The acidity in the oils of each of the treatments was determined using the official method AOAC [1]. The oil was obtained from each lyophilized sample using ether as a solvent and a Hahn vapor steam rotary evaporator in a 1:8 ratio of avocado-ether for 24 hours. Next, 7.0 g of the oil was mixed with 250 mL of neutralised alcohol and titrated with 0.1 NaOH normal solution using phenolphthalein as indicator. The acidity was reported as fatty acids based on oleic acid (factor, 0.0282).

Water Activity. The freeze-dried samples were passed through a mesh with a size of <1 mm to ensure uniform particle size. An Aqualab® Pre equipment was used.

Wettability. This technique was used to determine the ability of different lyophilised powders to dissolve in water. The method proposed by Gea [11] was used with modifications. Prior to testing, the powders were homogenised by screening to ensure a particle size of <1 mm. A 400 mL beaker containing 100 mL of deionised water at 25°C was used. A glass plate was used to hold 1 g of the powder. The powder-filled plate was positioned atop the beaker and inverted to allow the powder to meet the water, then a stopwatch was started. Wettability was determined by the time it takes for the final particle of dust to dissolve in the water without any agitation. The time was measured in seconds.

3.1. VOLATILE COMPOUNDS

Volatile compounds were detected in the four treatments, with a fresh avocado sample used as the control. The units of measurement were percentages corresponding to the area of each of the peaks. Two determinations were made for each of the tests. 30 g of the sample was placed in an amber glass vial and enriched with volatile compounds by stirring it at 100 rpm for 45 min in a water bath at 37°C. A metal needle containing a solid phase coated with a layer of divinyl-benzene-carboxen-polydimethylsiloxane (50/30) was then introduced. The microextraction fibre was exposed to the gas phase of the headspace for 30 min with agitation. After adsorption, the fibre was retracted, and the needle was immediately introduced into the gas injector of the chromatograph (Hewlett Packard 5890 Series II) coupled to a mass selective detector (HP 5972). The chromatographic conditions were as follows: the injector temperature was set to 180°C, and the fibre desorption time was 6 min in spitless mode. Helium was used as a carrier gas at a flow rate of 0.8 mL/min. The chromatographic column was an Agilent® J&W DB-5 with dimensions of 30 mm x 0.25 mm internal diameter. The initial temperature was set to 40°C and increased at a rate of 3°C/min until it reached 120°C. The total ion chromatograms (TIC) and the mass spectra corresponding to each compound were acquired with an impact electron ion (EI) at 70 eV at 1.6 scans/s. The identification of the compounds was done by comparing the spectrum of the compound with the Wily Spectra Library of the chromatograph's own equipment.

3.2. MICROSCOPY

The samples were prepared by cryo-embedding-compound and stored at -20°C for 24 h with a radius of 1.5 cm. Cutting was carried out using a Microm 505-n cryostat at 25 µm. Staining was performed using Malachite Green at 0.2% for 2 min to colour the cell wall (cellulose) green and Sudan Red III at 1% for 15 min to detect lipids that stain yellow. Finally, they were introduced to xylol for 5 min to fix the colour. The samples were observed using a Motic® model BA210 optical microscope with an integrated camera at 10x and 40x magnification.

3.3. STATISTICAL ANALYSIS

Statistical analysis was conducted using the JMP12 program. A one-way ANOVA analysis was performed to determine if there is a significant difference (α= 0.05) between the treatments, followed by a Tukey's test.

4. RESULTS AND DISCUSSION

4.1. RAW MATERIALS AND PROCESS

The avocados used in this study had an average weight of 150 ± 15 g. The avocados were chosen based on their firmness, which was measured at 13.5 ± 2.0 N. According to Márquez [18], the firmness of the avocado on day 1 ranges between 60-67 N, and decreases as the fruit ripens to final values around 5 N. This decrease is likely due to the hydrolysis of the pectic compounds of the cell wall by the action of pectinase, polygalacturonates, cellulases, and amylases. Optimal firmness is between 10-20 N, with over-maturity occurring from day 17 of storage.

4.2. HIGH PRESSURE

Before the size reduction process, the avocado pulps were divided into two batches: one treated with high pressure processing and one without. The purpose of applying high pressure was to observe any variations in the pulp that would occur during freeze-drying. The process conditions were 550 MPa at 20°C for 120 s. No tests were conducted to validate enzyme activity, but differences were observed in the physicochemical properties and volatile compounds between the samples treated with high pressure and those that were not.

According to Sarantakou [24], the avocado fruit contains endogenous pectolytic and cellulolytic enzymes, as well as lipolytic enzymes, which can cause oil hydrolysis and oxidation, including polyphenoloxidase and lipoxygenase; likewise high-pressure processing of avocado paste (600–700 MPa; 3–10 min) can lead to 50% inactivation of these enzymes, however, it was noted that both enzymes can reactivate during storage, resulting in undesirable quality deterioration. Qin [21], mentions that the freeze-dried avocado may still contain lipase enzyme activity, which can cause the degradation of oil and the destruction of valuable minor compounds.

In this study, it was observed in general that the HP treatments decreased the pH and increased the acidity of freeze-dried powders compared to control, avocado pulp without any treatment. Conversely, studies without HP treatment showed an increase in pH and a decrease in acidity. Regarding wettability, HP-treated samples exhibited longer times compared to untreated samples, while there was no significant difference in water activity levels.

4.3. SIZE REDUCTION

After the treatment with or without high pressures, the avocado pulps were reduced in size using two methods, compression (potato press) and friction (immersion blender). Size reduction is a common unit operation in food processing and can be critical in food technology. It involves activities such as cutting, slicing, grinding, or pulping. According to Souza [25], the mechanical properties of food are dependent on its composition and processing, and in the case of freeze-drying, the type of freezing used has a significant impact on the texture of the food, as well as the degree of maturity of the fruit and the processing parameters.

4.4. LYOPHILISATION

After undergoing size reduction treatments, either compression or friction, all products were freeze-dried using the same processing conditions. The information related to this process is not disclosed due to its confidential nature of the company SioSi Alimentos, Mexico. The moisture content of the fresh avocados was 63.5 ± 0.5%, while the freeze-dried products had a moisture content of 1.5 ± 0.2%. According to Rodiles-López [22], lyophilized avocado from Michoacán, Mexico, had a composition of 9.2% carbohydrates, 19.4% dietary fibre, 60.4% lipids, 3.9% proteins, and 7.1% ash on dry matter.

Figure 1 - Equipment for the reduction of size. Left side, equipment for friction process, Oster® brand immersion blender model M2609-13. Right side, equipment for compression process, Metaltex® brand potato press with a mesh size of 1 cm

4.5. PHYSICOCHEMICAL ANALYSIS

After freeze-drying, several physicochemical parameters of the powders were evaluated and compared to assess the effect of each treatment. Table I presents a summary of the different studies and their corresponding Tukey analysis.

4.5.1 Ph and acidity

The pH of the fresh avocado was 6.48, which is consistent with the values reported by Krumreicha [14] of 6.5 ± 0.1 and 6.49 according to Jacobo-Velázquez [13]. There was no significant difference observed between compression and friction in HP treatments, and these are different from control. Likewise, there is no difference between friction and compression in those not treated by HP. It is worth noting that there was no significant difference between the T1B treatment, compression and without HP, with respect to the control.

The acidity of fresh avocado was 0.353 on oleic acid. According to Krumreicha [14], the acidity of fresh avocado was measured at 0.5 ± 0.1 on oleic acid. The results show no difference between compression and

Table I - Physicochemical Analyses. Freeze-dried avocado pulp powders

Treatment	pH	Acidity (%)	Wettability (s)	Water activity
T	6.48 ± 0.11 ^{B}	0.353 ± 0.021 ^{AB}	57 ± 2 ^{BC}	0.968 ± 0.022 ^{A}
T1A	6.94 ± 0.14 ^{A}	0.309 ± 0.022 ^{B}	62 ± 3 ^{B}	0.352 ± 0.014 ^{B}
T1B	6.64 ± 0.13 ^{AB}	0.334 ± 0.018 ^{AB}	52 ± 2 ^{C}	0.311 ± 0.010 ^{C}
T2A	6.07 ± 0.10 ^{C}	0.384 ± 0.023 ^{A}	78 ± 4 ^{A}	0.350 ± 0.012 ^{B}
T2B	5.95 ± 0.12 ^{C}	0.389 ± 0.026 ^{A}	72 ± 3 ^{A}	0.327 ± 0.011 ^{BC}

Equal letters in the same column do not differ significantly (α = 0.05).

T = Witness; where pH and water activity correspond a fresh avocado, acidity correspond an avocado oil, and wettability a freeze-dried grapefruit powder; T1A = treatment without high pressure and friction; T1B = treatment without high pressure and compression; T2A = treatment with high pressure and friction; T2B = treatment with high pressure and compression. All analyses were performed in triplicate.

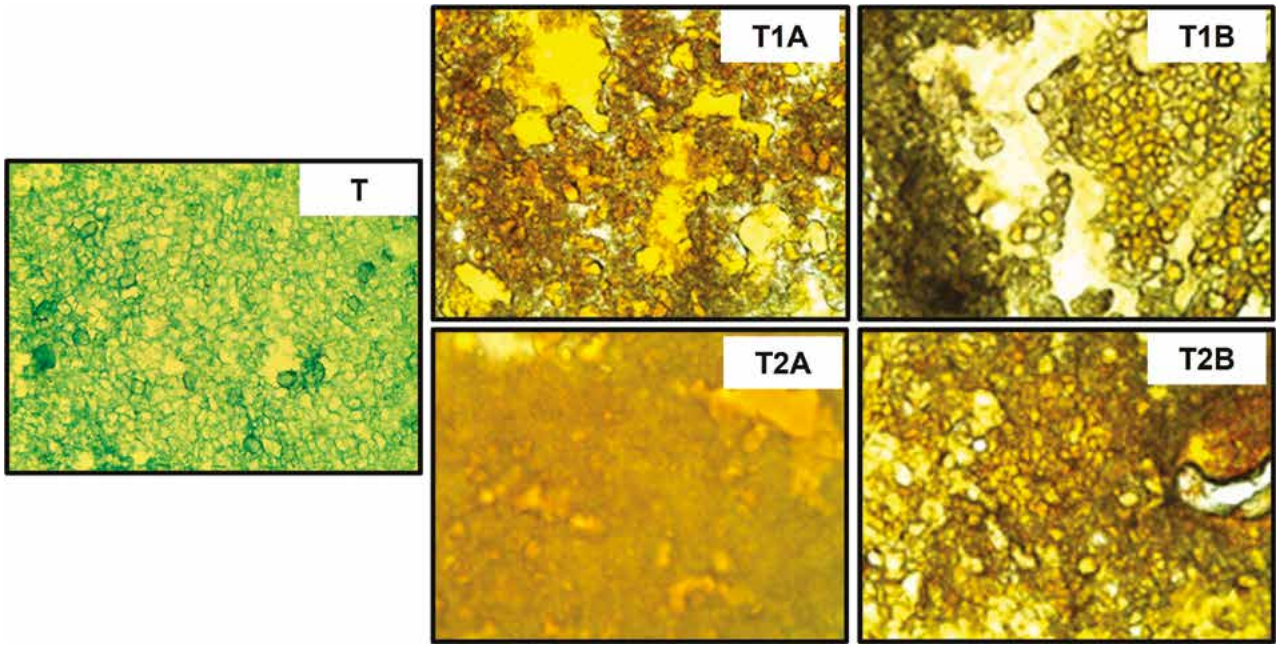

Figure 2 - Micrographs. 10x. T = Witness, fresh avocado; T1A = treatment without high pressure and friction; T1B = treatment without high pressure and compression; T2A = treatment with high pressure and friction; T2B = treatment with high pressure and compression

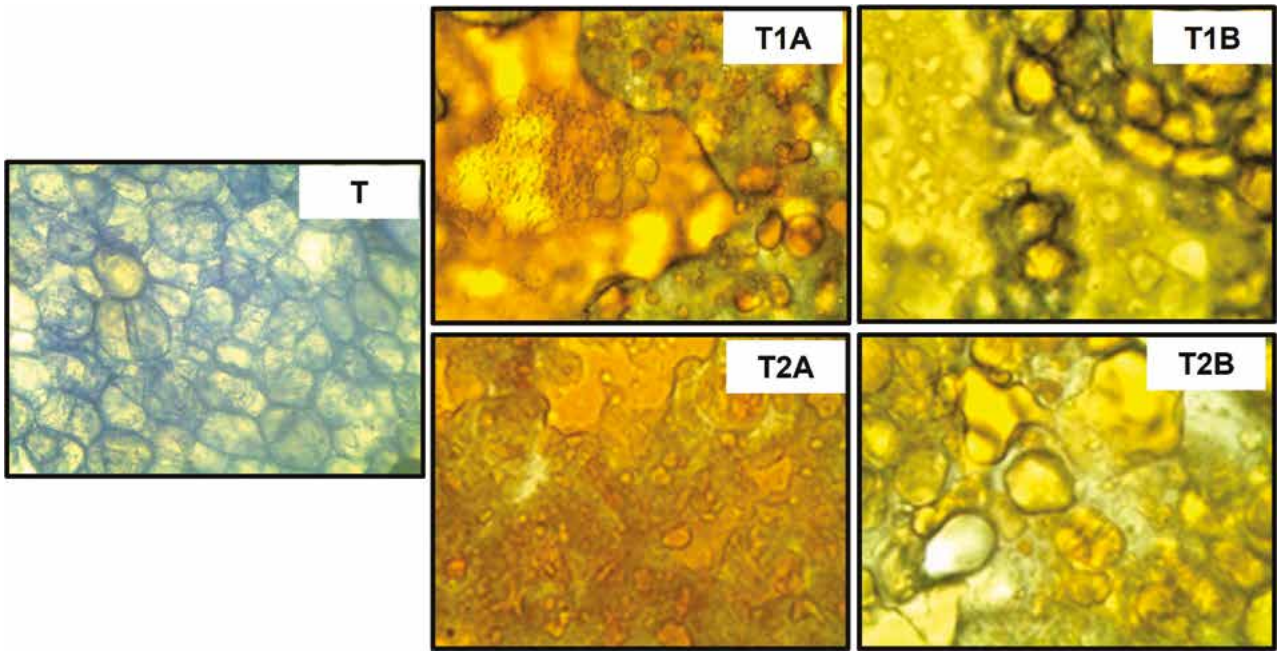

Figure 3 - Micrographs. 40x. T = Witness, fresh avocado; T1A = treatment without high pressure and friction; T1B = treatment without high pressure and compression; T2A = treatment with high pressure and friction; T2B = treatment with high pressure and compression

friction in HP treatments, and they are not different from the control. In non-HP, there is also no difference between friction and compression, and they are no different from control.
It can be concluded that the treatment which generated the least changes in comparison to the control was the sample without HP and compression, T1B. In this case, the pH changed by only 2.5% and the acidity by 5.5% in comparison to the witness, avocado fresh.

4.5.2 Humectability

In this case, it used a data reported by Egas-Astudillo [6] as witness, which corresponds to a freeze-dried grapefruit powder, 25°C. Shorter wettability time indicates that a powder is easier to solubilise in water. It observed that treatments with HP result in longer times compared to those without HP. Likewise, friction treatments have longer times compared to compression. Additionally, those treated with HP exhibit higher values than the control group and are statistically different. There is no difference between compression and friction without HP compared to the control group, but there is a difference between them. The batch without HP and compression had the shortest wettability time. Egas-Astudillo [6], state that freeze-dried powders have better wetting properties than those made by spraying, specifically in the case of grapefruit fruit; additionally, the freeze-dried powders exhibited shorter wetting times and lower hygroscopicity, but greater porosity. These properties could be further improved by adjusting particle size and shape.

4.5.3 Water activity

The fresh avocado had a water activity (aw) value of 0.968, while the average aw value of the freeze-dried powders in this study was 0.335 ± 0.020. Compression treatments resulted in lower aw values compared to friction, and the combination of no HP and compression had the lowest value. This batch also had the lowest variability in pH and acidity compared to the control, as well as the shortest wettability time. Water activity (aw) is a crucial factor in preventing or limiting microbial growth. Lower aw results in lower microbial growth and longer shelf life [26]. However, avocado is a fruit with high lipid content that is susceptible to oxidation reactions, and this behaviour varies with the water activity values. Vu [27], noted a J-shaped curve in the behaviour of aw regarding lipid oxidation. This curve can be divided into four regions. In the first region, where aw is less than 0.2, lipid oxidation rates are intermediate. In the second region, between 0.2 and 0.3, the lowest oxidation rates are observed. In the third region, between 0.3 and 0.9, lipid oxidation increases. Finally, in the fourth region, close to 1.0, lipid oxidation rates are again reduced.

4.6. VOLATILE COMPOUNDS

Gas chromatography was used to determine the volatile compounds in each of the lyophilised treatments and the fresh avocado, control. Changes were observed in comparison to the fresh avocado for each treatment. Table II summarises these compounds.
It found 26 volatile molecules in fresh avocado, with the top 10 being hexanal, 2-hexenal, caryophyllene, benzeneacetaldehyde, copaene, α-bergamotene, hexane-2,3-dimethyl, α-cubebene, hexane, and 3-carene, which make up 91% of the total compounds. The analysis of these 10 compounds reveals that the four treatments preserve hexanal, caryophyllene, α-cubebene, and 3-carene. However, benzeneacetaldehyde, hexane-2,3-dimethyl, hexane, and practically 2-hexanal are no longer present. Copaene disappears only in the treatment without HP and friction but remains in the other treatments. Lastly, α-bergamotene is maintained in HP treatments but disappears in non-HP treatments.
All treatments produced decano-2,6,8-trimethyl, heptane-2,2-dimethyl, and hexane-3,3-dimethyl, as well as ylangene, α-phellandrene, and octane-6-ethyl-2-methyl, along with traces of 1-nonene, 1-hexanol, and 1-octen-3-ol; and these compounds were absent in the control, fresh avocado. In contrast, all treatments disappear in relation to the control: pentanal-2,3-dimethyl, 2-heptenal, nonanal, α-amorphene, octanal, 3-nonen-2-one, dodecano-2,6,10-trimetil, and 2-heptylfuran.
Liu [17] detected 31 volatile compounds in fresh post-harvest avocado using gas chromatography-mass spectrometry (GC-MS), and they detected 8 alcohols, 6 ketones, 5 aldehydes, 3 acids, 4 heterocyclic compounds, 2 hydrocarbons, 1 amine, 1 ester, and 1 phenol.
According to Lara-García [15], the volatile compounds in 14 avocado genotypes were predominantly alcohols and aldehydes. This is due to the degradation of lipids, which are abundant in this fruit. The compounds present in the given sample are acetaldehyde, hexanal, 2-hexenal, α-cubebene, α-copaene, and β-caryophyllene. This author points out that acetaldehyde is known for its fresh fruit aroma, while hexanal and 2-hexenal have a grassy aroma and tend to decrease in concentration during ripening. α-cubebene has fruity aromas like citrus, whereas α-copaene and β-caryophyllene have spicy and woody notes.
Lipid oxidation processes, including auto-oxidation and enzymatic reactions, can lead to rancidity. The hydroperoxides formed during the oxidation of linoleic acid, which is the most abundant and oxidation-susceptible fatty acid in avocados, are rapidly broken down into several compounds that cause rancid odour and taste. These compounds include hexanal, 3-hexenal, and 2-hexenal [13].

Table II - Volatile compounds. Freeze-dried avocado powders and fresh avocado. Percentages

	T	T1A	T1B	T2A	T2B
Hexanal	24.4	18.5	19.9	56.7	33.6
2-hexenal	15.9	-	-	0.2	-
Cariofileno	15.8	11.8	13.6	7.0	10.9
Benzeneacetaldehyde	9.9	-	-	-	-
Copaene	9.1	-	8.7	4.3	7.9
α-bergamotene	8.5	-	-	2.5	4.7
Hexane, 2,3-dimethyl	2.5	-	-	-	-
α-cubebene	1.9	3.6	4.9	2.0	3.4
Hexane	1.5	-	-	-	-
3-carene	1.4	3.0	1.1	1.3	2.5
2-furanona	1.2	1.1	1.3	0.4	1.1
Pentanal, 2,3-dimethyl	0.9	-	-	-	-
Seychellene	0.8	-	0.1	-	0.1
Gurjunene	0.8	-	0.1	-	0.1
β-sesquiphellandrene	0.7	0.2	-	0.1	0.1
2-heptenal	0.7	-	-	-	-
Pentanal	0.6	-	-	2.9	1.4
Nonanal	0.5	-	-	-	-
α-amorphene	0.5	-	-	-	-
Octanal	0.5	-	-	-	-
1-hexene, 3,5-dimethyl	0.5	0.2	-	-	-
3-nonen-2-one	0.5	-	-	-	-
Acetic acid, methyl ester	0.4	5.0	6.2	-	3.9
Dodecane, 2,6,10-trimetil	0.2	-	-	-	-
2-heptylfuran	0.1	-	-	-	-
Neodihydrocarveol	0.1	0.1	-	-	-
Decano, 2,6,8-trimetil	-	2.1	1.8	1.5	1.6
Heptane, 2,2-dimethyl	-	8.0	7.0	2.8	6.2
α-copaene	-	8.0	-	-	-
Hexane, 2,3,5-trimethyl	-	6.6	0.3	-	0.3
Hexane, 3,3-dimethyl	-	5.4	4.4	2.3	0.3
3-pentanone	-	5.1	6.8	-	-
Heptane, 3,3-dimethyl	-	3.1	-	-	2.9
Ethyl acetate	-	2.7	-	0.8	2.0
5,6-decadien-3-yne, 5,7-diethyl	-	2.6	-	4.6	2.3
2,5-octadiyne, 4,4-diethyl	-	2.0	-	1.1	1.8
Furan, 2-pentyl	-	1.5	0.7	-	-
Ylangene	-	1.4	1.6	0.8	1.3
Heptane, 2,2,4,6,6-pentamethyl	-	1.4	0.7	-	-
α-caryophyllene	-	1.3	-	0.1	0.6
α-phellandrene	-	1.1	0.2	0.4	0.9
Octane, 6-ethyl-2-methyl	-	1.0	0.9	0.4	4.4
Decane, 2-methyl	-	0.8	-	-	2.1
Trans-α-bergamotene	-	0.5	-	0.4	0.5
1-nonene	-	0.4	0.4	0.2	0.3
1-hexanol	-	0.4	0.4	0.2	0.3
Octane, 2,2,6-trimethyl	-	0.3	1.0	-	0.7
Octane, 2,3,6,7-tetramethyl	-	0.2	1.6	-	-
β-myrcene	-	0.2	-	-	-

Table II (continue)

1-octen-3-ol	-	0.2	0.2	0.1	0.3
Hexane, 2,4-dimethyl	-	-	-	0.8	0.7
δ-cadinene	-	-	-	0.1	0.5
Hexane, 3-methyl-	-	-	0.2	-	0.3
1-octene	-	-	-	-	0.2
6-hepten-3-one, 5-hydroxy-4-methyl	-	-	3.2	-	-
Undecane, 2,8-dimethyl	-	-	3.0	-	-
α-humulene	-	-	2.2	-	-
Octane, 2,4,6-trimethyl	-	-	2.1	-	-
Hexane, 2,2,3-trimethyl	-	-	2.0	-	-
Decane, 2,5,9-trimethyl	-	-	1.8	0.7	-
Heptane, 2,2,3,5-tetramethyl	-	-	0.9	-	-
α-farnesene	-	-	0.5	-	-
Octane, 2,7-dimethyl	-	-	0.3	-	-
Pentane, 2,2,3,4-tetramethyl	-	-	-	2.5	-
1-hexene, 3,5,5-trimethyl	-	-	-	1.6	-
Heptanal	-	-	-	1.1	-
Hexane, 2,2,5,5-tetramethyl	-	-	-	0.2	-

The table presents the percentage of each compound with respect to the total of compounds present in each treatment. T = Witness, fresh avocado; T1A = treatment without high pressure and friction; T1B = treatment without high pressure and compression; T2A = treatment with high pressure and friction; T2B = treatment with high pressure and compression. All analyses were performed in duplicate.

The reduction in size increases the surface area. This could lead to increased oxidation, loss of nutrients and flavour compounds, and changes in volatile compounds [23]. The scent of avocados (*Persea americana Mill. cv. 'Hass'*) is determined by volatile compounds derived from fatty acids. These compounds change depending on the fruit's maturity stage, which is regulated by ethylene. They are the primary precursors of esters, alcohols, and aldehydes [9,10].

4.7. MICROSCOPY

A microscopic optical study was conducted to compare the different treatments with fresh avocado. The results are shown in Figure 2 (magnification of 10x) and Figure 3 (magnification of 40x). The images show the cell wall in green and the lipids in yellow. The control, fresh avocado, clearly shows the presence of the cell wall intact. In all treatments, the rupture of the cell wall and dispersed lipids is observed. However, the samples subjected to compression show less cell wall breakage than those subjected to friction.

Lipids are mainly present in the mesocarp of avocados, which is composed of numerous parenchyma cells and evenly scattered idioblasts. During ripening, the primary walls of the parenchyma cells can be degraded by the activity of cellulase and poligalacturonasas, but the suberised walls of the idioblastic cells remain intact due to the immunity of these enzymes. Lipids are released when the cell walls are broken by mechanical forces [21].

5. CONCLUSIONS

Prior to freeze-drying the Hass avocado pulp, a series of pre-treatments were conducted to determine the optimal methodology. The pulp was initially divided into two groups: one treated with high pressure and the other without. Each group was then subjected to two methods of particle reduction, compression, and friction. The freeze-dried products had a final humidity of 1.5 ± 0.2%. HP treatments decreased pH and increased acidity compared to untreated avocado pulp, while non-HP treatments increased pH and decreased acidity. Additionally, HP treatments resulted in longer wettability times than untreated samples. Powders treated with compression exhibited lower water activity compared to those treated with friction. The sample with the least change in pH and acidity compared to the control was the batch without HP treatment and with particle reduction by compression. Additionally, this batch had the best wettability time and lowest water activity. The comparison of volatile compounds among the four treatments revealed differences between them, and in comparison, to the profile of volatiles found in fresh avocado. Each treatment developed a unique profile that characterises it. Additionally, optical microscopy analysis showed that all treatments caused cell wall breakage and lipid dispersion, with compression treatments causing less damage.

Acknowledgments
The authors would like to express their gratitude to SioSi Company for providing the opportunity to conduct the freeze-drying processes.

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- Determinazione del **triptofano**

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Fatty Acids Contents & Nutritional Compositions of Grains of Different Barley Genotypes

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Received: October 23, 2023
Accepted: August 7, 2024

Barley is an important cereal in the world regarding its utilisation and extensive production as a feed and food grain in many countries. But little information is available on barley oil in terms of its composition. The aim of this study was to describe the importance of barley seeds by extracting their oil and determine its composition regarding lipids along with evaluations on composition, nutrient content, feed value and quality parameters. The crude ash, crude protein, ADF, NDF and ADL ratios of the seeds of barley genotypes varied between 3.5-7.1%; 8.3-13.4%; 13.5-26.0%; 23.8-36.3% and 1.99-8.78%, respectively. The DMD ratios, DMI ratios, RFV values, TDN ratios, DE and ME values of genotypes varied between 68.7-78.4%; 3.30-5.05%; 175.9-304.0%; 62.9-72.3%; 3.21-3.63 MJ/kg and 9.67-11.33 Mcal/kg, respectively. The total lipid content of the examined genotypes varied between 1.7 and 3.9%. Several fatty acids were detected; Palmitic, stearic and arachidic acids from saturated fatty acids were detected in the seeds of all barley genotypes examined in the study. The seed lipids of some barley genotypes contain palmitic (16.80-25.56%) and stearic (1.33-3.70%) acids as the major component of fatty acids, among the saturated acids, with small amounts of arachidic (0.24-0.54%) and behenic (0.06-0.90%) acids. The major unsaturated fatty acids found in the seed lipids were oleic (15.30-33.78%), linoleic (41.92-55.28%) and linolenic (2.84-5.43%) acids. Palmitoleic, erucic, docosahexaenoic and nervonic acids were shown to be lower than 1%. Eicosenoic and erucic acids were detected in all barley genotypes. Barley seeds may be used as a source of edible oil due to the presence of several unsaturated and essential fatty acids.

Keywords: Barley, nutrients, quality, fatty acids, saturated, unsaturated

1. INTRODUCTION

Cereal-based foods are the primary source of energy and nutrients for the vast majority of people worldwide [1]. Exploiting the nutritional potential of cereal grains, particularly their starch, protein, and lipid fractions, is receiving increased attention as the global need for nutrients, food, and feed increases [2]. There has been very little scientific interest in identifying the fatty acids and lipid fractions in barley grain and other cereals, even though lipids have a 7significant influence on the functional and storage characteristics of cereal products, on processing [1, 2]. From a nutritional and technological point of view, barley and other cereal grain lipids in general deserve a more focused interest than the few reports available [3]. Conventional barley offers superior nutritional value compared to other cereals in terms of protein, carbohydrate, and mineral content. The nutritional value of conventional barley is great; however, it contains fewer lipids than oat [2]. The largest lipid concentrations in cereals are in oat, with 2-18% lipids [4], and in maize, with 5-22% [5] of the whole grain weight. Barley has approximately 2-4% lipids of the total grain weight. Nevertheless, barley lipids deserve a focused

interest due to their fatty acid composition and their vitamin E composition [2]. When we look at the previous studies on the lipid content and fatty acid composition of cereal grains in Turkey; it was seen that the fatty acid contents were investigated in the seeds of selected cereals cultivated in Turkey [6], Turkish sorghum landrace [7], *Sorgum bicolor* genotypes [8], some pearl millet genotypes [9].
The aim of this study was to determine the lipid contents and fatty acid compositions of the seeds of some barley genotypes along with standard chemical analysis such as crude ash, crude protein, ADF, NDF, ADL, Relative feed value, dry matter digestibility and dry matter intake.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL

The names of some barley genotypes grown in the farmer's field in the 2020-2021 growing season in Elazig after being obtained from different institutions and organisations are given in Table I.

2.2. METHODS

2.2.1 Chemical Analyses

Seeds of the barley genotypes were ground in a mill and passed through 1 mm sieve for chemical analysis. Chemical analyses were performed in three repetitions. Crude ash ratio of barley grain samples was determined by burning at 550°Cfor 8 hours [10]. Crude protein analyses were performed by the methods specified in AOAC [11]. The ADF, NDF and ADL constituting the cell wall were performed by the method specified in Van Soest [12] and Van Soest and Wine [13]. Relative feed value (RFV), dry matter digestibility (DMD) and dry matter intake (DMI) of barley grains were calculated according to the following formulas [14].
DMD% = 88.9 - (0.779 × ADF%);
DMI% = 120 / NDF%;
RFV = (DDM% × DMI%) /1.29
The total digestible nutrients (TDN) ratios and metabolic energy (ME) values of the seeds of barley genotypes were determined according to the method specified by Moore and Undersander [15], and the digestible energy (DE) value was determined by Fonnesbeck et al. [16] according to the method specified.

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

Impurities were removed from the barley seeds, and the clean seeds were ground into powder using a ball mill. Lipids were extracted with hexane/isopropanol (3:2) [17]. 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.

2.2.3 Capillary GLC

Fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulfuric acid in methanol [18]. The fatty acid methyl esters were extracted with 2.5 ml hexane. Then the methyl esters were separated and quantified by gas chromatography and flame ionisation detection (Agilent brand 7890A model GC, 5975C model MS) coupled to a glass GC 10 software computing recorder. Chromatography was performed with a capillary column (100 m in length and 0.25 mm in diameter, BPX90: SGE 054596) using nitrogen as carrier gas (flow rate 3 ml/min). The temperatures of the column, detector, and injector valve were 120-250°C and 230-270°C, respectively. Identification of the individual method was performed by frequent comparison with authentic standard mixtures that were analysed under the same conditions.
The data obtained was analysed by one-way analysis of variance (ANOVA) with the Jump-Pro13 statistical package program, and the differences between the means were compared according to the Tukey test. Correlation analysis was performed between the examined features. Correlation analysis and colour map were made in Jump-Pro13 and Biplot graphics in Genstat 12th (Copyright 2011, VSN International Ltd.).

Table I - Names of barley genotypes used in the study

No	Genotypes	No	Genotypes	No	Genotypes	No	Genotypes
G1	Baris	G11	Onder	G21	Novosadski-565	G31	Bravo
G2	Caca Bey	G12	Burakbey	G22	Nonius	G32	Finola
G3	Akar	G13	Anka-11	G23	Dara	G33	Larende
G4	Cetin-2000	G14	Altikat	G24	Erginel-90	G34	Tosunpasa
G5	Lord	G15	Bozlak	G25	Altinay	G35	Bozlak
G6	Sladoran	G16	Altinorak	G26	Sentosa	G36	Anka-10
G7	Sur-93	G17	Scarpia	G27	Champie	G37	Aydan Hanim
G8	Anka-08	G18	Samyeli	G28	Inbat	G38	Sahin-91
G9	Anka-06	G19	Anka-09	G29	Kendal	G39	Ince-04
G10	Asil	G20	Hevsel	G30	Tarm-92	G40	Avci-2002
						G41	Unver

3. RESULTS AND DISCUSSION

3.1. BASIC CHEMICAL ANALYSIS

Crude ash, crude protein, acid detergent fibre (ADF), neutral detergent fjibre (NDF), acid detergent lignin (ADL), dry matter digestibility (DMD), dry matter intake (DMI) and total digestible nutrients (TDN) ratios and relative feed value (RFV), digestible energy (DE) and metabolic energy (ME) values determined in the grains of some barley genotypes were found to be statistically significant at the 1% level (Table II).

The crude ash and crude protein ratios of the seeds of barley genotypes varied between 3.5-7.1% and 8.3-13.4%, respectively. The highest crude ash content of barley genotypes was obtained in Altinay variety, followed by Kendal and Dara varieties, which are statistically in the same group. The highest crude protein ratios were found in Altikat and Burakbey varieties, which were in the same group statistically. On the other hand, the lowest crude ash and crude protein ratios in grains belonging to barley genotypes were statistically obtained in Aydan Hanim and Avci-2002 varieties, which are in the same group. While it was reported that crude protein ratios of hulled and hulless barley were obtained as 13.41% and 14.62-16.65%, respectively [19], crude protein ratios of winter barley grain were reported to obtained as 8.2-12.1% [20], and as 10-20% in barley seed [21]. On the other hand, crude ash and crude protein rates were obtained as 6.4% and 21.9%, respectively, in barley [22], as 2.0-2.7% and 9.6-11.5%, respectively, in batches of barley [23], as 2-4% and 11.4-14.3%, respectively, in Tunisian barley varieties [24], as 2.6% and 12.0%, respectively, in grain of barley [25], as 0.51-2.03% and 8.3-9.2%, respectively, in whole barley flour [26], as 2.01-4.65% and 8.68-10.74%, respectively, in highland barley flour [27].

The ADF, NDF and ADL ratios of the seeds of barley genotypes varied between 13.5-26.0%, 23.8-36.3% and 1.99-8.78%, respectively. While the lowest ADF, NDF and ADL ratios were obtained from Unver, Caca Bey and Aydan Hanim varieties, respectively; the highest ADF and NDF rates were found in Bozlak cultivar, and the highest ADL rate was found in Kendal, Dara, Sentosa and Larende cultivars, which are statistically in the same group. In the study in which the chemical composition of winter barley grain was examined, it was reported that the crude fibre, ADF, NDF and lignin values of barley seed were obtained as 45.6-53.4 g/kg DM, 57.2-69.1 g/kg DM, 186-259 g/kg DM and 8.7-13.1 g/kg DM, respectively [20], while in the study examining the nutritional values of some grain species, it was reported that the crude fibre, ADF, NDF and ADL values of barley grain were obtained as 50 g/kg DM, 105 g/kg DM, 253 g/kg DM and 25 g/kg DM, respectively [25]. On the other hand, crude fibre values were obtained as 38-64 g/kg DM in barley grain [23], as 4.21% in whole barley flour [26], as 11-34% in barley seeds [21].

The DMD and DMI ratios and RFV values of barley grains differed statistically by 1% among genotypes and varied between 68.7-78.4%, 3.30-5.05% and 175.9-304.0%, respectively. The highest DMD rate was obtained from the Unver cultivar, and the highest DMI rate and RFV value were obtained from the Caca Bey cultivar, while the lowest DMD, DMI rates and RFV value were determined from the Bozlak cultivar. The TDN ratio, DE and ME values of barley seeds differed statistically at the level of 1% among genotypes. The TDN ratios, DE and ME values of grains of barley genotypes varied between 62.9-72.3%, 3.21-3.63 MJ/kg and 9.67-11.33 Mcal/kg, respectively. While the highest TDN ratios and DE values were obtained from the Unver cultivar, the highest ME value was obtained from the Baris cultivar. The lowest TDN rate and DE value were determined from the Bozlak cultivar, and the lowest ME value from the Unver cultivar. While it has been reported that the ME of barley grain was obtained as 13.4 MJ/kg DM [22], the apparent metabolisable energy value of barley seeds was obtained as 10.5-13.7 Mcal/kg DM [23].

With biplot analysis methods, the relationship between genotypes and the characters examined in the research can be presented graphically. In these graphs, PC1 represents the efficiency of genotypes and PC2 represents the stability of genotypes [28]. For this reason, it is desired that the PC1 value of an ideal genotype/variety should be high in terms of the characters in question, and the PC2 value should be close to zero [29]. The study found that the total variation between genotypes and traits was 92.09%, where 80.33% was sourced from PC1 and 11.76% was sourced from PC2. Demirel et al. [30] showed that the PC1+PC2 variation was 73.32% in the graphics obtained from the biplot analysis in their study, Akcura et al. [31] 82.2%, Yorulmaz et al. [32] found that this value was 47.07%. Since the angle between the vectors representing the examined features DMI, RFV, DMD, TDN, DE and ME is lower than 90°, there is a high positive relationship between these parameters. In addition, there was a high negative relationship between ADF and NDF, ADL and ash content. The distance of the vectors expressing the features and the distance of these vectors from the centre point of the coordinate plane are due to the weakness of the relationship between these characters [33]. When the vector graphic of this study is examined; it can be seen that the variation between varieties was low in terms of ADL ratio, but the variation was high in terms of other characteristics. In addition, it was seen that RFV and DMI features were in the first mega environment, DMD, TDN, DE and ME features were located in the second mega environment, ash and protein features were located in the third mega environment and ADL, ADF and NDF features were located in the fourth mega environment.

Scatterplot biplot graphics provide a visual output by

Table II - Means of the examined characteristics

Genotype	CA	CP	ADF	NDF	ADL	DMD (Dry Matter Digestibility	DMI (Dry Matter Intake)	RFV (Relative Feed Value)	TDN (Total Digestible Nutrients)	DE (Digestible Energy)	ME (Metabolic Energy)
	(%)	(%)	(%)	(%)	(%)	(%)	(%)		(%)	(MJ/kg)	(Mcal/kg)
G1	5.2 k-m	11.0 kl	15.9 f-n	25.3 ı-m	5.03 n-q	76.5 a-ı	4.74 a-g	281.1 a-g	70.4 a-ı	3.54 a-ı	11.33 a
G2	4.3 rs	10.4 mn	14.6 l-n	23.8 m	4.07 r	77.6 a-c	5.05 a	304.0 a	71.4 a-c	3.59 a-c	11.29 ab
G3	5.8 g-ı	12.0 ef	18.3 d-j	27.5 d-k	8.33 cd	74.7 e-k	4.36 f-k	252.4 f-k	68.7 e-k	3.47 e-k	11.29 ab
G4	5.7g-j	12.8 bc	15.7 h-n	26.6 f-m	7.62 f	77.0 a-g	4.51 b-ı	268.0 a-ı	70.6 a-g	3.55 a-g	11.26 a-c
G5	5.5 ı-k	10.3 no	16.0 f-n	26.0 g-m	5.34 l-n	76.5 a-ı	4.61 a-h	273.5 a-ı	70.4 a-ı	3.54 a-ı	11.19 a-c
G6	5.4 j-l	11.1 jk	13.8 mn	24.2 lm	3.81 r	78.2 ab	4.97 a-c	301.4 a-d	72.0 ab	3.62 ab	11.13 a-d
G7	6.2 ef	10.7 lm	17.1 e-l	25.4 ı-m	5.33 l-n	75.5 c-j	4.73 a-g	277.3 a-h	69.5 c-j	3.50 c-j	11.13 a-d
G8	5.2 lm	9.5 dt	18.4 d-ı	27.6 d-j	6.35 ıj	74.5 f-k	4.35 f-k	251.7 f-k	68.5 f-k	3.46 f-k	11.11 a-e
G9	5.5 g-j	12.3 de	16.7 f-m	27.1 d-l	5.31 l-n	75.8 b-ı	4.42 e-k	260.2 e-j	69.8 b-ı	3.52 b-ı	11.11 a-e
G10	5.4 j-l	11.7 f-h	16.7 f-m	27.1 d-l	6.85 gh	75.9 b-ı	4.43 d-j	260.4 e-j	69.8 b-ı	3.52 b-ı	11.05 a-f
G11	5.9 f-h	13.0 b	16.1 f-n	25.1 ı-m	5.68 k	76.3 a-ı	4.78 a-g	283.0 a-g	70.3 a-ı	3.54 a-ı	11.04 a-g
G12	5.8 g-ı	13.1 ab	16.8 e-m	27.2 d-l	4.81 q	75.8 b-j	4.41 e-k	259.0 e-j	69.7 b-j	3.51 b-j	11.03 a-h
G13	4.6 qr	11.7 fg	15.8 g-n	26.1 f-m	5.21 l-o	76.6 a-h	4.60 a-h	273.1 a-ı	70.5 a-h	3.55 a-h	11.02 a-h
G14	6.5 c-e	13.4 a	18.8 c-g	29.2 b-f	8.04 de	74.2 h-l	4.11 h-l	236.3 ı-l	68.2 h-l	3.45 h-l	11.00 a-ı
G15	5.9 fg	10.6 m	26.0 a	36.3 a	6.04 j	68.7 n	3.30 m	175.9 m	62.9 n	3.21 n	11.00 a-ı
G16	5.7 g-j	10.7 lm	16.6 f-n	27.2 d-l	6.48 ı	76.0 a-ı	4.42 e-k	260.5 e-j	69.9 a-ı	3.52 a-ı	10.99 a-ı
G17	6.2 de	12.0 ef	17.0 e-l	25.9 h-m	6.65 hı	75.6 c-j	4.63 a-h	271.4 a-ı	69.6 c-j	3.51 c-j	10.98 a-ı
G18	6.5 c-e	11.5 g-ı	19.9 b-e	30.3 b-d	8.06 de	73.4 j-m	3.96 j-l	225.2 j-l	67.4 j-m	3.41 j-m	10.98 a-ı
G19	4.6 p-r	10.2 no	14.9 k-n	25.3 ı-m	4.85 pq	77.2 a-d	4.74 a-g	284.1 a-g	71.1 a-d	3.58 a-d	10.92 a-ı
G20	6.3 de	11.1 k	17.9 d-k	28.3 c-ı	7.13 g	75.0 d-k	4.25 g-l	247.0 f-l	68.9 d-k	3.48 d-k	10.90 b-ı
G21	5.4 j-l	11.1 k	16.9 e-m	27.3 d-l	6.12 j	75.7 b-j	4.40 e-k	258.1 e-j	69.7 b-j	3.51 b-j	10.90 b-ı
G22	6.5 cd	11.8 fg	17.9 d-k	28.3 c-ı	7.84 ef	75.0 d-k	4.25 g-l	247.1 f-l	69.0 d-k	3.48 d-k	10.90 b-ı
G23	6.9 ab	11.2 ı-k	22.1 b	30.9 bc	8.76 ab	71.6 m	3.88 kl	215.6 k-m	65.7 m	3.34 m	10.89 b-ı
G24	6.7 bc	13.0 b	18.8 c-h	29.2 b-g	7.92 ef	74.2 g-l	4.12 h-l	237.0 h-l	68.2 g-l	3.45 g-l	10.88 b-j
G25	7.1 a	12.6 cd	19.1 b-f	28.0 c-j	8.34 cd	74.0 ı-m	4.29 f-k	246.6 g-l	68.1 ı-m	3.44 ı-m	10.87 b-j
G26	5.5 hıj	10.0 o-q	17.0 e-l	26.1 f-m	8.67 ab	75.6 c-j	4.60 a-h	269.5 a-ı	69.6 c-j	3.51 c-j	10.86 c-j
G27	6.2 de	10.2 n-p	20.6 b-d	29.0 b-h	8.44 bc	72.8 k-m	4.14 h-l	233.8 ı-l	66.9 k-m	3.39 k-m	10.86 c-j
G28	5.2 lm	11.8 f	16.1 f-n	27.1 d-l	5.16 m-p	76.3 a-ı	4.42 e-k	261.8 c-j	70.2 a-ı	3.54 a-ı	10.84 c-j
G29	7.0 ab	11.7 f-h	21.8 bc	32.1 b	8.78 a	71.9 lm	3.73 lm	208.3 lm	66.0 lm	3.35 lm	10.75 d-k
G30	4.9 m-p	10.6 m	15.8 g-n	26.2 f-m	1.99 u	76.6 a-h	4.58 a-h	271.9 a-ı	70.5 a-h	3.55 a-h	10.75 d-k
G31	4.7 o-q	9.6 r-t	15.6 ı-n	24.2 lm	3.86 r	76.8 a-f	4.97 a-d	295.9 a-e	70.7 a-f	3.56 a-f	10.69 e-k
G32	5.0 m-o	9.3 t	16.0 f-n	27.1 d-l	3.34 s	76.4 a-ı	4.42 e-k	262.1 b-j	70.3 a-ı	3.54 a-ı	10.67 f-k
G33	4.8 n-q	9.7 q-s	20.6 b-d	30.0 b-e	8.65 a-c	72.9 k-m	4.01 ı-l	226.5 j-l	66.9 k-m	3.39 k-m	10.62 g-l
G34	4.1 s	9.9 p-r	15.1 j-n	25.0 j-m	5.32 l-n	77.1 a-e	4.81 a-f	287.6 a-f	71.0 a-e	3.57 a-e	10.62 h-l
G35	4.8 n-q	10.2 no	13.8 mn	24.1 lm	5.04 n-q	78.2 ab	4.98 ab	301.9 a-c	72.0 ab	3.62 ab	10.59 ı-m
G36	5.0 mn	12.3 de	15.1 j-n	25.0 j-m	3.06 s	77.1 a-e	4.81 a-f	287.6 a-f	71.0 a-e	3.57 a-e	10.47 j-m
G37	3.7 t	8.3 u	14.0 l-n	24.4 k-m	2.66 t	78.0 a-c	4.93 a-e	298.1 a-e	71.8 a-c	3.61 a-c	10.39 k-m
G38	5.4 j-l	11.4 h-j	17.0 f-m	27.1 e-l	7.85 ef	75.9 b-ı	4.44 b-j	261.1 d-j	69.8 b-ı	3.52 b-ı	10.38 k-m
G39	5.1 mn	11.7 f-h	16.7 f-m	27.1 e-l	4.94 o-q	75.9 b-ı	4.43 c-j	260.7 d-j	69.8 b-ı	3.52 b-ı	10.23 lm
G40	3.5 t	8.4 u	15.0 k-n	25.0 j-m	5.48 kl	77.2 a-d	4.80 a-f	287.4 a-f	71.1 a-d	3.58 a-d	10.18 m
G41	4.6 p-r	9.4 t	13.5 n	24.1 lm	5.36 k-m	78.4 a	4.98 a-c	302.6 ab	72.3 a	3.63 a	9.67 n
Av.	**5.5**	**11.1**	**17.1**	**27.0**	**6.06**	**75.6**	**4.50**	**262.6**	**69.5**	**3.50**	**10.85**
TUKEY$_{(0.05)}$	**0.080****	**0.079****	**0.783****	**0.789****	**0.078****	**0.610****	**0.134****	**10.059****	**0.589****	**0.026****	**0.103****
CV (%)	**1.64**	**0.815**	**5.555**	**3.548**	**1.567**	**0.979**	**3.570**	**4.691**	**1.350**	**0.857**	**1.107**

** significant at the P≤0.01 level. There is no statistical difference between the averages shown with the same letter.

evaluating the relationship between genotypes based on average data. However, it cannot provide information about the importance level of the relationship between features. In addition to biplot graphics, our research was supported by a pairwise correlation table and a correlation colour map resulting from the correlation to reveal the importance of the relationship between features (Table III; Figure 3). While there was a positive and high relationship between the research CA rate and CP, ADF, NDF and ADL, a negative and high relationship was found between other features (Table III).

In the biplot chart (Figure 2), which makes it easy to express which genotype is at the forefront at which point, in terms of the parameters examined, it has been determined that varieties that stand out were: 1) Variety number 15 (Bozlak) in terms of ADF, NDF, 2) Variety numbers 23 (Dara) and 29 (Kendal) in terms of ADF, NDF and ADL, 3) Variety numbers 21 (Novosadski-565) and 25 (Altinay) in terms of protein and ash content. The absence of any feature in the regions with varieties 4 (Cetin-2000), 6 (Sladoron), 11 (Onder), 15 (Bozlak) and 37 (Aydan Hanim) located at the diagonal points of the polygon indicates that these varieties are not in an ideal environment for any feature. Numbers 2 (Caca Bey), 5 (Lord), 13 (Anka-11), 18 (Samyeli), 25 (Altinay), 28 (Inbat), 29 (Kendal) and 35 (Bozlak) were located close to the centre point of the coordinate plane. It

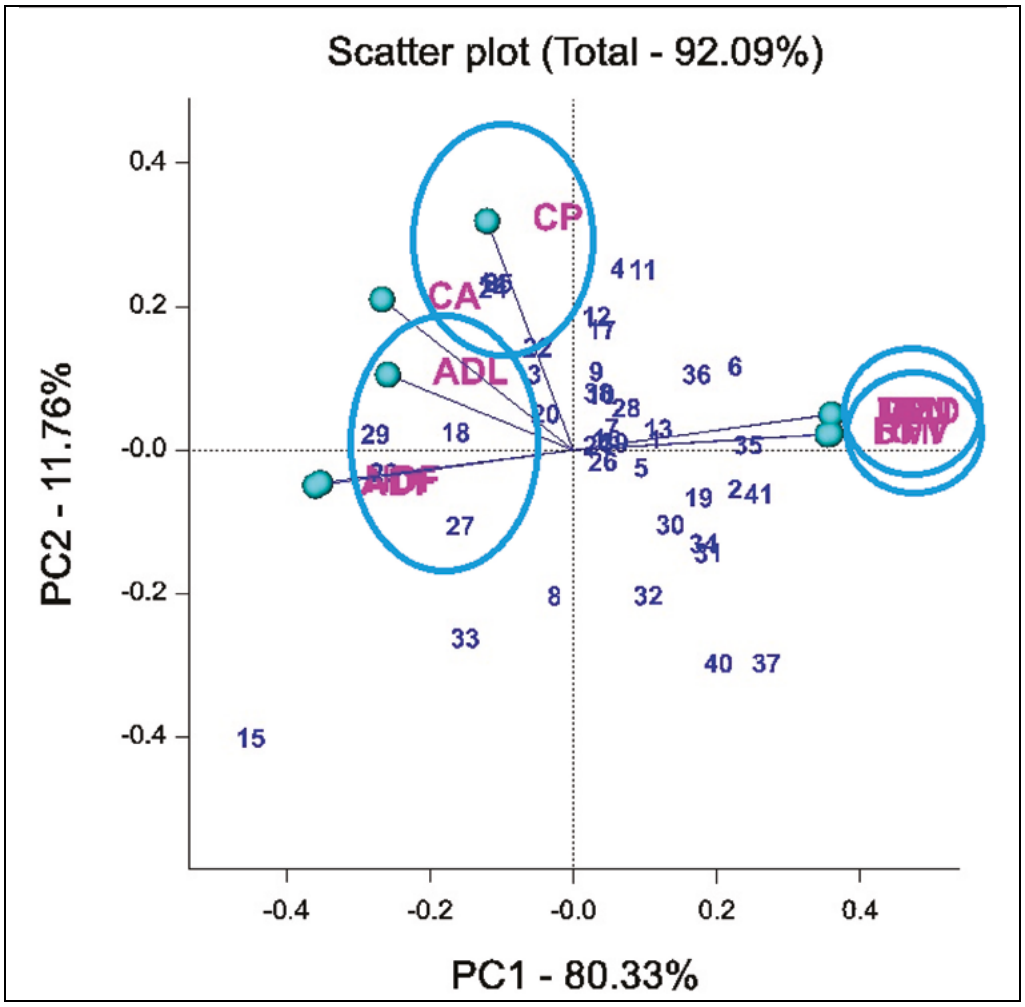

Fig. 1 - Vectoral representation of the relationship between the features examined in terms of average data. Abbreviations: CP; crude protein, ADF; Acid detergent fiber, NDF; Nötral detergent fiber, ADL; Acid detergent lignin, DMD; Dry Matter Digestibility, DMI; Dry Matter Intake, RFV; Relative Feed Value, TDN ;Total Digestible Nutrients, DE; Digestible Energy, ME; Metabolic Energy.

shows that the varieties have reasonable results in all examined traits and give higher than average values.
There is a positive and significant relationship between ADL and CP, a positive and significant relationship between ADF and NDF and ADL, and a negative and significant relationship between other features. A positive and significant relationship was found between NDF and ADL, a negative relationship between NDF and other features, and a negative and high relationship between ADL and other features. A direct (R=1) relationship was detected between DMD and DMI, RFV, TDN, DE and ME. A positive and high relationship was found between DMI and RFV, TDN, DE and ME, and a positive and high relationship was found between RFV and TDN, DE and ME. A direct relationship (R=1) was found between TDN and DE and ME, and between DE and ME (Table III). In the colour mapping system obtained based on the correlation between features, those with a correlation R value equal to 1 are dark red, while as they approach 0, their colour becomes lighter. As the R value moves away from 0, the colours turn dark blue (Figure 3).

3.2. LIPID CONTENTS AND FATTY ACID COMPOSITION

Lipid contents and fatty acid composition of 41 genotypes of barley were determined and the results are shown in Table IV.
The lipid content of the examined genotypes varied between 1.7 and 3.9% (Table IV). Scarpia (3.9%), Kendal (3.9%), Hevsel (3.8%), Onder (3.7%), Sahin-91 (3.7%) and Erginel-90 (3.6%) varieties were the highest for lipid content. The lowest percentages of lipid content were in Tarm-92 and Aydan Hanim varieties. It was reported that the lipid contents of 21 different barley strains grown in Ottawa varied between 2.5-3.1% [34], while the oil contents extracted from different barley grains varied between 1.90-2.87% [35]. In addition, it was reported that the oil content was obtained as 1.27% in the Larende barley variety grown in Turkey [6], the oil content was obtained as 1.73% in the barley grains obtained from a farmer's field in Konya [36] and oil content ranged from 3.71 to 4.69% in hulless and

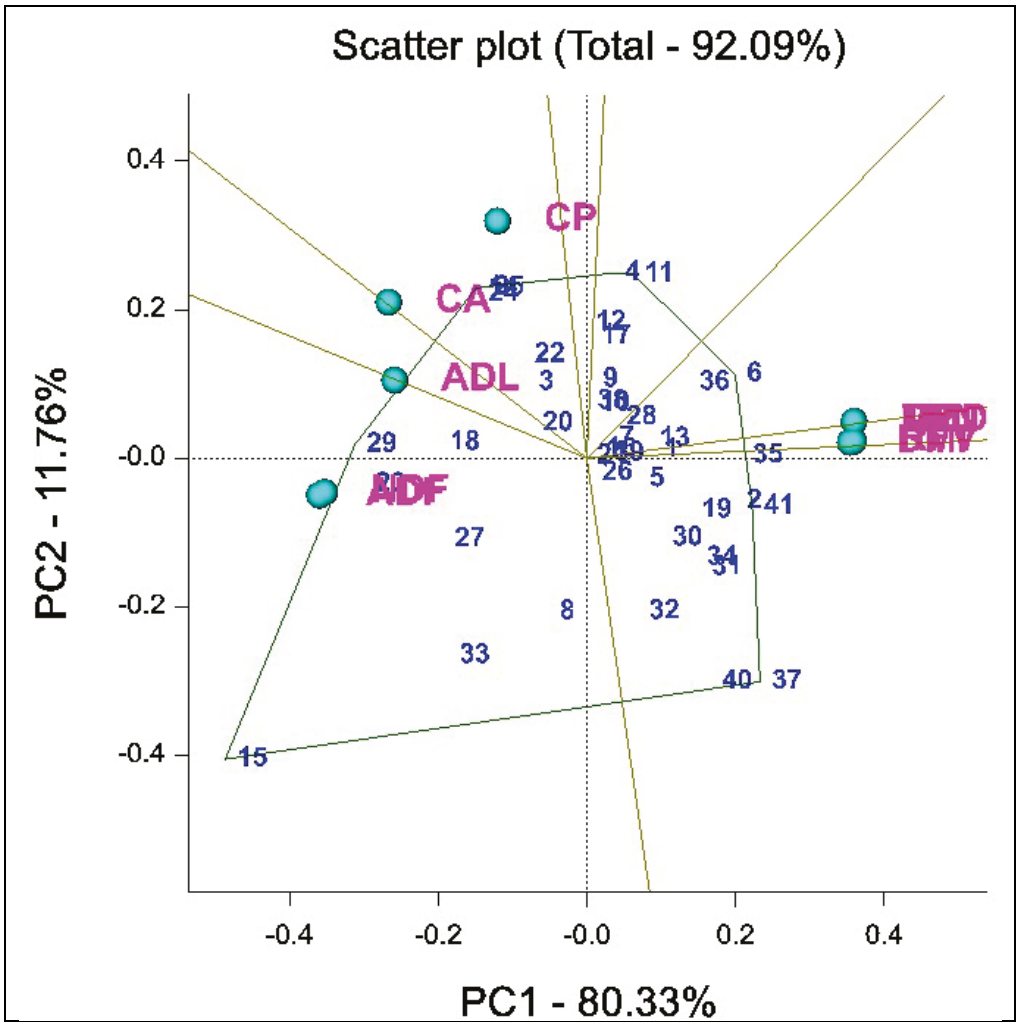

Fig. 2 - Fig. 2. Representation of the relationship between the examined features in terms of average data with polygon and sectors. Abbreviations: CP; crude protein, ADF; Acid detergent fiber, NDF; Nötral detergent fiber, ADL; Acid detergent lignin, DMD; Dry Matter Digestibility, DMI; Dry Matter Intake, RFV; Relative Feed Value, TDN ;Total Digestible Nutrients, DE; Digestible Energy, ME; Metabolic Energy.

Table III - Pairwise correlation analysis results of the relationship between features

	CA	CP	ADF	NDF	ADL	DMD	DMI	RFV	TDN	DE
CP	0.6425**	1								
ADF	0.6331**	0.1728	1							
NDF	0.5926**	0.2177	0.9538**	1						
ADL	0.6667**	0.3222*	0.6413**	0.5847**	1					
DMD	-0.6331**	-0.1728**	-1.0000**	-0.9538**	-0.6413**	1				
DMI	-0.6337**	-0.2621	-0.9412**	-0.9902**	-0.6363**	0.9412**	1			
RFV	-0.6497**	-0.2541	-0.9615**	-0.9885**	-0.6551**	0.9615**	0.9973**	1		
TDN	-0.6331**	-0.1728	-1.0000**	-0.9538**	-0.6413**	1.0000**	0.9412**	0.9615**	1	
DE	-0.6331**	-0.1728	-1.0000**	-0.9538**	-0.6413**	1.0000**	0.9412**	0.9615**	1.0000**	1
ME	-0.6331**	-0.1728	-1.0000**	-0.9538**	-0.6413**	1.0000**	0.9412**	0.9615**	1.0000**	1.0000**

* Significant at level P≤0.05; ** Significant at level P≤0.01

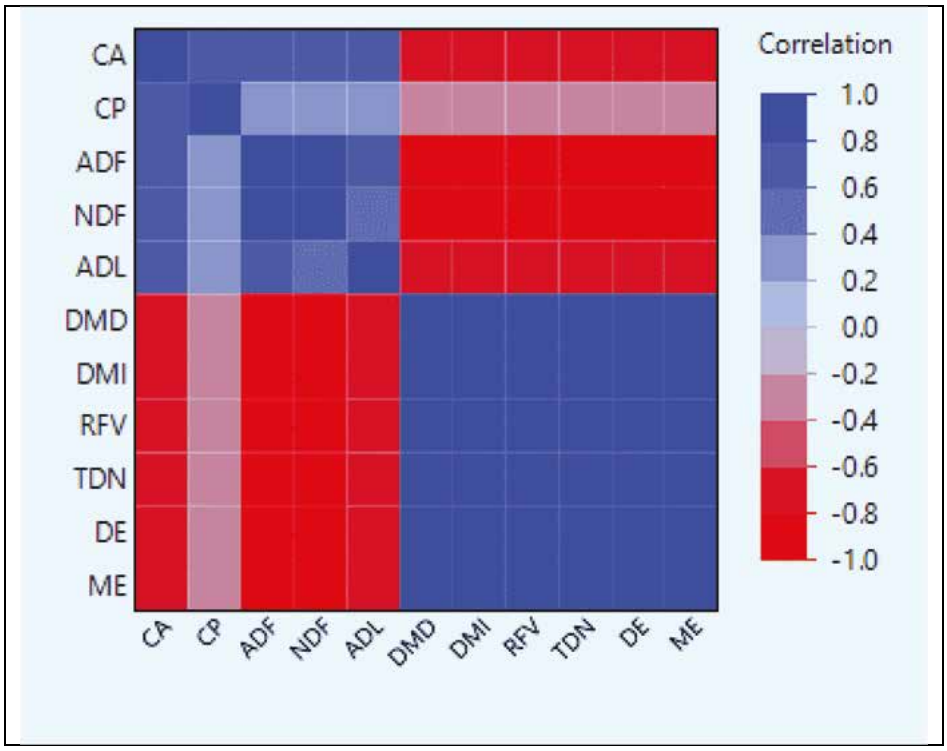

Fig. 3. Representation of pairwise correlation between features with color mapping system. Abbreviations: CP; crude protein, ADF; Acid detergent fiber, NDF; Nötral detergent fiber, ADL; Acid detergent lignin, DMD; Dry Matter Digestibility, DMI; Dry Matter Intake, RFV; Relative Feed Value, TDN ;Total Digestible Nutrients, DE; Digestible Energy, ME; Metabolic Energy.

covered barley grain under organic and conventional management regimens [37].

The seed lipids of some barley genotypes contain palmitic (16.80-25.56%) and stearic (1.33-3.70%) acids as the major component fatty acids, among the saturated acids, with small amounts of arachidic (0.24-0.54%) and behenic (0.06-0.90%) acids (Table IV). The major unsaturated fatty acids found in the seed lipids were oleic (15.30-33.78%), linoleic (41.92-55.28%) and linolenic (2.84-5.43%) acids (Table IV). Palmitoleic, erucic, docosahexaenoic and nervonic acids were lower than 1% in Table IV. In this study, the saturated fatty acids of some barley genotypes were between 19.48 and 28.54%, while the amounts of unsaturated fatty acids were between 71.46 and 80.52%.

Palmitic, stearic and arachidic acids from saturated fatty acids were detected in the seeds of all barley genotypes examined in the study. The highest palmitic, stearic and arachidic acids were in Akar (25.56%), Avci-2002 (3.70%) and Bravo (0.54%) varieties, respectively, while the lowest palmitic, stearic and arachidic acids were in Lord (16.80%), Tarm-92 (1.33%) and Finola (0.24%), respectively. It has been reported that the palmitic and stearic acids of winter and spring barley varieties vary between 21.7-23.6% and 0.59-1.81%, respectively [38], and the palmitic and stearic acids of 21 different barley strains vary between 18.3-27.0% and 2.5-3.1%, respectively [34], and the palmitic and stearic acids of barley oils are 120 g/kg and 6.9 g/kg, respectively [39]. While the palmitic and stearic acids of some barley cultivars were found to vary between 17.72-23.79% and 0.28-4.58%, respectively [35], the palmitic and stearic acids of Larende barley seeds in Turkey were reported to be 20.41% and 1.25%, respectively [6]. On the other hand, it was reported that the palmitic and stearic acids were obtained as 18.53 and 1.85%, respectively, in the barley grains obtained from a farmer's field in Konya [36] and, palmitic and stearic acids were ranged from 10.5 to 22.0% and from 0.3 to 1.1%, respectively, in hulless and covered barley grain under organic and conventional management regimens [37].

Palmitoleic acid was detected in all genotypes except Akar, Sladoran, Anka-08 and Burakbey genotypes; the highest level (0.15%) was found in the Dara variety, while the lowest level (0.07%) was found in the seeds of the Novosadski-565 variety. While the palmitoleic acid content of seeds of some barley cultivars was reported to vary between 0.31-2.87% [35], the palmitoleic acid content of Larende barley cultivar seeds was determined as 0.13% [6].

The major unsaturated fatty acids in the seed lipids of all barley genotypes were oleic, linoleic, and linolenic acids. The oleic, linoleic and linolenic acid contents were the highest in the seeds of Lord (33.78%), Bravo (55.28%) and Champie (5.43%) varieties, respectively and the lowest oleic, linoleic and linolenic acids in Champie (15.30%), Lord (41.92%) and Anka-10 (2.84%) genotypes, respectively. It has been reported

Table IV - The lipid contents and fatty acid compositions of seeds of some barley genotypes

No	Lipid	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	22:6	24:1	SFA	USFA
G1	3.1	21.90	0.11	1.56	16.21	54.01	4.35	0.36	1.07	0.09	0.22	0.12	-	23.91	76.09
G2	2.5	21.07	0.08	2.44	18.20	52.12	4.07	0.46	1.00	0.07	0.23	0.18	0.07	24.05	75.95
G3	3.1	25.56	-	2.54	20.59	45.41	3.87	0.44	0.95	-	0.20	0.23	0.19	28.54	71.46
G4	3.4	18.11	0.12	1.58	26.80	48.20	3.36	0.29	0.90	0.10	0.28	0.11	0.16	20.08	79.92
G5	3.3	16.80	0.12	2.67	33.78	41.92	3.13	0.35	0.74	0.08	0.24	0.10	0.07	19.90	80.10
G6	2.6	19.25	-	2.55	17.43	55.17	4.11	0.43	0.81	0.10	0.15	-	-	22.33	77.67
G7	3.2	19.38	0.11	1.59	20.31	52.55	4.38	0.34	1.07	0.08	0.19	-	-	21.39	78.61
G8	2.6	19.48	-	1.53	25.80	47.32	4.21	0.29	1.04	-	0.17	0.15	-	21.30	78.70
G9	3.1	19.82	0.09	2.34	18.55	53.22	4.15	0.40	0.87	0.07	0.20	0.29	-	22.63	77.37
G10	2.8	20.13	0.10	1.51	18.71	54.45	3.23	0.32	0.93	0.11	0.15	0.36	-	22.07	77.93
G11	3.7	19.46	0.10	3.04	18.23	52.93	4.34	0.53	0.85	0.07	0.23	0.21	-	23.11	76.89
G12	3.2	20.31	-	2.18	18.49	52.78	4.25	0.39	0.85	-	0.19	0.55	-	22.88	77.12
G13	2.4	19.86	0.08	2.07	18.27	53.06	4.62	0.40	0.95	0.08	0.22	0.38	-	22.41	77.59
G14	3.1	19.38	0.10	1.74	18.52	54.63	3.61	0.36	0.98	0.08	0.19	0.30	0.10	21.57	78.43
G15	2.7	19.60	0.09	2.58	18.30	53.15	4.40	0.42	0.88	0.10	0.21	0.19	0.07	22.71	77.29
G16	2.2	19.63	0.10	2.65	17.18	53.95	4.71	0.48	0.89	0.06	0.22	0.12	-	22.83	77.17
G17	3.9	20.38	0.09	2.71	16.97	54.24	3.98	0.39	0.84	0.08	0.19	0.12	-	23.56	76.44
G18	3.4	20.17	0.13	1.54	17.80	53.70	4.93	0.29	1.04	0.10	0.18	0.12	-	22.10	77.90
G19	3.0	20.26	0.11	1.38	17.45	53.68	5.24	0.26	1.10	0.08	0.14	0.17	0.12	21.98	78.02
G20	3.8	19.51	0.13	1.50	17.49	54.19	5.21	0.31	1.22	0.09	0.16	0.09	0.10	21.41	78.59
G21	2.4	20.14	0.07	2.18	17.03	54.44	4.54	0.40	0.91	0.08	0.20	-	-	22.80	77.20
G22	3.4	19.93	0.08	2.62	17.13	54.38	4.22	0.49	0.85	0.09	0.19	-	-	23.14	76.86
G23	3.2	21.06	0.15	1.96	23.22	47.99	3.60	0.31	1.00	0.08	0.22	0.28	0.13	23.41	76.59
G24	3.6	20.16	0.13	1.43	18.26	53.67	4.57	0.27	1.09	0.10	0.17	0.16	-	21.96	78.04
G25	3.4	20.67	0.11	2.64	23.63	47.51	3.43	0.48	0.87	0.08	0.30	0.15	0.13	23.87	76.13
G26	3.0	21.10	0.11	1.40	17.21	53.60	4.75	0.26	1.07	0.09	0.18	0.12	0.11	22.85	77.15
G27	2.9	21.57	0.10	2.15	15.30	53.73	5.43	0.32	1.00	0.11	0.19	0.10	-	24.16	75.84
G28	2.5	18.92	0.11	1.75	24.65	48.89	3.47	0.30	0.86	0.13	0.27	0.19	0.46	21.10	78.90
G29	3.9	17.17	0.09	1.89	29.62	45.71	3.85	0.24	0.78	0.18	0.27	0.20	-	19.48	80.52
G30	1.7	20.84	0.11	1.33	18.98	52.59	4.14	0.26	1.18	0.09	0.17	0.17	0.15	22.51	77.49
G31	2.2	19.10	0.09	2.72	16.60	55.28	4.24	0.54	0.85	0.09	0.28	0.12	0.08	22.45	77.55
G32	1.9	21.29	0.12	1.41	17.46	53.86	4.05	0.24	1.13	0.10	0.17	0.10	0.07	23.05	76.95
G33	2.9	19.36	0.09	1.56	18.06	54.87	4.14	0.37	0.93	0.25	0.15	0.14	0.08	21.53	78.47
G34	2.5	21.28	0.10	2.74	17.27	52.47	4.26	0.48	0.81	0.12	0.21	0.18	0.06	24.63	75.37
G35	2.5	20.73	0.10	3.00	17.07	53.02	4.08	0.52	0.80	0.14	0.26	0.20	0.07	24.40	75.60
G36	1.9	22.28	0.12	2.03	22.52	48.20	2.84	0.38	1.06	0.08	0.21	0.14	0.14	24.76	75.24
G37	1.7	20.58	0.11	3.05	17.42	52.91	3.91	0.52	0.79	0.11	0.29	0.21	0.09	24.26	75.74
G38	3.7	18.62	0.11	1.60	20.06	53.75	3.20	0.36	0.99	0.90	0.23	0.18	-	21.48	78.52
G39	2.5	21.24	0.10	1.46	17.29	53.61	4.41	0.29	1.03	0.13	0.16	0.17	0.10	23.12	76.88
G40	2.6	22.05	0.10	3.70	16.26	52.51	3.64	0.47	0.74	0.14	0.25	0.14	-	26.36	73.64
G41	3.0	21.00	0.11	1.71	19.60	51.10	4.43	0.37	1.01	0.13	0.24	0.18	0.11	23.21	76.79

C16:0 Palmitic acid; C16:1 Palmitoleic 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, C22:6 Docosahexaenoic acid, C24:1 Nervonic acid; SFA: Saturated fatty acid; USFA: Unsaturated fatty acid

that oleic, linoleic and linolenic acids in the seeds of winter and spring barley varieties vary between 9.4-12.6%, 58.2%-58.9% and 5.16-7.78%, respectively [38]. On the other hand, oleic, linoleic and linolenic acids were obtained as 12.2-21.2%, 50.7-58.5% and 4.3-7.1%, respectively, in 21 different barley strains [34], as 91 g/kg, 237 g/kg and 16.0 g/kg, respectively, in barley oils [39], as 13.96-22.40%, 39.49-53.40% and 4.65-25.07%, respectively, in grains of some barley varieties [35], as 17.08%, 55.20% and 4.69%, respectively, in Larende barley variety [6], as 19.94%, 51.74% and 0.97%, respectively, in barley oils [36], as 15.8-25.6%, 50.6-71.2% and 2.2-5.2%, respectively, in hulless and covered barley grain [37].

Eicosenoic and erucic acids were detected in all barley genotypes; the highest level was found in Hevsel (1.22%) and Altinay (0.30%), respectively, while the lowest level was found in the seeds of Lord and Avci-2002 (0.74%), and Anka-09 (0.14%) genotypes, respectively. Behenic acid was detected in all genotypes except Akar, Anka-08 and Burakbey genotypes, and docosahexaenoic acid was detected in all genotypes except Sladoran, Sur-93, Novosadski-565 and Nonius varieties. While the highest behenic and docosahexaenoic acids were found in Sahin-91 (0.90%) and Burakbey (0.55%), respectively, the lowest behenic and docosahexaenoic acids were detected in the seeds of Altinorak (0.06%) and Hevsel (0.09%) genotypes, respectively. On the other hand, Nervonic acid was detected in only 22 genotypes examined in the study;

While the highest value was found in Inbat (0.46%), the lowest value was determined in Tosunpasa (0.06%) genotype. While arachidic acid was detected in trace amounts in barley oil [39], it was found as 0.30% in Larende barley seeds [6] and as 0.31% in barley grains [36]. In the study carried out to determine the effects of malt processing steps on the bioactive properties and fatty acid composition of barley, green malt and malt grains, the behenic acid content was determined as 0.18% in barley grain, 0.33% in green malt and 0.25% in malt [36].

The saturated fatty acids (SFA) of barley genotypes were between 19.48 and 28.54%. The seeds of the Kendal variety had the lowest level of total saturated acid, and the seeds of the Akar variety had the highest saturated fatty acid (SFA) concentration. The unsaturated fatty acids (USFA) of barley genotypes were between 71.46 and 80.52%. The highest unsaturated fatty acid contents were detected in the seeds of the Kendal (80.52%), Lord (80.10%), Cetin-2000 (79.92%), Inbat (78.90%), Anka-08 (78.70%), Sur-93 (78.61%), Hevsel (78.59%) and Sahin-91 (78.52%) genotypes, respectively (Table IV).

4. CONCLUSIONS

The crude ash, crude protein, ADF, NDF and ADL ratios of the seeds of barley genotypes varied between 3.5-7.1%; 8.3-13.4%; 13.5-26.0%; 23.8-36.3% and 1.99-8.78%, respectively. The DMD ratios, DMI ratios, RFV values, TDN ratios, DE and ME values of genotypes varied between 68.7-78.4%; 3.30-5.05%; 175.9-304.0%; 62.9-72.3%; 3.21-3.63 MJ/kg and 9.67-11.33 Mcal/kg, respectively. The lipid content of the examined genotypes varied between 1.7 and 3.9%. Palmitic, stearic and arachidic acids from saturated fatty acids were detected in the seeds of all barley genotypes examined in the study. The seed lipids of some barley genotypes contain palmitic (16.80-25.56%) and stearic (1.33-3.70%) acids as the major component fatty acids, among the saturated acids, with small amounts of arachidic (0.24-0.54%) and behenic (0.06-0.90%) acids. The major unsaturated fatty acids found in the seed lipids were oleic (15.30-33.78%), linoleic (41.92-55.28%) and linolenic (2.84-5.43%) acids. Palmitoleic, erucic, docosahexaenoic and nervonic acids were shown to be lower than 1%. Eicosenoic and erucic acids were detected in all barley genotypes.

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Enhancing the properties and oxidative stability of margarine through *Moringa* oil enrichment

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Received: February 8, 2024
Accepted: September 10, 2024

Reports on moringa oil being a great food source. Because of the high nutritional content and possible health benefits associated with its chemical makeup, moringa oil is categorised as a nutraceutical food. This study aimed to valorise *Moringa oleifera oil* and evaluate the effect of its incorporation at different concentrations on the properties and oxidative stability of margarine during a one-month refrigerated storage.
Moringa oleifera seeds were extracted in a cold press, which have undergone preparation operations and degreased by passing through a continuous-feed screw press (horizontal press) of the Xeoleo screw press. The Moringa oil was incorporated into margarine at the following two levels: 5% (MO5%) and 15% (MO15%).
The best way to preserve the high biochemical quality of MO, with good concentrations of fatty acids and triglycerides, particularly oleic acid, was to extract the oil by cold pressing. MO extracted for this investigation has high levels of β-sitosterol (49.85%) and α-tocopherol (243.02 mg/kg). The results showed that this oil presented respective peroxide value, acidity and iodine index of about 2.47 meq O_2/Kg, 0.84 mg KOH/g and 64.53 g/100g, with good contents of polyphenols (0.163 mg/kg) and carotenoids (3.26 mg/kg). Additionally, the efficacy of including MO in high-fat diets was validated by preventing margarine's oxidative stability while it was refrigerated in comparison to the control. The strong antioxidant activity of the recently created margarine qualifies it as a functional product. Nonetheless, significant water content was the outcome of the 15% moringa oil dosage.
Moringa oil is extremely resistant to autoxidation and can be used as an antioxidant for the long-term stabilisation of commercial edible oils.

Keywords: *Moringa oleifera; oil; antioxidant; margarine; durability of oxidation*

1. INTRODUCTION

Moringa oleifera (MO) is a member of the Moringaceae family, cultivated worldwide for its interesting numerous properties including nutritional, medical and industrial potential [1]. All parts of the MO tree have good nutritional and therapeutic values [2]. Proteins, minerals, β-carotene, and naturally occurring antioxidants are abundant in the leaves. They are used not only for human and animal nutrition, but also in traditional medicine [3]. On the other hand, MO seeds have been used to cure or prevent inflammation, rheumatism, bacterial and fungal infections, constipation, arthritis and hypertension [4]. In addition, the seed is a source of protein, minerals such as zinc and magnesium, oleic acid, as well as antioxidants [5]. Because of its qualities, this oil can be used for cosmetic and pharmacological applications as well as for human consumption. In fact, MO oil could be a good substitute for olive oil in human food as well as non-food applications, such as biodiesel, cosmetics and a lubricant for fine machinery

Salama et al. (2018) [6] revealed that MO greatly increased the content of fatty acids (oleic acid > 70%) as well as triglycerides (triolein > 30%). Gharsallah et al. [7] state that β-sitosterol and α-tocopherol are the two most compounds.
Today's consumers are increasingly concerned about food quality and their nutritional attributes. To meet consumer needs and create functional foods that are beneficial to human health, it is now necessary to enrich foods with nutrients to improve their nutritional content. Due to its widespread commercialisation, lower cost, use in the bakery and confectionery industries, and seasonal independence, margarine is currently experiencing a global market expansion [8]. *trans*-fatty acids are being phased out of industrial margarines by the World Health Organisation due to their established link to an increased risk of cardiovascular disease. To accomplish this, producers are currently working to create new margarines that include non-lipid ingredients and additional phytosterols to guarantee a nutritious and useful product [8]. However, oxidation is one of the most widespread phenomena in the food industry particularly in fat-rich products. Among these goods, margarine is a good illustration of one that is oxidation-prone because it contains 80% fat [9]. The aim of this work was to evaluate if MO oil might be a novel antioxidant for stability and preservation in the oxidation of margarine. Hence, two amounts of 5% and 15% of this oil was incorporated in the margarine, after a month of storage at +7°C, the physicochemical parameters, including oxidative stability and antioxidant activity, were assessed.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL AND MORINGA OIL EXTRACTION

The seeds of *Moringa* were harvested just after ripening from the Chams Industrial (Sidi Thabet, Ariana) during 2021. The seeds were extracted in a cold press, which have been degreased by being inserted through a continuous-feed screw press of the Xeoleo screw. Centrifugation at 5000 rpm for 15 min is used after sieving to extract the oil.

2.2. FEATURES OF PHYSIOCHEMISTRY OF MORINGA OLEIFERA OIL

The official protocols were followed to determine the extracted MO's acid, peroxide, iodine, saponification, refractive at 20°C, and specific absorptivity values K232 et K270 [10]. Using the hunter L*, a*, and b*, a Chromameter (Konika Minolta, Sensing INC, Japan) measured the surface colour of the cookie samples under study. Black (0) to white (100) is measured by the L* values, and a* values quantity (+100) and greenness (-100), and b* values measure yellowness (+100) and blueness (-100). Chlorophyll and carotenoid compounds were determined in oil samples according to the method described by Haddada et al. [11] using a by a Cary 60 UV–vis spectrophotometer at wavelengths of 470 nm and 670 nm, respectively.

2.3. FATTY ACID AND TRIACYLGLYCEROL ANALYSIS

The composition of fatty acids was performed using gas chromatography (GC/FID) according to the method described in the European Commission Regulation (EU No 2015/1833). After the transesterification of the fatty acids, the obtained fatty acid methyl esters (FAMEs) were analysed using GC (Agilent system 7890A) with a capillary column HP-5MS (30 m × 0.25 mm × 0.25 µm). The separation was involved with a programmed temperature (110 °C held for 5 min, increase of 3 °C/min to 150 °C and held for 16.33 min, increase of 4 °C /min to 230 °C and held for 27 min) and the flame ionisation detector (FID) temperature was 150 °C. FAs were identified by comparing retention times to standard compounds.
To produce the triacylglycerol (TAG) profile, high-performance liquid chromatography (HPLC) equipped with a reversed-phase C18 column (250 x 4.5 mm and 5 µm particle size) was used according to the International Olive Council. (2010).

2.4. STEROL AND TOCOPHEROL ANALYSIS

The International Olive Oil Council's standard technique was used to determine the sterol composition. The silylated sterol fraction was separated and quantified using capillary gas chromatography (GC2010, Shimadzu, Japan) with a flame ionisation detector (FID) and a Supelco (SPBTM-5 24034, Bellefonte, USA) capillary column (30 m, 0.25 mm, i.d. and 0.25 mm film thickness). The temperature in the column was 260°C. The temperatures of the injector and detector were 280°C and 290°C, respectively. A split ratio of 50:1 was employed with helium serving as the carrier gas, with a flow rate of 1 ml/min.
The International Olive Oil Council's standard technique was used to determine the sterol composition. The silylated sterol fraction was separated and quantified using capillary gas chromatography with a flame ionisation detector and a Supelco capillary column (30 m, 0.25 mm, i.d. and 0.25 mm film thickness) coated with a stationary phase formed by 5% of biphenyl and 95% of dimethylpolysiloxane. The analytical conditions were vector gas: Helium; flow rate: 35 mL/min; column temperature: 235 °C; Injector temperature: 255 °C; detector temperature: 290 °C; quantity injected: 1 µL.
Tocopherol extraction and analysis were determined according to the method described in the International Olive Council. (2010) using a

high-performance liquid chromatography (HPLC) equipped with Agilent HP1100 (Agilent Technologies, Palo Alto, CA, USA).

2.5. QUANTIFICATION OF CONDENSED TANNIN (CT), TOTAL FLAVOINOIDS (TF), AND TOTAL POLYPHENOLS (TP)

CT was calculated using the method described by Salar and Purewal's [13]. The amount of CT was expressed as mg catechin equivalent CE/g oil. To determine TF, the method of Páramo-Calderón et al. [14] was used. Results were expressed as mg quercetin equivalent QE/g oil. TP was estimated using Folin-Ciocalteu colorimetric method [14]. Results were expressed as mg gallic acid equivalent GA/g oil.

2.6. ANTIRADICAL ACTION

Ceylan et al. [15] approach was utilised to measure the radical scavenging activities of MO. Three millilitres of a DPPH solution (0.004% in methanol) were combined with half a millilitre of samples at varying concentrations in microtubes. After 30 min of standing at room temperature in the dark, the mixture's absorbance was measured at 517 nm. The DPPH free radical's inhibition (Ih%) was calculated using the following formula:

Ih% = [(absorbance of the control– absorbance of the trial)/ absorbance of the control]×100, The concentration of extract (IC50) that could scavenge 50% of the DPPH radicals was determined.

2.7. MARGARINE SAMPLES PREPARATIONS

To study the effect of the incorporation of MO on the quality of commercial margarine (Goldina), purchased from a local market, an amount of 5% and 15% of this oil was added. The integration of MO in margarine was made according to the procedure outlined by Nadeem et al. [16]. The physicochemical properties (moisture, acidity, refractive index and peroxide value) and the evolution of the antioxidant activity were evaluated for the formulated and control margarines, after a month of preservation at +7°C.

2.8. ANALYTICAL STATISTICS

Every extraction and calculation were carried out three times. The mean and standard deviation (SD) of the data are expressed. Using SPSS 23.0 (SPSS IBM2017), an analysis of variance (ANOVA) was conducted.

3. RESULTS AND DISCUSSION

3.1. PHYSICOCHEMICAL CHRACTERISATION OF MO

Our oil was classified as non-siccative with an index of refraction (IR) that is 1.465 (Table 1). This finding is nearly comparable to Gharsallah et al. [7] that reported that the IR from the MO was 1.462. The cold press MO's acid index is 0.848 mg KOH/g oil. The results showed that a peroxide index of 2.47 meq O2/Kg. This value is lower than required by the Codex Alimentarius. Khemakhem et al. [17], were recorded that the peroxide ranging from 3.3 to 4.5 meq O2/Kg in oil of pomegranate seeds.

The measured saponification index for the MO is 188 mg KOH/g oil, which is comparable to the olive oil that is between 184 and 196 mg KOH/g oil. The saponification value for use as an alternative fuel oil is 183.20 mg KOH/kg. This is within the range of 170-195 mg KOH/kg recommended in literature [18].

The iodine value is 64.53 g/100g. Our result is lower than that found by Gharsallah et al. [7] reporting 67.42 g/100g. However, the value found is lower than that of olive oil which varies between 75 and 94 g/100g. Our sample's K270 and K232, are lower, coming in at 0.0576 and 1.2345, respectively (Table 1). These results indicate that secondary oxidation is limited by the natural antioxidants in our oil. The results obtained are comparable to those found by Gharsallah et al. [7].

3.2. COMPOSITION OF CHLOROPHYLL AND CAROTENOID OF MO

The results showed that the carotenoid content of MO studied is 3.26 mg/kg (Table 1). According to Zhuang et al. [19], carotenoid levels depend on the degree of maturation and their protective role against oxidation. Conversely, the chlorophyll content is 1.56 mg/kg. Based on studies by Conesa et al. [20] the content of chlorophyll is negligible and almost absent for MO oil. It should be noted that chlorophyll and carotenoid give the extracted oil the green and yellow coloration,

Table I - Physiochemical characteristics of *Moringa oleifera* oil

Properties	Values
Oil yield (%)	14±0.87
Refractive index (20°C)	1.46±0.02
Acide value (mg kOH/g oil)	0.84±0.03
Peroxide value (meq O_2/kg oil)	2.47±0.32
Saponification value (mg KOH/g oil)	188±2.14
Iodine value (g I2/100g oil)	64.53±0.25
K232	1.23±0.02
K270	0.057±0.01
Color	
L*	98.49±0.03
a*	-1.44±1.07
b*	58.64±4.5
Carotenoid (mg/Kg)	3.26±0.12
Chlorophyll (mg/Kg)	1.56±0.03
Total polyphenol (mg GA/g oil)	0.163±0.01
Total Flavonoid (mg QE/g oil)	0.355±0.03
Condensed tannin (mg CE/g oil)	0.041±0.5
IC_{50} (µg/ml)	81±1.23

K232 and K270: Specific extinctions coefficients at 232 and 270 nm, Values are means ±standards deviations.

respectively, and that their contents depend mainly on the seed maturity.
Table 1 showed that the brightness L* of MO is 98.49 which indicates that our oil is quite clear. Regarding the parameter a*, we recorded a negative value of -1.44 highlighting a colour showing the colour of MO changing to green. As for parameter b*, the results showed a higher value of the yellow colouration (58.64). These values are partially consistent with those recorded by Gharsallah et al. [7]. Indeed, it has been reported that the major pigments of oils obtained by cold press are carotenoids and chlorophylls [7].

3.3. TP, TF AND CT

TP is 0.163 mg AG/g oil, as shown in Table 1. This level is higher than what was discovered by Gharsallah et al. [7], who reported a TPC of 0.102 mg AG/g oil. However, this level is lower than that recorded for olive oil, which was 0.32 mg EAG/g oil [21].
It should be noted that the TP in the oil is considered a natural antioxidant that prevents oxidation and provides a better storage stability, bitter flavour and pungency [22]. TP also prevents cardiovascular disease and cancer [7] and this encourages its incorporation in food products.
The TF in MO is 0.355 mg QE/g oil (Table 1). In fact, this level is higher than olive oil (0.174 mg EC/g oil) [21].
Based on Table 1, the CT in MO is 0.041 mg CE/g oil. The presence of tannins suggests the ability of our plant to play an important role as an antimicrobial and antioxidant.
The IC50 is 81 µg/mL (Table 1). Similarly, our recorded value is higher than that found by Bhatnagar and Krishna [23] who reported an IC50 of 35.5 µg/ml of MO from India. It is evident from the IC50 value that MO is among the oils with the highest concentration of naturally occurring antioxidants.

3.4. COMPOSITION OF FATTY ACID, TOCOPHEROL, STEROL AND TRIGGLYCERIDES PROFILES

Eleven fatty acids were identified where oleic acid was predominant (82.32%) (Table 2). These findings bear some resemblance to those of Gharsallah et al. [7], where oleic acid (73.36%) was predominant. Because of its high oleic acid content, MO has a higher nutritional value and is more palatable due to its superior holding and stability during heating and frying. In fact, this oil oxidises less than other oils rich in polyunsaturated fatty acids. Besides, the saturated fatty acid content did not exceed 15% (Table 2).
According to the findings, the sterol fraction of MO was β-sitosterol (49.85%), stigmasterol (25.74%), campesterol (23.15%) representing 90% of total

Table II - Fatty acids, sterols, triacylglycerols and tocopherols composition of *Moringa oleifera* oil

Fatty acids (%)		**Sterols (%)**		**Triacylglycerol (TAG,%)**		**Tocopherols (mg/kg oil)**	
Myristic acid C14:0	0.10±0.01	Cholesterol	0.10±0.02	OOO	39.21±1.23	α-Tocopherol	243.02±2.14
Palmitic acid C16:0	5.90±0.03	Stigmasterol	25.74±1.23	POO	13.53±1.03	γ-Tocopherol	112.7±1.26
Palmitoleic acid C16:1n−7	1.24±0.04	Campesterol	23.15±1.45	SOO	11.28±1.11	δ-Tocopherol	11.7±0.55
Heptadecanoic acid C17: 0	0.05±0.01	β-Sitosterol	49.85±2.31	OOA	7.39±0.45		
Ginkgolic acid C17: 1	0.03±0.00	Chlerosterol	0.40±0.05	POL	4.8±0.07		
Stearic acid C18:0	5.28±0.02	Δ^7-Avenasterol	0.29±0.02	PLS	2.48±0.04		
Oleic acid C18:1n−9	82.32±1.82	Δ^5,23 Stigmastadienol	0.27±0.03	POS+SLS	2.37±0.03		
Linoleic acid C18:2n−6	0.68±0.03	Δ^7-Stigmastenol	0.20±0.02	SOS	2.11±0.05		
α-Linolenic acid C18:3n−3	0.13±0.01	Erythrodiol	0.10±0.01	LLL	0.31±0.01		
Arachidic acid C20:0	2.98±0.02	Uvaol	0.04±0.01	OLLn	0.18±0.00		
Gadoleic acid C20:1n−9	1.30±0.01			PLLn	0.16±0.01		
Σ SFA	14±0.56			Others	16.83±1.21		
Σ MUFA	84.89±1.35						
Σ PUFA	0.81±0.03						
Ratio unsaturated / saturated	6.12						

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. P: radical palmityl. PO: radical palmitoleyl. O: radical oleyl.
L: linoleyl radical. Ln: linolenyl radical. S: stearyl radical. All measurements were done in triplicate and results were expressed as means ± SD.

sterols (Table 2). These sterols are known for their cholesterol-lowering effect and health-promoting effects [24].
These interesting results highlight the oil's high nutritional content and pertain to the examination of cold-pressed MO. The outcomes obtained allowed that α-tocopherol is predominant of 243.02 mg/kg oil followed by γ-tocopherol (112.7 mg/kg oil) and δ-tocopherol (11.7 mg/kg of oil) (Table 2). The content of α-tocopherol in MO agreed with those reported for soybean, groundnut and palm oils. These values were higher than those detected by Pluháčková et al. [25]. Literature revealed that α-isomer of tocopherol has a greater vitamin E potency, whereas δ-isomer of tocopherol has a greater antioxidant efficacy than either γ-or α-tocopherols. Salama et al. [6], reported α-tocopherol levels of 164.2 mg/kg oil.
As shown in Table 2, twelve triglycerides were detected mainly OOO (39.21%), POO (13.53%) and SOO (11.28%). Our results are consistent with those of Salama et al. [6] who highlighted that the most TAG was OOO (34.81%), followed by OOP (14.50%) and SOO (11.25%). The analysis of TAG allowed confirming our results of fatty acids.

3.5. EFFECT OF MO INCORPORATION ON MARGARINE

3.5.1 Moisture, acidity and refractive index

The moisture of the margarine (MC), as shown in Table 3, is 6.10%. Thus, the water content of the MC and those supplemented with MO did not exceed the standard required by the Codex Alimentarius, who emphasised that the content should be between 16% and 18%. After 15 days of storage, the water content of MC and MO5% increased to 9.54% and 10.037%, respectively, without exceeding the norm. In return, margarine with 15% of MO (MO15%) was higher in in water (13.64%) showing that the incorporation of a high concentration of MO induced a significant increase ($p<0.05$) during the conservation.
Silva et al. [8] emphasised that the water content of margarine is a parameter that affects its physical characteristics without having a major impact on its stability. Therefore, the addition of MO5% showed a water content the closest to that of the control with a significant difference ($p<0.05$) throughout the time spent refrigerated.
Every sample that was examined revealed a steady rise in acidity over time while being stored (Table 3). After 30 days, the control's acidity rose from its initial value of 0.14% to 0.18%. Acidity measured for MC, MO5% and MO15% were 0.14%, 0.13% and 0.12% respectively (Table 3). Our results are higher than those recorded by Nadeem and Imran [16] reporting initial values not exceeding 0.11%. Following the conservation, we took notes on acidities in the order of MC (0.18%), MO5% (0.15%) and MO15% (0.14%). These values did not exceed the limit value (0.2%) recommended by Nadeem et al. [16]. This result showed that higher the dose of MO, the more the acidity decreases confirming our findings on the antioxidant activity of this oil due to its richness in polyphenols and some pigments [23].
From Table 3, the refractive index increases with the dose of MO. Initially, the refractive index was 1.496 (MC) and 1.499 (MO15%). These results are in line with those of Nadeem et al. [16]. This index decreased significantly for all margarines analysed over 30 days of storage. However, no significant difference ($p<0.05$) was observed between the control and fortified margarines throughout their shelf life.

3.5.2 Peroxide index

Initially, MO15% had the lowest peroxide value measured, 0.3 O2/Kg (Figure 1). All initial PI values are lower than the standard (5 O2/kg). This result confirms the antioxidant activity of MO and its effectiveness in improving oxidative stability in the preservation of high-fat foods such as margarine [23]. In fact, margarines

Table III - Physicochemical composition of margarine enriched with *Moringa oleifera* oil during a refrigerated storage

Storage period (days)	Samples	Moisture (%)	Acidity (%)	Refractive index
0	MC	6.10 ± 0.001^{aA}	0.14 ± 0.01^{aA}	1.49 ± 0.002^{aA}
	MO5%	7.37 ± 0.003^{bA}	0.13 ± 0.02^{bA}	1.49 ± 0.002^{aA}
	MO15%	9.30 ± 0.005^{cA}	0.12 ± 0.01^{cA}	1.49 ± 0.003^{aA}
15	MC	9.54 ± 0.02^{aB}	0.16 ± 0.01^{aB}	1.49 ± 0.01^{aA}
	MO5%	10.03 ± 0.001^{aB}	0.14 ± 0.02^{aB}	1.49 ± 0.02^{aA}
	MO15%	13.64 ± 0.05^{bB}	0.13 ± 0.03^{bB}	1.49 ± 0.01^{aA}
30	MC	13.88 ± 0.03^{aC}	0.18 ± 0.01^{aB}	1.49 ± 0.001^{aA}
	MO5%	15.81 ± 0.05^{bC}	0.15 ± 0.02^{bB}	1.49 ± 0.003^{aA}
	MO15%	18.51 ± 0.01^{cC}	0.14 ± 0.02^{cC}	1.49 ± 0.002^{aA}

MC: Control margarine; MO5%: Margarine with 5% *Moringa oleifera* oil added; MO15%: Margarine with 15% *Moringa oleifera* oil added. All measurements were done in triplicate and results were expressed as means ± SD. Values followed different letters (a-c) in the same column indicated significant differences by the Duncan test different at $P < 0.05$.

enriched with MO were more resistant to peroxidation compared to the control. Indeed, MO15% had better oxidation resistance than MO5%. The outcomes are logical since our oil showed an important IC50 of 81 µg/ml. However, the PI values found are lower than those reported by Silva et al. [8]. These results show that MO can be used to improve the oxidative stability of commercial edible oils [16].

3.5.3 Inhibition percentage

The DPPH activity was found to be 81 µg/ml, indicating that forid-pressed is a highly potent natural antioxidant. It has a powerful DPPH°-neutralising capacity at low doses.

A significant difference was observed between the different margarine samples analysed (Figure 2). In fact, the best percentage inhibition (35.25%) was noted for margarine MO15%. This parameter underwent a significant increase ($p<0.05$) during refrigerated margarine storage. In fact, the inhibition percentage of margarine (MC) increased from 26.47% to attain a value of 42.52%. As for the MO 5% sample, a change in this parameter was noted, after 30 days, from 31.53% to 49.45%. Also, the best inhibition (57.56%) was recorded for the MH15 margarine, after being kept at 7°C for 30 days.

These findings corroborated those of the peroxide indices, which showed that even at modest dosages (5%), the inclusion of MO enhanced the margarine's antioxidant ability.

3.5.4 Colour

Changes in the colour parameters L* (lightness), a* (redness) and b* (yellowness) of all MO during storage are illustrated in Figure 3. The colour L* varied significantly during storage after treatment with MO. For the

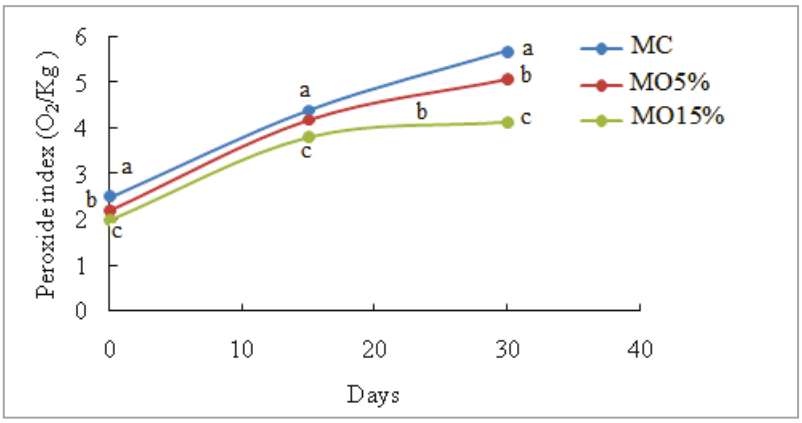

Figure 1 - Evolution of the peroxide value of control and enriched margarines during one month of refrigerated storage. MC: Control margarine; MO5%: Margarine with 5% *Moringa oleifera* oil added; MO15%: Margarine with 15% *Moringa oleifera* oil added

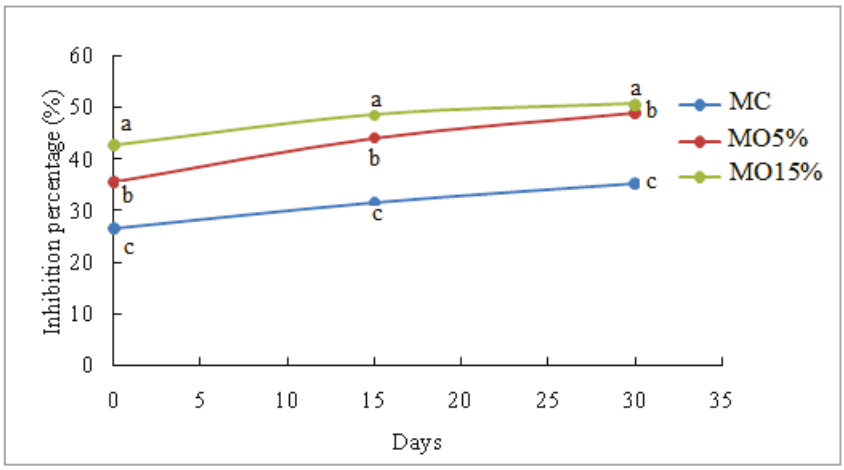

Figure 2 - Percentage change in inhibition of control and enriched margarines during one month of refrigerated storage. MC: Control margarine; MO5%: Margarine with 5% *Moringa oleifera* oil added; MO15%: Margarine with 15% *Moringa oleifera* oil added. All measurements were done in triplicate and results were expressed as means ± SD. Values followed different letters (a-c) in the same column indicated significant differences by the Duncan test different at $P < 0.05$.

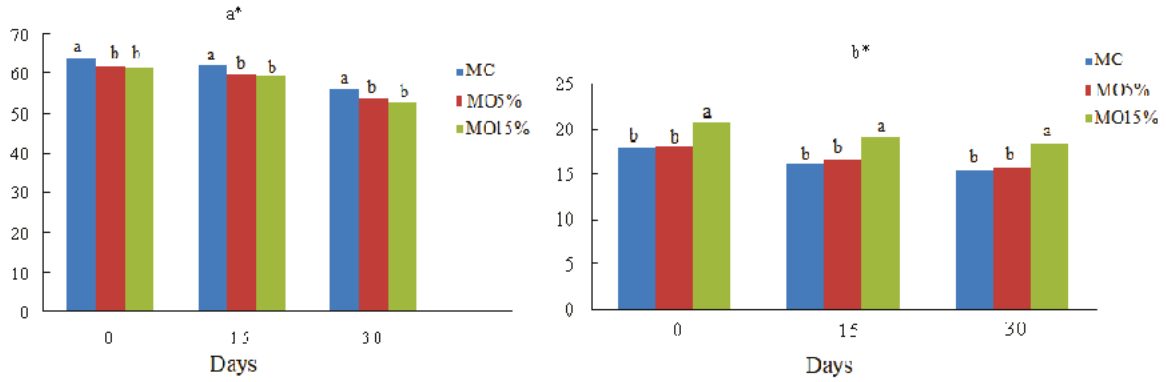

Figure 3 - Changes in color parameters of control and enriched margarines during one month of refrigerated storage.
MC: Control margarine; MO5%: Margarine with 5% *Moringa oleifera* oil added; MO15%: Margarine with 15% *Moringa oleifera* oil added. All measurements were done in triplicate and results were expressed as means ± SD. Values followed different letters (a-b) in the same column indicated significant differences by the Duncan test different at $P < 0.05$.

untreated MC, the value of L* decreased slightly with the dose of MO during storage. In fact, the luminosity of the control and fortified margarines decreased during storage. It was observed that the value of b* increases with the increasing concentration of MO (Figure 3). In fact, the typical colour of margarines is due to the richness of MO in carotenoids. After 30 days, the highest b* (18.467) was found for MO15%. This result is due to the oxidation of the carotenoids, which leads to a decrease in the yellow colour of the margarine. MO is extremely resistant to autoxidation and can be used as an antioxidant for the long-term stabilisation of commercial edible oils. MO's high oleic content may have the ability to increase beneficial HDL cholesterol and decrease serum cholesterol and triglycerides [16].

4. CONCLUSION

During storage, margarine with MO15% showed better resistance to oxidation, with the lowest peroxide value and the highest inhibition percentage, due to the high content of TP in MO, which are very powerful natural antioxidants. These results were also confirmed by the determination of acidity, which was the lowest at 0.14% in MO15%. However, this same enriched margarine showed a high-water content. The findings indicated that the high carotenoid content of the MO contributed to an increase of the yellow hue with an increasing added concentration. Similarly, the colour parameter b* decreased during storage in all the margarines analysed, due to the oxidation of the carotenoids responsible for the typical colour.

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
The authors reported that there has been no funding for the work featured in this article.

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From used cooking oils to P3HB biopolymers, a techno-economic feasibility study for production plants in Italy to foster the creation of a new value chain

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This study conducted a comprehensive economic feasibility analysis of a biotechnological plant that converts used cooking oils into the poly-3-hydroxybutyrate (P3HB) polymer used as an ingredient by the cosmetics industry, especially as a UV filter. Its findings offer critical insights for stakeholders, investors, and industry practitioners regarding the economic viability and financial sustainability of the project. The study opens with an assessment of the compatibility of used cooking oils (UCOs) collected in the Lazio Region (Italy) with the biotechnological process, though chemical-physical analyses that were conducted on crude UCOs and treated UCOs. Technological feasibility and the estimated process yield were confirmed, followed by a simulation of Operational Expenditures (OPEX) to evaluate how profitability varies with production capacity. The smallest profitable production capacity was identified, and the corresponding plant was modelled for the economic feasibility analysis. The study concludes that UCOs from Lazio households are suitable feedstock for the Hydal® biotechnology producing P3HB for cosmetic applications, with a B2B market value of around 50 €/kg for the lowest product grades. Treated UCOs are preferred over crude UCOs due to their superior performance in fermentation and smoother environmental licensing processes. Financial indicators, including OPEX, ROI, and payback period, indicate that a 350 t/y capacity plant, starting with a two-year phase of 175 t/y, offers a promising business opportunity, with an average yearly ROI of 14.5% and a payback period of 4.1 years. Market fluctuations in demand and supply were analysed for robustness. While the costs of treated UCOs, electricity, and natural gas do not significantly impact OPEX and other financial indicators, another two cost items do so to a critical degree: labour costs and interest rates. Consequently, they must be thoroughly monitored to avoid falls in profitability, if P3HB selling prices remain unchanged. Nevertheless, the positive market outlook for P3HB in cosmetics, supported by EU legislation and the forecast growth in the relevant segments, underscores the project's potential. The market for P3HB-based cosmetic products, with segments such as upcycled ingredients, waterless cosmetics, UV protection, and natural and organic cosmetics, is forecast to grow significantly, reaching over 60 billion euros by 2024.

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Received: June 28, 2024
Accepted: September 30, 2024

1. INTRODUCTION

In Italy, approximately 90-95,000 tons of used cooking oils (UCOs) are collected and exploited, making up one third of the total UCOs generated. [1] Used cooking oils, when properly collected, are one of the most widely recycled biowaste streams, mainly through the production of biodiesel. However biodiesel production from UCOs will face regulatory challenges in the near future [2] and new value chains are expected to devise and launch new UCO-derived products on the market. This is the case with the biodegradable and biocompatible biopolymer poly-3-hydroxybutyrate (P3HB) for cosmetics applications, of which the patented [3] and scalable production process from UCOs delivers a

high-added value product, with remarkable economic revenues (B2B market price: 50,000-100,000 € / ton) and market reach. The production process starts with untreated UCOs and is free of toxins and GMOs. The biopolymer particles obtained display many remarkable properties: high UV absorption rates, non-toxicity, high biodegradability rates [4]–[6]. This technology is completely in accordance with sustainability goals and regulations, both current and predicted. [7]–[10]
The yield of the production process is around 52% and can be up to 70%, meaning the P3HB Italian market demand for cosmetics applications would require a small percentage of currently collected UCOs in Italy. This opens up the possibility of creating and expanding the value chain gradually without disrupting the currently established chains, even considering the growing market demand for natural and waterless cosmetics.
Within the framework of a project development assistance initiative for the Lazio Region (Italy), we performed a techno-economic feasibility study into the establishment of a full-scale industrial production plant in Lazio, building on the process and market parameters available from the demonstration plant currently operating in Prague in the Czech Republic. The demonstration plant's production capacity is insufficient to meet current market demand and the expansion of operations in Italy is being explored.
This expansion would involve the construction of a production plant able to convert more than 660 tonnes per year (t/y) of UCOs into 350 t of P3HB, preceded by a preliminary phase with a production capacity of 175 t/y (from 330 of t/y UCOs). There are several reasons for the implementation of a preliminary 175 t/y production phase in Italy. Firstly, the demand of the Italian market for specific P3HB particles must be tested and consolidated. Based on that, the production process parameters, operational intricacies and logistics considerations will be fine-tuned.
The purpose of this work is to conduct a comprehensive economic feasibility analysis encompassing both the initial 175-t/y phase and the subsequent proposed expansion to double production capacity. The findings of this research are expected to provide stakeholders, investors, and industry practitioners with crucial insights, facilitating informed decisions concerning the economic viability, financial outlook and sustainability of the proposed steps in plant construction.

2. METHODOLOGY

The authors first evaluated the compatibility of used cooking oils collected in Lazio with the biotechnological process required to convert them into P3HB. This was achieved through the chemical-physical analysis of two samples, one of crude UCOs and another of UCOs treated according to the Italian framework for the conversion of collected UCOs into secondary raw material for recycling processes. Details can be found in paragraph 2.1.
Once the technological feasibility and the estimated process yield were ensured, the authors followed the methodology laid out in paragraph 2.2 to simulate OPerational Expenditures (OPEX) and to understand how profitability varies with production capacity. Subsequently, the smallest profitable production capacity was identified and the corresponding plant was modelled and submitted to an economic feasibility analysis (details in paragraph 2.3).

2.1. CHEMICAL-PHYSICAL ANALYSIS

Two 1-kg samples of UCOs, one crude and one treated, were submitted to the following characterisation: acid value, saponification value, iodine value, peroxide values, solids content, aqueous phase content. Two replicates were conducted for each analysis. These values were monitored on fermentation entry as they have a profound impact on the fermentation yield and duration.

- Acid value: a precisely measured amount of 2 g of oil sample was dissolved in 10 ml of diethyl ether. Phenolphthalein was added and the sample solution was titrated with standardised 0.01M KOH solution in MeOH.
- Saponification value: a precisely measured amount of 0.3 g of oil sample was weighted in a closed cap tube, 5 mL of approx. 1M KOH in MeOH was added. The closed tube was placed for 1 hour in a water bath at 65°C and periodically shaken. On cooling down, the content was quantitatively transferred to an Erlenmeyer flask for titration, while 2x10 mL of EtOH was used for flushing the residues from the tube. The sample solution was then titrated using approx. 0.5M H_2SO_4, a solution preferred over the 0.5M HCl laid down by the standard methods ISO 3657:2020 and ASTM D5558 because, in our experience, it was more stable. Phenolphthalein was used as an indicator. Blank titration values were subtracted from the titration measurements of the samples.
- Iodine Value was determined according to the Wijs method in accordance with ISO 3961:2018.
- Peroxide value was determined in accordance with "Peroxide value of oils and fats 965.33.12. Official methods of analysis of AOAC international".

2.2. OPEX CALCULATION

OPEX was calculated on the simulations of three P3HB production capacities: 35 t/y, 175 t/y and 350 t/y. OPEX was obtained by updating existing

economic studies performed with the Peters *et al.* model [11], which is described in detail in the next section, for the capacities considered and using late-2021 and early 2022 Czech market values. The following assumptions were made:

- the industrial site is rented (for all three plant capacities considered)
- the microbiological unit of the quality control laboratory and seed training lab and its personnel are always included
- some workers operate in shifts
- the process waste flow, a solution containing the residues of bacterial cells and cultivation broth, including residual UCOs, is treated off-site in an anaerobic digestion plant.

2.3. COST AND FINANCIAL EVALUATION OF A PRODUCTION PLANT

Once the production capacity was established on the basis of the OPEX analysis, a plant was simulated by Nafigate on the basis of their knowledge of the process and the facilities, acquired from the operation and the design optimization of the demonstration plant currently running in Prague (CZ) and the small-scale capacity (35t/y) plant under construction in Ostrava (CZ). The production plant for the selected capacity was submitted to economic and financial assessment in accordance with the model proposed by Peters *et al.* in "Plant Design and Economics for Chemical Engineers, 5th edition" [11]. This particular model's strongpoint is its conciseness, encapsulating all the necessary factors to build and run a chemical plant in its four input sheets ("Capital Investment", "Material and Labour", "Annual Total Product Cost" and "Utilities"). The output sheets deliver an "annual total product cost at 100% capacity", "economic evaluation" with Return on Investment (ROI), payback period, net return, discounted cash flow rate. The additional sheet "Year-0 $" is similar to the Evaluation sheet but delivers a more accurate view of the time value of money and its influence on project profitability and financial gains.

As well as the proper balance between data quantity and accuracy, the model offers a time resolution of 1 year, making it particularly suitable for the simulation of the economic and financial aspects of the initial stages of a production plant, be it a demo or a full-scale plant.

The model for chemical plants developed by Peters *et al*. [11] was considered the most appropriate of those for which suitability has been explored in the past, despite three main drawbacks: i) the conversion of UCOs into P3HB is a biotechnological process, not a chemical one, ii) the model does not accommodate highly specialized scenarios and iii) the model is dated and uses 2002 parameters. The authors addressed the first drawback by modifying weighing factors of cost items on the "Capital investment" sheet and including inputs for the biotechnology process among the rest of the input sheets. The second drawback was not detrimental since consideration was only given to the scenario of solely manufacturing of P3HB as material for use by others without the complication of a more complex business case, while the third was addressed by using economic data from projects conducted in the last couple of years and 2023 market values, updating financial parameters like loan interest and inflation rates. A list of all the modifications to Peters *et al*.'s model [11] is given in Table I.

In the initial phase, the model was filled with inputs from the Czech case, in CZK currency converted to EUR in accordance with the conversion rate in November 2023 (1 CZK = 0.0408 EUR). Later, to adapt the data to the specifics of the Italian case, a recalibration was undertaken by applying factors to entries like labour costs or energy cost, while other entries were left unchanged if they referred to the international market (i.e.: prices are consistent across Europe). This was the case with the equipment and machinery, raw material and inputs sold on the EU chemical commodity market, such as $MgSO_4 \cdot 7H_2O$, mineral nutrient solution, NH_3 24%, H_2SO_4 96%, NaOH 50%, KOH 45%, H_3PO_4 85%, HCl 35% and the bacterial seed.

The recalibration process ensures that the economic model and cost estimates reflect the unique conditions and considerations associated with the Italian context. All modifications required for the Italian case are also reported in Table I.

2.4. FINANCIAL MODELLING BASED ON CONSTRUCTED TECHNO-ECONOMICAL MODEL

In addition to the direct outputs of economical feasibility, taken directly from the constructed model in accordance with the inputs gathered, alternative scenarios were explored and tested in which the input costs might be different. For this assessment, the cost categories with the greatest operational impact were first selected based on the simple comparison of their percentage contribution to Annual Total Production Costs (TPC). The impact of these categories was further explored by varying the values used to assess the extent of each category's impact. To facilitate the comparison of the effect of each cost category under consideration, the initial values were assigned as 100% and then the same percentage changes were made to each category during impact modelling. The values of only one category were changed at a time, so the other values remained constant when generating the modelling outputs. The average annual Return On Investment (ROI) was used as a simplified output to cover all the relevant financial issues. The results are presented in section 3.3.

Table I - Model inputs specific for a P3HB production plant. (n.a. = not applicable)

Data type	Specificities for P3HB production plant against a chemical plant (Czech case)		Conversions for the Italian case (Dec 2023)	
	location in the calculation sheet	Rationale for the specificity	Conversion factor or direct cost	Rationale behind the change
Purchased equipment	Capital investment	Based on data acquired from an inquiry procedure conducted across Europe in the middle of 2023	n.a.	n.a.
Purchased equipment installation	Capital investment	Expenditures computed based on data acquired from an inquiry procedure conducted in the CZ in 2021 and updated by inflation	1.79	Ratio between labour costs in Italy and CZ. [12]
Instrumentation & Controls(installed), Piping (installed), Electrical systems (installed)	Capital investment	Expenditures computed based on data acquired from an inquiry procedure conducted in the CZ in 2021 and updated by inflation	n.a.	n.a.
Buildings (including services),	Capital investment	The coefficients relevant for CZ are drawn from Roušar's manual on Czech Project management of technological buildings [13]	1.25	Ratio between building costs/m^2 in IT vs CZ (on residential buildings). No data available for industrial buildings. [14], [15]
Yard improvements	Capital investment	The coefficients relevant for CZ are drawn from Roušar's manual on Czech Project management of technological buildings [13]	1.25	Ratio between building costs/m^2 in IT vs CZ (on residential buildings). No data available for industrial buildings. [14], [15]
Service facilities (installed)	Capital investment	Expenditures computed based on data acquired from an inquiry procedure conducted in the CZ in 2021	n.a.	n.a.
Engineering and Supervision	Capital investment	Expenditures computed based on data acquired from an inquiry procedure conducted in the CZ in 2021 and updated by inflation	1.30	Ratio between labour costs for engineers in IT and CZ. [16]–[18]
Construction Expenses	Capital investment	The coefficients relevant for CZ are drawn from Roušar [13]	1.25	Ration between building costs/m^2 in IT and CZ (on residential buildings) No data available for industrial buildings. [14], [15]
Legal Expenses	Capital investment	As per the *Peter et al*. model [11]	1.14	Ratio between labour costs for lawyers in IT [19] and CZ. [20]
Engineering/Construction Contractor's fee	Capital investment	As per market fees in CZ	n.a.	n.a.
Contingency	Capital investment	Chosen value – a low value of 5% was chosen due to high confidence in estimates backed by industrial reality at the time the study was performed	1.2	The average of conversion factors applied to any entries, that range from 1.00 to 1.79
Raw materials	Materials & Labour	Based on data acquired from an inquiry procedure conducted in the CZ in the middle of 2023	0.85 €/kg Used Cooking Oil	Survey CONOE associates - Feb 2023
Operating Labour	Materials & Labour	The hourly rate was chosen according to average salary in Moravian-Silesian (CZ), in the middle of 2023	20.83 €/h	The operator hour rate in Italy is calculated by dividing the estimated labour costs for shift labour (3000 €/month), divided by the average amount (144) of working hours/month
Process Air, Instrument Air Prices	Utilities	The costs were calculated as (solely) electricity spending for compressor operation, i.e. included in the electricity item	n.a.	n.a.
Electricity	Utilities	Prices in CZ, in the middle of 2023	0.134 € / KWh	Price of electricity for industry in Italy [21], [22]
Natural Gas	Utilities	Prices in CZ, in the middle of 2023	30.65 € / K	Price of Natural Gas in Nov 2023
Non-hazardous waste disposal	Utilities	As per budgetary information, in CZ, 2023	0.110 € / Kg	Average from various waste management companies.
Process Water	Utilities	The total costs per cubic metre as per listed prices in Ostrava, CZ, in the middle of 2023	0.03440 €/Kg	Water prices ACEA [2023] (it includes sewage services and aqueduct usage fee) [23]

Table I - Continues

Data type	Specificities for P3HB production plant against a chemical plant (Czech case)		Conversions for the Italian case (Dec 2023)	
	location in the calculation sheet	Rationale for the specificity	Conversion factor or direct cost	Rationale behind the change
Operating Supervision Costs	Annual TPC	Calculated using the estimated respective technology needs, used an average salary on such a position in Moravian-Silesian Region of CZ, in the middle of 2023	n.a.	n.a.
Maintenance & Repairs cost coeficient	Annual TPC	Value was established by technology type and chosen according to Roušar (2008) [13]	n.a.	n.a.
Operating supplies	Annual TPC	As per Nafigate company internal estimates	n.a.	n.a.
Laboratory Staff	Annual TPC	The numbers were estimated combining the amount of personnel needed by the respective technology,	28'500 €/y	Average gross salary for a quality control technician
Process and Quality Analyses Lab	Annual TPC	Technology provider's internal knowledge of analyses costs are used	n.a.	n.a.
Quality Control [QC] staff	Annual TPC	The numbers were estimated combining the amount of personnel needed by the respective technology,	40'000 €/y	Average gross salary for a quality control supervisor
Property Tax	Annual TPC	Estimated average of property tax rate applied in CZ in 2023	0.00 €/m^2	From 2023, in Italy the property tax on estates (IMU) is fully deductible for industrial buildings
Financing – interest rate	Annual TPC	A value obtained in a 2022 R&D project was used.	n.a.	The interest on loans is similar for the two countries (4,5%)
Insurance	Annual TPC	Value was thougt by technology type and chosen according to Roušar (2008) [13]	n.a.	n.a
Rent	Annual TPC	The specific value per unit area from a 2022 R&D project was used and multiplied by area required for the respective technology unit.	n.a.	n.a
Plant overhead, general	Annual TPC	Project-specific internal estimate,		
Cost of Scraps	Annual TPC	Estimated as as 5% of variable costs	n.a.	n.a
Administration	Annual TPC	Calculated using estimate of number of human resources needed and multiplied by respective average salaries on such positions in CZ, 2023	n.a.	The model estimates these values from labour costs (already updated)
Distribution & Selling	Annual TPC	Calculated using estimate of number of human resources needed and multiplied by respective average salaries on such positions in CZ, 2023	n.a.	The model estimates these values from labour costs (already updated)
Construction, product price, and TPC inflation rates	Evaluation	Industry long-term expectations in early 2023, in CZ	n.a.	n.a
Income tax rate	Evaluation	21% used as CZ relevant value from 2024 onwards	n.a.	n.a
Minimum acceptable rate of return	Evaluation	Set on 8% as minimal value considered by investors during long-term discussions	n.a.	n.a

3. RESULTS AND DISCUSSION

The following section reports the results and the discussion of the three consecutive evaluation steps of a P3HB production plant that uses UCOs as feedstock in Italy, that is, the technical feasibility, the OPEX calculation and the economic feasibility.

3.1. CHEMICAL PHYSICAL ANALYSIS

Table II presents the characterization of two samples of UCOs collected from households in the Lazio Region, one crude (not treated according to the Italian framework for the conversion of collected UCOs into secondary raw material for recycling processes) and the second treated according to the aforementioned regulatory framework in order to obtain end-of-waste status for conversion into a secondary raw material. Table II also presents the reference value ranges suggested by the technology provider.

Acid Value is a measure of triglycerides hydrolysed into free fatty acids (FFA) and glycerol. While a certain amount of FFA in the oil is beneficial for the P3HB production process, process yield may be reduced over a limit of 150 mg KOH / g. The Acid Value for crude samples corresponds to degraded cooking oils and, although slightly higher than usual in Europe, they would probably perform well in the fermentation process, given the robust nature of the process against this parameter. The treated oil has slightly reduced acidity due to the water separation process it undergoes in order to remove part of the amphiphilic free fatty acids.

The saponification value is a measure of the average chain length of the fatty acids in the sample in the form of glycerides. The values recorded indicate a slight shortening of the chains after treatment, with no negative effects on the conversion process. The values for both samples are in the same range as oils like olive oil and canola oil, which indicates that the oils are suitable to effectively undergo the fermentative process.

As with the iodine values, both samples lie within a range corresponding to “non-drying oils” (i.e. oils that do not polymerize in presence of oxygen and so do not create solid films). The iodine value is an important way of measuring unsaturation in fats and oils. Double and triple bonds in fatty acid chains reduce energy content while boosting polymerization. The amount of unsaturation demonstrated by the measured value implies that the characterised oils are not likely to polymerize on the surface of reactor components such as valves, sensors, etc. thereby limiting the occurrence of related process setbacks. Considering that bacteria metabolise only unpolymerized UCOs, this reduced tendency to polymerization also means reduced losses of substrate that can be used for fermentation during long-term storage of the oil.

The peroxide value is a measure of fat oxidation. The measured low values for both samples indicate low levels of rancidification and therefore a lower level of oil deterioration that would otherwise lead to limited process yields.

The content of the aqueous phase must be less than 3% in weight before introducing the UCOs in the fermenter. The crude sample does not comply with this requirement, but water can be removed with a simple physical treatment. It is worth considering that this removal would be achieved through the installation of a UCO pre-treatment unit.

Even though both crude and treated oils from the Lazio region are potentially suitable for fermentation and conversion into P3HB, the authors consider the best choice to be treated oils since they facilitate the attainment of end-of-waste status, making it easier to obtain environmental permits for the plant installation and operation.

3.2. OPEX CALCULATION AND CAPACITY PRODUCTION SELECTION

OPEX calculations were performed for different production capacities and are presented in Table III. The production plant scenario is expensive due to its biotechnological nature, and draws little benefit from economies of scale since production units are modular. The bigger the plant, the higher the number of biological reactors that must be installed. This is reflected in the OPEX for plants of different capacities, which still decreases even after doubling already significant production capacities. On the other hand, the modular

Table II - Chemical physical analysis (in duplicates) of Italian samples of used cooking oils from households

Characterization item	Italian sample, crude [average of 2 replicates]	Italian Sample, treated [average of 2 replicates]	Reference values as suggested by the biotechnology provider
Acid Value [mg KOH / g]	7,6 ± 0,1	4,9 ± 0,1	0,5-150
Saponification Value [mg KOH / g]	180 ± 3	192 ± 1	120-220
Iodine Value [g I_2 / 100 g]	74 ± 1	75 ± 2	15-130
Peroxide value [µmol O_2 / kg]	6 ± 0.4	12 ± 0.4	0-300
Content of aqueous phase [% by weight]	~ 20%	~1.5	< 3 %
Solids [% by weight]	< 1 %	< 1 %	< 1 %

Table III - OPEX for three different plants, depending on production capacity [EUR, converted from Czech market]

Cost item	OPEX [EUR / Kg P3HB]		
	35 t/y	175 t/y	350 t/y
Material costs for fermentation and isolation	3,060	3,200	3,154
Labour	12,615	4,931	2,718
Energy	7,881	6,026	5,948
Waste disposal at an anaerobic digestion plant	2,385	2,132	2,078
Maintenance and other costs	1,919	1,246	1,045
Site Rental	7,470	0,889	0,611
Quality control (laboratory consumables)	1,173	0,586	0,586
Corporate civil liability insurance	0,640	0,415	0,697
Scrap costs	1,828	0,985	0,868
Total OPEX	38,971	20,410	17,705

layout makes it possible to easily expand operations while retaining existing facilities.

Considering that the business to business (B2B) market value of P3HB as an ingredient in cosmetics applications is 40-55 €/kg, the construction of a 35 t/y capacity production plant would not be viable for a profitable business. A small-scale operation would only be attractive if supported by grants and/or funds, or if the production process is aimed at a biomedical application with a higher value (above 90 €/kg). Production capacities of 175 t/y and 350 t/y must be the target if a profitable business is to be created for the production of P3HB as an ingredient in cosmetics applications. In the following section, we demonstrate that, in the Italian case, a production capacity of 350 t/y, with an initial two-year period of halved production capacity (175t/y), would be a potentially profitable business opportunity.

3.3. ECONOMIC FEASIBILITY

Based on the considerations in section 3.1, we modelled production plants with feedstock of treated used cooking oils. Costs linked to the procurement of this secondary raw material were considered and the absence of a dewatering pre-treatment unit in the plant was assumed. This model is referred to in the OPEX evaluation in section 3.2.

For a plant located in Italy, the model gives an average yearly return on investment (ROI) of ~14.5%, with a payback period of 4.1 years. These are quite good values for the chemical industry in manufacturing raw material for further production. A conservative estimate of a 6% inflation rate was used in the model and, if the European economy remains stable, the profitability indicators would be even more advantageous.

In order to identify production costs that might disrupt the project's economic feasibility, we analysed the weight of each production cost on the TPC. As shown in Figure 1, the cost categories that most affect TPC are financing (17.9%), input materials (17.0%), labour (15.5% - both in operations and oversight) and maintenance and repairs (13,7%).

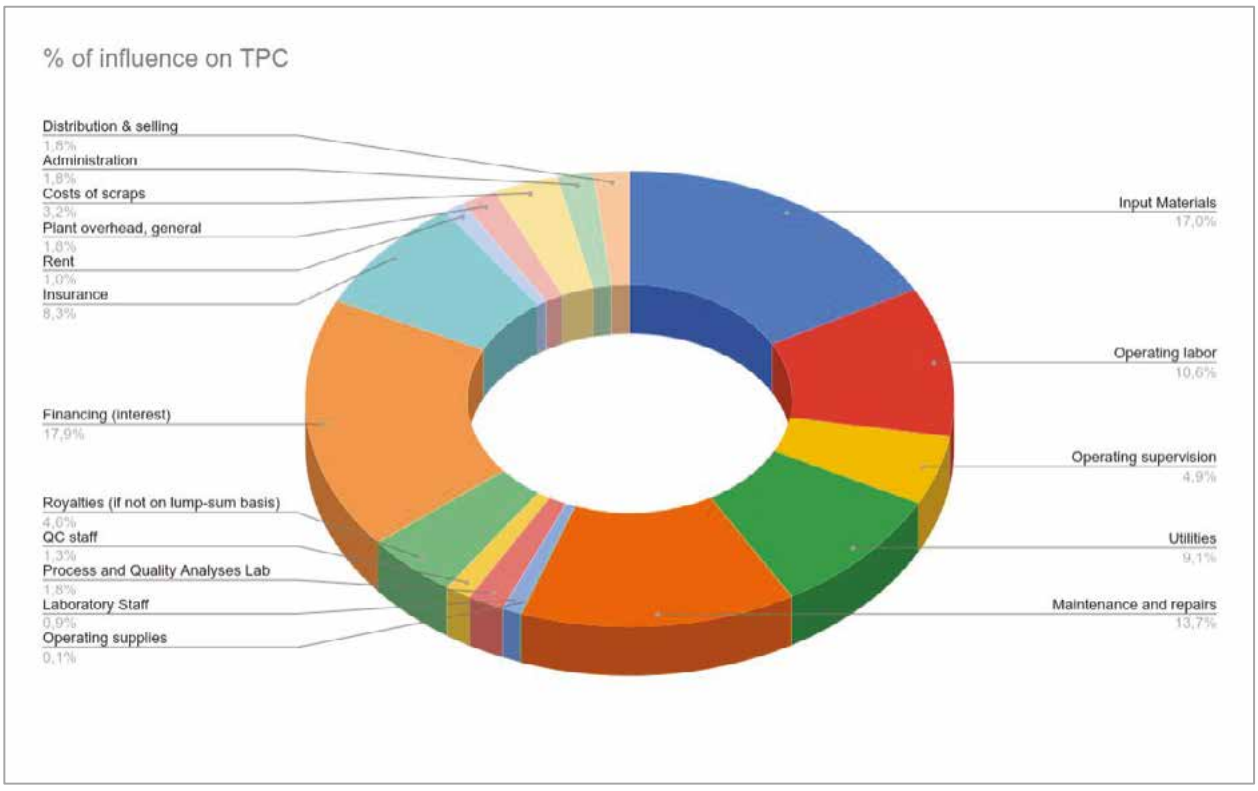

Figure 1 - TPC share among the different cost categories

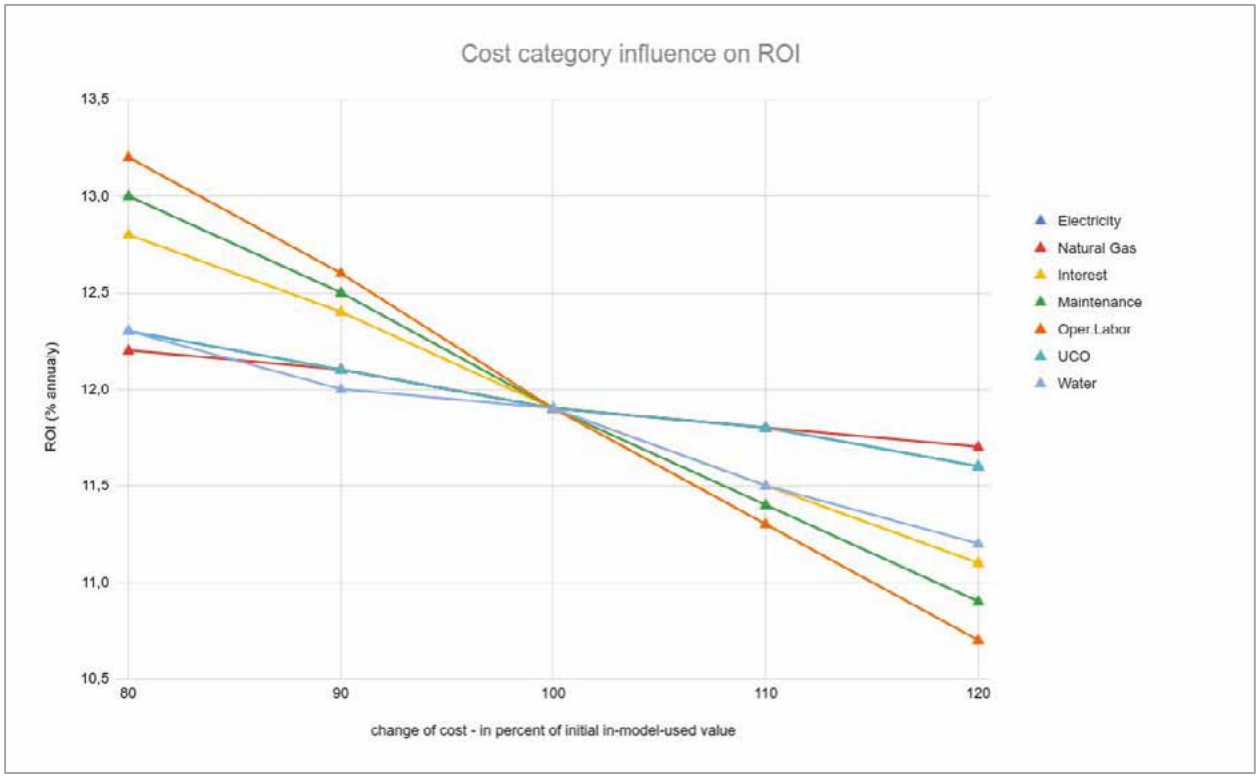

Figure 2 - Graph of impact of proportional changes of selected items on ROI (% yearly average)

A closer examination of the cost category "input materials" shows that UCOs and water make up 70% of the cost item (i.e.: 11.9% of total TPC) while the commodity items mentioned in Section 2.3, if taken individually, have lesser significance. Rising inflation, however, as in the last few years, can affect all prices and disrupt the weight factors of cost categories, reducing net return and profitability. Among the process inputs, electricity and natural gas are also significant. The increasing costs of electricity and gas may therefore also disrupt the economic model. To reduce the sensitivity of the model to energy market fluctuations in 2023, the authors drew on costs from late 2022, when energy costs hit record prices.

After identifying the cost categories with greatest impact on TPC, an assessment of the cost items' impact was performed by modifying in turn the value of the items with greatest weight on TPC (ranging from 8% to 20%) to values equivalent to 80%, 90%, 110% and 120% of the original default value. To this end, the most relevant subcategories were extracted from the significant categories of Utilities and Material Inputs and submitted for assessment together with Operating Labour, Maintenance and Interest. These are Electricity, Natural Gas, Used Cooking Oil (UCO) and Water. The results are presented in Figure 2.

The cost categories with the least impact are Electricity, Natural Gas, UCO and Water. Operating Labour Costs, Maintenance and Interest have the greatest impact, in that order.

Maintenance rates are, for the most part, an estimate for the new technology and do not usually vary much with the fluctuations of the economy and so Operation Labour Costs is the most significant cost category to be taken into account when choosing the right site for the plant in Italy. The lowest possible interest rate must be obtained from the banks or the amount of the loan required reduced, for example, by seeking as much equity investment as possible. The rest of the cost categories have much less significance and can be omitted in initial stages of site selection, to be used for later for the optimization of the plant site economy.

4. CONCLUSION

The authors conclude that the samples of UCOs collected from households in the Lazio Region are suitable as feedstock for the Hydal® biotechnology that produces P3HB as an ingredient used in cosmetics applications, with a B2B market value starting at ~50 €/kg for the lowest product grades. The authors suggest consideration of the treated used cooking oils available on the secondary raw material market rather than crude, collected but untreated oil. As well as improved performance in the fermentation process, treated UCOs are useful for obtaining end-of-waste status, thereby making it easier to obtain environmental permits for the plant installation and operation.

When it comes to production capacity, financial indicators like OPEX, ROI and payback period show that 350t/y, with an initial two-year period of halved

production capacity (175t/y), is a potentially sound business opportunity, with an average yearly ROI of 14,5% and with a payback period of 4.1 years. However, the authors are aware that the market for P3HB for cosmetics applications in Italy is not yet sufficiently mature to sustain the business case.

Market fluctuations in the demand and supply sides were considered in order to check the robustness of the Italian case. Although significant fluctuations in the cost of treated UCOs, electricity and natural gas, barely affect OPEX or other financial indicators, rises in Operation Labour Costs and Interest rates would affect the profitability of the business if the P3HB selling price remains unchanged.

However, the very favourable market outlook for P3HB as an ingredient for use in cosmetics applications and the current and future EU legislative framework are very positive. [7]–[10] The relevant cosmetics segments for P3HB-based products are forecast to enjoy strong growth, namely upcycled cosmetics ingredient 6.5% CAGR, waterless cosmetics 9,6% CAGR, UV protection 9.1% CAGR and Natural and Organics cosmetics of 9.5% CAGR. Overall, these markets were worth more than 60 billion euros in 2024. [24]

Acknowledgments

This study was co-funded by the European Union, under the Horizon 2020 project HOOP (Grant Agreement N°101000836). The authors would also like to thank Ms Aneta Vacula for the chemical-physical analysis of the UCO sample and key Italian stakeholders of the used cooking oil valorisation value chain, for their essential market insights: i) Consorzio Italiano Oli Esausti (CONOE), and in particular its director Francesco Mancini; ii) FriggiOil Service, in particular Benedetta Raponi; iii) Mario Stancanelli from SIMAD Rifiuti S.R.L., Roma; iv) Maurizio Spreca from S.A.A. s.r.l, Vallerano (VT). The contribution of Giorgio Scavino and Andrea Vignoli from ANCI Lazio in reaching the aforementioned stakeholders is also acknowledged.

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Unlocking the Therapeutic Potential of the Genus *Senecio* (Asteraceae): Essential Oil Composition and Pharmacological Insights

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Received: October 18, 2023
Accepted: January 25, 2025

Senecio is the largest and most complex genus in the family of the Asteraceae with more than 1,500 species distributed widely throughout the world. A comprehensive search of the electronic databases (1986–2023) using the keywords of '*Senecio*' and 'essential oil' revealed that an essential oils composition breakdown is available for 57 species, with α-pinene, α-farnesene, germacrene D, p-cymene, myrcene, α-terpinene, and caryophyllene oxide being the most identified components. The pharmacological activities have been summarized of different species including antimicrobial, antioxidant, repellent, antifungal, acaricidal, anti-inflammatory, cytotoxicity, phytotoxic, anticholinesterase, allelopathic, nematicidal, antimalarial, antileishmanial, α-glucosidase, anticorrosive, analgesic, and toxicity. This review is expected to lay the foundation for further studies of this genus and provides guidance for selecting accessions of species with the best chemical profiles.

Keywords: Asteraceae, *Senecio*, essential oil, composition, α-pinene, antimicrobial.

1. INTRODUCTION

Plants have been used as medicines since ancient times and were effectively recognised as bactericides, fungicides, virucides, antiparasitics, and pesticides. In several cases, their properties are mainly attributed to their essential oils (EOs). EOs from aromatic plants are considered a vital source of medicine that contains unique bioactive compounds. They are widely used in the pharmaceutical, agricultural, cosmetic, and food industries due to their pharmacological properties [1-3]. Investigations into the potential uses of plant EOs are again exciting the interest of scientists in further research. *Senecio*, the largest and most widespread genus of the family Asteraceae, includes more than 1,500 species. Among them, 270 species are reported in Argentina, 22 species are found in Morocco, and 6 occur in Egypt. The genus is also widespread in the temperate regions of Europe, North America, Asia, and South Africa . *Senecio* is derived from the Latin word *senex*, which literally means 'old man', referring to the white egrets that alight on the achenes during plant fructification. Several species of *Senecio* have been documented in the literature to date, some of which are distributed worldwide (*Senecio vulgaris*), while others can only be found in restricted areas (e.g., *Senecio rosinae*, reported only on the island of Corsica) . Other species of the genus *Senecio* have been used in traditional medicine for the treatment of asthma, coughs, bronchitis, eczema, and for healing wounds . Several phytochemical studies have investigated EOs of *Senecio* and discovered significant chemical variability.
To appreciate the potential of the genus *Senecio,* a review is therefore required of their traditional uses, chemical compositions, and the pharmacological activities of its EOs.

2. SEARCH STRATEGY

The protocol for performing this study was developed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) : (a) the first step was to exclude duplicate articles, (b) titles and abstracts were then read and the inclusion and exclusion criteria were applied, (c) all articles resulting from this stage were read in full, and the inclusion and exclusion criteria were applied again. Figure 1 shows the flow diagram of the identification and selection of articles. Following this step, we selected the articles for this study. This systematic review was conducted through searches using Scopus, PubMed, Science Direct, SciFinder, and Google Scholar. The keywords used were '*Senecio*', 'essential oil', and 'biological activity' to find articles over the period from the beginning of the database until October 2023. The inclusion of articles was based on the following criteria: (1) type of publication - original research articles, (2) only articles in English, (3) articles must present the chemical composition of *Senecio* essential oils, (4) articles must discuss the bioactivity of the essential oils. The following were the exclusion criteria : (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 EOs.

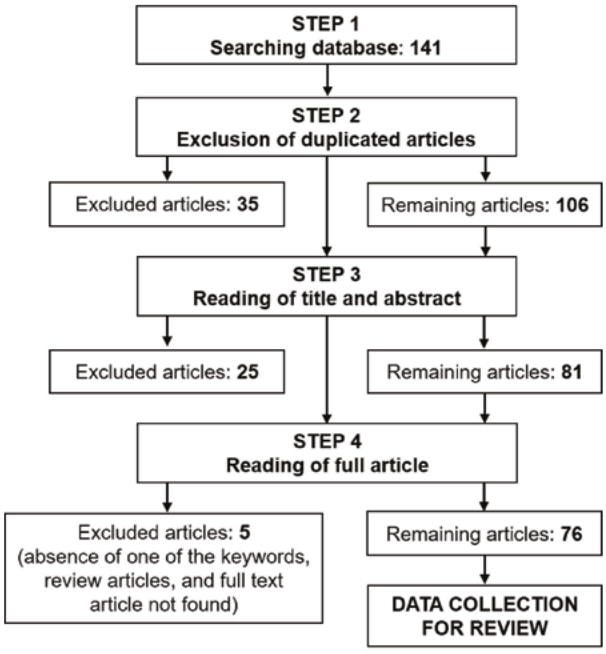

Figure 1 - PRISMA flow diagram of included studies

3. TRADITIONAL USES

The traditional uses of various *Senecio* species highlight their ethnomedicinal importance in different cultures [9-23]. The leaves of *S. ambavilla* are used for treating wounds, boils, and skin diseases. An infusion of its leaves is also traditionally used for managing rheumatism, gout, and gastrointestinal complaints . Similarly, *S. anteuphorbium* is applied as a sedative for abdominal or back pain and is also employed to treat rheumatism, wounds, and injuries [10], while its poultices offer sedative relief for abdominal and dorsal issues [11]. In traditional Chinese medicine, *S. cannabifolius* is valued for the treatment of viral influenza, icteric hepatitis, and stomach ailments [12]. *S. cineraria* has been recognized for its role in alleviating eye problems , while *S. filaginoides* is used to address rheumatic pains and toothaches [13]. *S. flammeus* is another species popular in Chinese folk medicine, primarily for treating inflammation and ulcers [14]. *S. glaucus* is known for managing respiratory and hepatic conditions, including coughs, fever, colds, bronchitis, and asthma, as well as for treating eczema and wounds [15]. The water extract of *S. graciliflorus* leaves is traditionally used for treating skin rashes and eruptions [16]. *S. graveolens* has distinctive medicinal properties: it is reputed to counteract mountain sickness and act as an emmenagogue, a digestive aid, and a cough suppressant [17]. Similarly, *S. nudicaulis* is employed for its ability to treat colic, fever, and various skin diseases, including conjunctivitis [18,19]. *S. nutans* has a wide range of uses, such as lowering blood pressure, alleviating altitude sickness, and relieving cold-related discomfort, bronchitis, whooping cough, asthma, stomach aches, and fever [20]. *S. pogonias* is applied in the treatment of hepatic disorders, fever, coughs, and colds [17], while *S. serpens* serves both medicinal and ornamental purposes in regions like Portugal, Morocco, and Egypt [21]. Lastly, *S. ventanensis* is known for its role in treating wounds and as an anti-emetic, anti-inflammatory, and vasodilatory agent [22]. *S. vulgaris*, with its broad spectrum of uses, acts as a diaphoretic, antiscorbutic, purgative, diuretic, and an anthelmintic [23]. It is also used to expel kidneys gravel. These diverse applications underline the vast medicinal potential of the *Senecio* genus in traditional practices.

4. CHEMICAL COMPOSITION

The analysis of EOs from *Senecio* species involves various techniques designed to extract, identify, and characterize the volatile compounds present. One of the most widely used methods of extraction is steam distillation. This technique is effective in obtaining oils in a relatively pure form and is commonly used for aromatic species. However, it can cause the degradation of heat-sensitive compounds and often requires a large amount of plant material. Another method is solvent extraction, which is often

employed when steam distillation is not effective or when higher yields are needed. Hydrodistillation, a variant of steam distillation, involves directly boiling plant material in water. This method is effective for both dried and fresh plant samples and is simpler than steam distillation. However, it can lead to the loss of volatile compounds if not carefully controlled and requires larger quantities of plant material.

Various techniques are used to analyze their chemical composition. Gas chromatography (GC), often coupled with mass spectrometry (GC-MS), is one of the most commonly used methods for identifying and quantifying volatile compounds in EOs. It offers high sensitivity and resolution, allowing for the detailed identification of terpenes, aldehydes, ketones, and other compounds. However, it requires sophisticated equipment and expertise, and preparing samples can be time-consuming. For analyzing non-volatile or thermally unstable compounds, high-performance liquid chromatography (HPLC) is sometimes used. In addition to chromatographic techniques, Fourier Transform Infrared (FTIR) spectroscopy is employed to study the functional groups present in EOs. FTIR provides rapid, non-destructive analysis of chemical compositions and is especially useful for confirming the presence of functional groups like alcohols, aldehydes, or esters. However, it provides less detailed structural information compared to techniques like GC-MS.

Numerous investigations have explored the chemical composition of EOs of the *Senecio* genus. Table I shows the details of the isolated EOs, including the main components [24-83]. In this context, it appears that monoterpenes and sesquiterpenes (hydrocarbons and oxygenated) constitute the main components of the *Senecio* EOs. EOs of *S. glaucus* [36], *S. pogonias*, *S. oreophyton* [43], *S. giganteus* [47], *S. graciliflorus* [52], *S. mustersii*, *S. subpanduratus* [38], and *S. farfarifolis* [73] were found to contain α-pinene at high concentrations. Meanwhile, EOs of *S. nutans* [31], *S. polyanthemoides* [68], *S. subulatus* [33], and S. *squalidus* [74] were characterized by an abundance of *p*-cymene. In another study, α-farnesene was notable for its richness in the EOs of *S. cannabifolius* [26] and *S. flammeus* [51], while β-farnesene was found in the EOs of *S. racemosus* and *S. trapezuntinus* [67]. In addition, germacrene D was also present as a major component in EOs of *S. rufinervis* [61-62], *S. crassiflorus* [71], and *S. argunensis* [83]. Several *Senecio* EOs presented an entirely different chemical profile compared to other species of the *Senecio* genus. 9,10-Dehydrofukinone, 1-nonene, 1-undecanol, 1-tridecene, and 1,10β-epoxy-6-oxofuranoeremophilane were found to be the main components of the EOs of *S. viridis* [29], *S. filaginoides* [37], *S. belgaumensis* [59], *S. coincyi* [65], and *S. royleanus* [66], respectively. There was significant intra- and interspecies variation in the chemical compositions of EO extracted from *Senecio* species, which appears to be influenced by the environmental factors of plant cultivation. Indeed, it has been reported that the chemical profiles of EO could vary with season, plant age, soil composition, collection time, and geographic origin. This variability might also be correlated to the genetic characteristics of the plant and/or to the source of the EOs [84-85].

5. PHARMACOLOGICAL ACTIVITIES

Senecio species have been used for centuries as a folk remedy because of their diverse pharmacological activities. The genus is a rich source of bioactive components, which are implicated in the reported pharmacological activities of the genus *Senecio*. EOs also have several promising pharmacological activities, briefly described here.

5.1. ANTIMICROBIAL ACTIVITY

Different microbes, bacteria, fungi and yeast are associated with the antimicrobial activity of EOs derived from several species of *Senecio.* Details of the activity are summarized in Table II [86-90]. The EOs from various *Senecio* species exhibit notable antimicrobial activity, particularly against significant bacterial and fungal pathogens. *S. graveolens* demonstrates strong activity with MIC values of 8.73 mg/mL for *Micrococcus luteus*, 10.91 mg/mL for *Staphylococcus aureus*, and an impressive 0.02 mg/mL for *Candida albicans* [86]. Similarly, *S. nemorensis* shows potent inhibition against *Bacillus cereus* (20 mm), *Staphylococcus aureus* (17 mm), and *Enterococcus faecalis* (18 mm), making it highly effective against Gram-positive bacteria [87]. Other significant examples include *S. pandurifolius*, which displays remarkable activity against *Mycobacterium smegmatis* within a large inhibition zone of 30 mm [60], and *S. nutans*, which effectively inhibits *Vibrio cholerae* within a 22 mm inhibition zone and a low MIC of 0.4 mg/mL [30]. *S. belgaumensis* also stands out for its strong antimicrobial effects against *S. faecalis*, *Aspergillus fumigatus*, and *A. niger*, with MIC values ranging from 0.015 to 0.104 mg/mL [88]. These findings highlight the significant antimicrobial potential of certain *Senecio* species, especially against major pathogens like *S. aureus*, *V. cholerae*, and *C. albicans*. This underscores their potential in developing natural antimicrobial agents and merits further exploration.

5.2. ANTIOXIDANT ACTIVITY

The antioxidant activity of *S. anteuphorbuim* EO was determined by the ability of the EO to inhibit

the bleaching of β-carotene by peroxide generation along linoleic acid oxidation. The EO presented a percentage rate of 49.42% at 4 mg/mL [79]. Furthermore, *S. massaicus* EO exhibited antioxidant in reducing power and ABTS assays with A0.50 value 93.0 µg/mL and IC50 value of 88.7 µg/mL respectively, compared to the CUPRAC test A0.50 value of 116.5 µg/mL. For the DPPH method, the EO did not display radical activity where the inhibition rate did not exceed 37% in the high concentration tested (200 µg/mL) [80]. Furthermore, the *S. glaucus* EO showed a strong antioxidant effect with EC50 values for DPPH radical scavenging of 1.6 and 1.9 µL/mL for capetula and shoot EOs, respectively [34]. In another study, *S. nudicaulis* EO was found to exhibit significant activity by scavenging DPPH, ABTS and nitric oxide radicals with IC50 values of 10.61, 11.85, and 11.29 µg/mL, respectively [45]. Similarly, *S. graciliflorus* flower EO exhibited a strong antioxidant potential, displaying IC50 values of 21.6 and 26.0 µg/mL in DPPH and hydroxyl radical assays, respectively [52].

The DPPH assay was chosen to evaluate the antioxidant potential of EOs from the genus *Senecio* because it is simple, reliable, and well-suited to this purpose. It directly measures the ability of antioxidants to neutralize free radicals, which is highly relevant when studying the radical-scavenging activity of EOs. The assay is quick and does not require complex equipment, making it practical for testing complex mixtures like EOs. Unlike other assays, such as TEAC and FRAP, the DPPH assay works well with both hydrophilic and lipophilic antioxidants, making it more suitable for EOs that are rich in hydrophobic compounds. TEAC involves additional steps to generate radicals, which can introduce variability, while FRAP measures the reducing power rather than directly assessing free radical scavenging. Other assays like ORAC and CUPRAC are more complex and time-consuming. Overall, the DPPH assay provides a straightforward and accurate way to evaluate the antioxidant properties of *Senecio* EOs, ensuring reliable results while avoiding the limitations of other methods.

5.3. REPELLENT EFFECT

The *S. scandens* EO decreased its repellent effect against *L. serricorne* from 78.63 to 0.13 nL/cm^2 and the effect of EO on *L. bostrychophila* fell from 63.17 to 12.63 nL/cm^2, The percentage of repellency value of EO against *L. bostrychophila* was similar to DEET (N,N-diethyl-meta-toluamide), which indicated that EO had great potential insecticidal activity for *L. bostrychophila* [26]. In another study, *S. glaucus* EO repelled *Tetranychus urticae* adults after 24, 48, and 72 h of exposure. After 72 h, a low repellent effect was observed in adults with *T. urticae* at the highest concentration tested, with a repellency index of 24% [36]. Furthermore, *S. pogonias* and *S. oreophyton* EOs showed good repellent properties against *Triatoma infestans* with repellency percentage values of 76% and 36% at 24 h, respectively [43].

5.4. ANTIFUNGAL ACTIVITY

The *S. nutans* and *S. viridis* EOs displayed moderate activity against *Fusarium graminearum* and *F. verticillioides* (MIC value of 1.2 mg/mL) and there was a weak effect against *Aspergillus carbonarius* and *A. niger* (MIC values of >1.2 mg/mL) [29]. The antifungal activity of *S. glaucus* EO was tested against the phase method (VF) and the poisoned food method (PF). The EO recorded an inhibition of 83% at 16 µL/mL using the PF method, while the VF method recorded 86% inhibition of mycelial growth of *B. cinerea* at 0.8 µL/ml air [91]. In another work, the EO of *S. amplexicaulis* exhibited significant activity against five phytopathogenic fungi, *Sclerotium rolfsii*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Pythium debaryanum* and *Fusarium oxysporum,* with EC50 values of 164.9, 157.0, 199.2, 187.4, and 159.0 µg/mL, respectively [49].

The EOs contain bioactive compounds like terpenoids and phenolic acids, which are effective against both Gram-positive and Gram-negative bacteria. These compounds work by disrupting bacterial membranes, interfering with enzymes, and preventing biofilm formation. *Senecio* EOs are particularly effective against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, which have simpler cell membranes that are easily disrupted. They also active against Gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa*. Although Gram-negative bacteria have an extra outer membrane for protection, the oils can penetrate and damage their cell structure.

5.5. ACARICIDAL ACTIVITY

The efficacy of *S. cannabifolius* EO against females and larvae of *Rhipicephalus microplus* was assessed by the adult immersion test (AIT) and the larval immersion test (LIT). LIT revealed that EO achieved 100% mortality at a concentration of 1.6% wt/vol, whereas biochemical assays indicated that the EO can significantly reduce the overall detoxification enzyme activities in engorged females and larvae at a high concentration (≥0.4% wt/vol) [27]. Furthermore, *S. glaucus* EO recorded significant mortality in adults 24, 48, and 72 h after treatment. The mortality rate ranged from 32 to 100%, and the probit analysis indicated an LD50 of 5843 ppm [91].

5.6. ANTI-INFLAMMATORY ACTIVITY

The EOs of the capetula and shoots of *S. glaucus* exerted the highest anti-inflammatory effect after 3

and 4 hours of carrageenan injection with percentage inhibition of 43.3% and 44.3%, respectively [34]. Furthermore, *S. flammeus* EO significantly reduced carrageenan-induced paw oedema by 17.42%, 52.90% and 66.45%, 4 h after carrageenan injection, respectively, and significantly reduced myeloperoxidase activity. The EO (10, 30, and 90 mg/kg) also produced a significant dose-dependent response in reducing TPA-induced ear oedema by 20.27%, 33.06%, and 53.90%, respectively. Furthermore, EO produced significant dose-response anti-inflammatory activity against cotton pellet-induced granuloma that peaked at the highest dose of 90 mg/kg (49.08% wet weight and 47.29% dry weight) [51].

5.7. CYTOTOXICITY STUDIES

S. glaucus capetula EO was found to be the most potent against human breast cancer cells (MCF-7) with an IC50 value of 1.5%, followed by shoot EO against the same cell line with an IC50 value of 2.1% [34]. In addition, as regards the inhibitory activity against hepatocellular carcinoma cells (HePG2), EO exhibited a promising activity with an IC50 value of 5.63 µg/mL [39]. In another study, the flower and root EO of *S. graciliflorus* showed strong activity against lung cell lines (A-549) with IC50 values of 19.1 and 21.3 µg/mL, respectively [52]. *S. rowleyanus* EO showed significant activity against brain tumour cell line (U251) and liver carcinoma cell line (MCF-8) with IC50 values of 5.0 and 2.6 µg/mL, respectively [70], while *S. leucanthemifolius* EO displayed significant activity against the human cervix uteri cancerous cell line (HeLa) with an IC50 value of 1.15 µL/mL [50].

5.8. PHYTOTOXIC ACTIVITY

The *S. amplexicaulis* EO inhibited the germination of *Triticum aestivum* (65.0%) and *Phalaris minor* (58.3%) at 500 µg/mL compared to the control. At a higher concentration (500 µg/mL), EO inhibited the shoot and root lengths of both test crops [49]. Another study on the EO of *S. erucifolius* reported the length of roots of *Medicago sativa*, *Urtica cannabina* and *Amaranthus retroflexus* increased by 21.00, 10.46, and 2.53%, respectively, after treatment with EO at the lowest concentration of 0.125 mg/mL and decreased by 9.36, 23.00, and 19.53% after treatment at the highest concentration of 4 mg/mL [82].

5.9. ANTICHOLINESTERASE ACTIVITY

The *S. massaicus* EO exhibited strong inhibitory activity against butyrylcholinesterase (BChE: IC50 value 13.85 µg/mL) and acetylcholinesterase (AChE: IC50 value 10.34 µg/mL) [80]. Furthermore, *S. ventanensis* EO was found to exert a weak activity in the AChE assay with a percentage inhibition of 8.9% (conc. 2.5%), compared to the positive control serine with 99.0% [48].

5.10. ALLELOPATHIC EFFECT

The allelopathic effect of *S. anteuphorbium* EO was evaluated by studying the inhibition of the germination and growth of *Lactuca sativa* seeds. At a high concentration of EO (0.28 mg/mL), the growth of the shoots and roots was reduced by 87.60% and 78.88%, respectively. The EO recorded an IC50 value of 0.12 mg/mL for shoot growth and 0.15 µg/mL for root growth, respectively [24].

5.11. NEMATICIDAL ACTIVITY

The nematicidal activity of *S. glaucus* EO was tested using two bioassays aimed at the mortality of second-stage juveniles (J2) and the inhibition of *Meloidogyne javanica* eggs. The effect exerted by the EO was nematostatic with a percentage inhibition of 95%, immobility of J2 and 92% inhibition of egg hatch at 16,000 ppm [91].

5.12. ANTIMALARIAL ACTIVITY

The *S. acaulis* EO showed moderate activity against chloroquine-sensitive *Plasmodium falciparum* (D6) and chloroquine-resistant *Plasmodium falciparum* (W2) with IC50 values of 8020.3 and 5785.4 µg/mL, respectively [42].

5.13. ANTILEISHMANIAL ACTIVITY

The *S. acaulis* EO exerted a weak activity against *Leishmania donovani* with IC50 and IC90 values of 24.3 and 34.3 µg/mL, respectively [42].

5.14. α-GLUCOSIDASE ACTIVITY

The *S. scandens* EO displayed strong inhibitory activity on α-glucosidase with an IC50 value of 0.1304 mg/mL, compared to the positive control acarbose of 23.4 mg/mL [92].

5.15. ANTICORROSIVE ACTIVITY

The *S. inaequidens* EO showed promising activity against mild steel in 1M HCl. The EO was found to inhibit 90.56% at 2g/L [55].

5.16. ANALGESIC ACTIVITY

The *S. rufinervis* EO produced a significant and dose-dependent inhibition of acetic acid-induced writhing (85% at 75 mg). The effect was more significant than the standard drug pentazocine (72.3% at dose 75 mg/kg) [62].

5.17. TOXICITY STUDIES

The *S. argunensis* EO exhibited acute lethal toxicity (25%) against *Artemia salina* larvae at a concentration of 1 mg/mL [83]. Additionally, the EO of *S. scandens* showed strong contact toxicity with *Tribolium castaneum*, *Lasioderma serricorne* and *Liposcelis bostrychophila* with LD50 values of 18.01, 20.11, and 72.14 µg/cm^2, respectively [26].

Table I - Major chemical components identified from *Senecio* essential oils

Species	Locality	Part	Yield (%)	Identification (No. %)	Major components
S. anteuphorbium	Morocco	Aerial parts	0.30	46, 85.62	Bicyclogermacrene (22.75%), spathulenol (25.26%), epi-β-eudesmol (6.8%), and selina-4,11-diene (5.08%) [24]
		Aerial parts	0.15	21, 99.60	γ-Selinene (27.2%), cyperene (21.7%), γ-cadinene (11.4%), and α-cyperone (8.1%) [25]
S. scandens	China	Aerial parts	0.84	20, 88.03	Humulene epoxide II (18.05%), 1,4,7-cycloundecatriene-1,5,9,9-tetramethyl-Z,Z,Z- (17.69%), caryophyllene oxide (16.24%), linalool (7.16%), and caryophyllene (4.52%) [26]
S. cannabifolius	China	Aerial parts	0.83	68, 99.2	Eucalyptol (13%), camphor (10.6%), germacrene D (6.0%), and caryophyllene oxide (6.1%) [27[
		Leaves	0.503	26, NM	α-Farnesene (13.37%), n-hexadecanoic acid (8.62%), 2,6-dimethyl-6-(4-methyl-3-pentenyl)-bicyclohept-2-ene (6.93%), and caryophyllene (5.32%) [26]
		Stems	0.036	36, NM	n-Hexadecanoic acid (24.12%), caryophyllene oxide (11.16%), 1-methyl-2-pentyl-cyclopropane (9.55%), and caryophyllene (5.78%) [26]
S. pedunculatus	India	Leaves	0.05	20, 93.44	Caryophyllene oxide (23.5%), δ-cadinene (10.4%), humulene epoxide II (9.4%), and myrcene (8.2%) [28]
S. nutans	Argentina	Aerial parts	1.42	25, 89.50	Sabinene (27.6%), α-phellandrene (15.7%), o-cymene (9.6%), and β-pinene (6.1%) [29]
	Chile	Leaves	0.37	19, 100	Methyl cinnamate (44.9%), p-cymenol (27.2%), terpinen-4-ol (6.8%), AND α-terpineol (4.1%) [30]
	Peru	Leaves	0.15	60, 93.70	p-Cymene (51.7%), α-phellandrene (21.8%), and (Z)-methyl cinnamate (3.7%) [31]
	Italy	Aerial parts	0.16	21, 94.30	α-Phellandrene (15.5%), α-terpinene (15.1%), sabinene (13.3%), δ-3-carene (8.8%), and p-cymene (8.8%) [32]
S. viridis	Argentina	Aerial parts	0.14	12, 97.50	9,10-Dehydrofukinone (92.7%) [29]
			0.10	14, 94.80	α-Thujene (31.7%), β-phellandrene (15.7%), α-pinene (9.0%), camphene (8.9%), and sabinene (7.0%) [33]
S. glaucus	Egypt	Capetula (flower)	0.50	33, 97.10	m-Mentha-1(7),8-diene (31.4%), cis-m-mentha-2,8-diene (22.9%), and dehydrofukinone (17.2%) [34]
		Shoots	0.25	33, 96.00	m-Mentha-1(7),8-diene (25.6%), dehydrofukinone (19.9%), and *cis*-m-mentha-2,8-diene (8.2%) [34]
		Aerial parts	0.25	80, 90.00	Myrcene (24.0%), dehydrofukinone I (21.0%), and p-cymene (9.9%) [35]
	Morocco	whole plants	0.20	84, 94.30	α-Pinene (26.2%), myrcene (11.4%), and p-cymene (9.9%) [36]
S. filaginoides	USA	Aerial parts	0.34	56, 96.1-97.6%	1-Nonene (2.0-4.7%), α-pinene (28.3-40.5%), sabinene (1.5-1.9%), β-pinene (4.7-5.4%), and δ-3-carene (1.8-5.7%) [37]
	Argentina	Aerial parts	NM	7, 60.90	β-Terpinene (28.0%), α-pinene (20.0%), and α-terpinene (4.4%) [38]
S. vulgaris	Egypt	Whole plants	NM	14, 98.53	Butylated hydroxytoluene (63.87%), 4,4'-ethylenebis(2,6-di-tert-butylphenol) (8.29% [39]
	France	Aerial parts	0.13-0.16	54, 95.20	α-Humulene (57.3%), (E)-β-caryophyllene (5.6%), terpinolene (5.3%), ar-curcumene (4.3%), and geranyl linalool (3.4%) [40]
S. serpens	Egypt	Aerial parts	0.09	38, 99.9	α-Myrcene (26.0%), α-pinene (22.81%), β-pinene (16.44%), germacrene D (8.94%), and 1-nonene (8.88%) [41]

Table I - continue

S. acaulis	Egypt	Aerial parts	0.11	22, 81.08	Limonene (13.32%), β-pinene (11.54%), sabinene (10.79%), and cryptone (10.13%) [42]
S. pogonias	Argentina	Aerial parts	0.40	19, 97.60	α-Pinene (48.0%), α-phellandrene (22.0%), p-cymene (7.1%), and β-pinene (5.9%) [43]
S. oreophyton	Argentina	Aerial parts	1.00	18, 97.30	α-Pinene (40.0%), p-mentha-1(7), 8-diene (31.0%), and β-phellandrene (5.3%) [43]
S. nudicaulis	India	Aerial parts	0.1-0.6	8, 96.30	α-Humulene (30.2%), germacrene D (26.3%), β-caryophyllene (22.3%), and linoleic acid (9.7%) [44]
			NM	30, 95.30	Caryophyllene oxide (24.99%), humulene epoxide II (21.25%), α-humulene (18.75%), β-caryophyllene (9.67%) [45]
S. giganteus	Algeria	Aerial parts	0.02	40, 92.38	Hexadecanoic acid (17.80%), isophytol (12.43%), 3-methyl pentanol (7.28%), phytol (6, 66%), and the spathulenol (4.47%) [46]
			0.80	18, 82.80	α-Pinene (19.4%), 6,10,14-trimethyl-2-pentadecanone (19.1%), pentacosane (16.9%), and tricosane (11.9 %) [47]
S. ventanensis	Argentina	Aerial parts	0.015	23, 98.50	α-Terpinene (12.2%), limonene (11.9%), α-humulene (10.5%), sabinene (9.1%), terpinolene (8.8%), and p-cymene (8.1%) [48]
S. amplexicaulis	India	Roots	0.66	18, 94.70	α-Phellandrene (48.57%), o-cymene (16.80%) and β-ocimene (7.61%) [49]
S. leucanthemifolius	Morocco	Whole plant	NM	NM	α-Hydroxy-p-cymen (27.3%), carvacrol (12.2%), nerol (10.9%), carveol (9.2%), and *cis*-α-bisabolene (7.0%) [50]
S. flammeus	China	Aerial parts	0.38	48, 98.41	α-Farnesene (11.26%), caryophyllene (8.69%), n-hexadecanoic acid (7.23%), and α-pinene (6.36%) [51]
S. graciliflorus	India	Flower	0.08	17, 99.90	α-Pinene (33.97%), cis-ocimene (26.83%), β-pinene (11.9%), and 1,2,3-trimethylcyclohexane(6.37%) [52]
		Leaf	0.07	20, 95.50	*cis*-Ocimene (24.14%), α-Pinene (18.36%), 1,2,3-trimethylcyclohexane(14.11%), and β-pinene (6.41%) [52]
		Stem	0.04	19, 98.93	*cis*-Ocimene (24.97%), α-pinene (24.66%), 1,2,3-trimethylcyclohexane(11.15%), and β-pinene (9.05%) [52]
		Root	0.04	17, 95.96	α-Pinene (36.36%), 1,2,3-trimethylcyclohexane (13.32%), *cis*-ocimene (11.15%), and β-pinene (10.84%) [52]
S. bombayensis	India	Flower	0.20	46, 98.20	Linalool (26.3%), β-cedrene (14.5%), E-β-farnesene (10.8%), 2,5-dimethoxy-p-cymene (7.0%), (E)-β-ocimene (5.9%), terpinen-4-ol (5.1%), and Z-β-ocimene (4.7%) [53]
S. selloi	Brazil	Aerial parts	0.0035	20, 71.3	α-Zingiberene (54.0%) and α-isolimonene (16.0%) [54]
S. inaequidens	France	Aerial parts	NM	60, 98.8	Myrcene (21.4%), (Z)-β-ocimene (17.6%), α-pinene (12.5%), limonene (8.1%), and cacalohastine (6.8%) [55]
S. vernalis	Turkey	Aerial parts	0.40	39, 91.50	β-Phellandrene (12.6%), 1,8-cineole (9.2%), caryophyllene oxide (7.3%), β-selinene (6.3%), and limonene (6.2%) [56]
		Flower	0.16	69, 93.40	β-Pinene (13.0%), (E)-caryophyllene (28.6%), δ-3-carene (10.4%), germacrene D (8.6%), α-phellandrene (8.3%), (Z)-β-ocimene (4.7%), and α-humulene (4.5%) [57]
	Iran	Aerial parts	0.16	10, 98.90	Spathulenol (37.1%), 1,8-cineol (19.0%), m-cymene (16.6%), and isobicyclo-germacrenal (15.2%) [58]

Table I - continue

S. belgaumensis	India	Flower	0.20	48, 91.50	1-Undecanol (19.5%), β-caryophyllene (18.9%), caryophyllene oxide (10.4%), and γ-terpinene (9.2%) [59]
S. pandurifolius	Turkey	Flower	0.24	45, 90.10	α-Cuprenene (30.7%), borneol (11.9%), β-eudesmol (9.3%), 1-undecene (7.4%), (E)-caryophyllene (6.0%), nonadecane (4.4%), and hexadecane(4.0 %) [60]
		Leaves	0.15	60, 88.00	α-Zingiberene (16.1%), borneol (13.4%), 1-undecene (8.3%), (E)-γ-bisabolene (6.4%), β-eudesmol (5.3%), and bicyclogermacrene (4.5%) [60]
		Stems	0.19	42, 89.00	γ-Curcumene (14.9%), undecane (12.0%), α-zingiberene (9.0%), (E,E)-α-farnesene (8.8%), (E)-caryophyllene (7.2%), and 6-methoxy-2-(1-buten-3-yl)-naphthalene (6.5%) [60]
S. rufinervis	India	Leaves	0.50	11, 70.17	Germacrene D (40.19%), β-pinene (12.33%), and p-cymene (4.15%) [61]
		Roots	0.40	15, 72.14	Germacrene D (24.95%), α-cubebene (8.14%), and α-longipinene (6.46%) [61]
		Leaves	0.50	11, 78.18	Germacrene D (40.19%), β-pinene (12.23%), β-caryophyllene (6.21%), and β-longipinene (4.15%) [62]
		Leaves	0.60	92.50	Germacrene D (33.7%), δ-cadinene (5.5%), γ-cadinene (5.5%), germacrene D-4-ol (5.4%), α-cadinol (4.9%), and β-longipinene (4.0%) [63]
		Roots	0.50	89.10	Germacrene D (32.9%), germacrene A (19.5%), δ-elemene (7.6%), α-cubebene (4.9%), and β-eudesmol (3.0%) [63]
S. atacamensis	Chile	Leaves	1.04	19, 75.69	α-Terpinene (36.05%), α-phellandrene (27.79%), and p-cymene (11.85%) [64]
		Stems	0.92	24, 68.49	α-Phellandrene (25.37%), p-cymene (22.55%), and α-terpinene (20.57%) [64]
S. coincyi	Spain	Leaves	0.01	38, 60.00	1-Tridecene (28.1%), β-bisabolene (6.1%), and 1-pentadecene (6.0%) [65]
S. royleanus	India	Aerial parts	0.24	43, 96.50	1,10β-Epoxy-6-oxofuranoeremophilane (69.2%) and 1β-10-epoxyfuranoeremophilane (3.3%) [66]
		Flowers	0.18	44, 97.10	1,10β-Epoxy-6-oxofuranoeremophilane (39.4%), (E)-β-ocimene (17.0%), 1β-10-epoxyfuranoer-emophilane (8.2%), and α-pinene (7.6%) [66]
		Leaves	0.30	44, 95.50	1,10β-Epoxy-6-oxofuranoeremophilane (50.3%) and 1β-10-epoxyfuranoeremophilane (25.2%) [66]
		Stems	0.14	41, 95.00	1,10β-Epoxy-6-oxofuranoeremophilane (54.9%), γ-muurolene (9.8%), and (E)-β-ocimene (7.9%) [66]
S. othonnae	Turkey	Flowers	0.12	56, 83.10	Caryophyllene oxide (18.6%), (E)-caryophyllene (13.7%), α-cadinol (7.4%), and 1-undecene (6.8%) [67]
S. racemosus	Turkey	Flowers	0.08	38, 97.70	(E)-β-Farnesene (21.6%), (E)-caryophyllene (20.0%), γ-amorphene (19.1%), and 1-undecene (11.4%) [67]
S. nemorensis	Turkey	Flowers	0.12	37, 86.80	γ-Curcumene (42.8%), (E)-β-Farnesene (25.2%), and β-curcumene (6.2%) [67]
S. mustersii	Argentina	Aerial parts	0.81	24, 95.20	α-Pinene (53.3%) and β-pinene (21.2%) [38]
S. subpanduratus	Argentina	Aerial parts	0.71	21, 92.90	α-Pinene (22.1%), β-pinene (11.9%), sabinene (23.8%), terpinen-4-ol (10.2%) and p-cymene (8.7%) [38]

Table I - continue

S. polyanthemoides	South Africa	Flower	0.10	13, 97.00	p-Cymene (24.7%), limonene (18.3%), and myrcene (15.7%) [68]
		Leaves	0.23	8, 94.10	β-Selinene (32.7%), caryophyllene oxide (13.4%), α-pinene (11.8%), and 1,8-cineole (11.4%) [68]
		Stems	0.17	8, 99.60	α-Pinene (21.4%), p-cymene (18.7%), limonene (18.1%), β-pinene (12.4%), and 1,8-cineole (9.3%) [68]
S. platyphyllus	Turkey	Flowers	0.12	48, 94.40	(E)-Caryophyllene (28.6%), germacrene D (23.4%), and (*E*)-β-farnesene (6.8%) [57]
S. adenotrichius	Chile	Aerial parts	0.36	11, 91.30	Dehydrofukinone (70.9%), (E)-β-ocimene (5.8%), and α-terpinene (4.5%) [69]
S. zoellneri	Chile	Aerial parts	0.41	21, 97.00	δ-3-Carene (19.5%), β-phellandrene (18.0%), β-pinene (16.4%), and α-pinene (10.8%) [69]
S. rowleyanus	Egypt	Aerial parts	0.10	25, 99.95	Spathulenol (22.9%), myrcene (12.80%), germacrene B (12.4%), viridiflorol (10.99%), and *trans*-caryophyllene (8.42%) [70]
S. subulatus var. *salsus*	Argentina	Aerial parts	0.10	18, 95.50	p-Cymene (33.3%), β-pinene (31.2%), γ-terpinene (15.6%), and α-thujene (5.0%) [33]
S. subulatus var. *erectus*	Argentina	Aerial parts	0.10	13, 97.50	γ-Terpinene (53.6%), p-cymene (18.3%), β-pinene (17.3%), and α-thujene (5.2%) [33]
S. argophylloides	Argentina	Aerial parts	0.20	13, 99.80	Camphene (52.7%), sabinene (12.3%), and β-phellandrene (10.7%) [33]
S. trapezuntinus	Turkey	Flowers	0.15	34, 91.70	(E)-β-Farnesene (26.3%), β-selinene (11.8%), eremophilane (9.2%), 14-hydroxy-9-epi-(E)-caryophyllene (7.8%), γ-gurjunene (7.7%), and (E)-caryophyllene (7.1%) [67]
		Leaves	0.12	26, 86.20	(E)-β-farnesene (16.9%), β-selinene (11.6%), 14-hydroxy-9-epi-(E)-caryophyllene (10.5%), eremophilane (9.3%), (E)-caryophyllene (8.7%), and δ-amorphene (7.3%) [67]
		Stems	0.10	12, 73.50	(E)-β-farnesene (31.2%), eremophilane (9.9%), γ-gurjunene (8.1%), and (E)-caryophyllene (5.4%) [67]
S. crassiflorus	Brazil	Leaves	0.023	15, 98.90	α-Cadinol (56.0%), t-muurolol (25.0%), and germacrene D (6.1%) [71]
		Aerial parts	0.023	14, 98.80	Germacrene D (59.0%) and germacrene B (22.0%) [71]
		Stems	0.033	17, 97.70	Germacrene D (48.0%), germacrene B (24.0%), α-cadinol (5.7%) [71]
S. leucostachys	Iran	Leaves	0.80	31, 98.60	Sabinene (20.7%), α-phellandrene (19.7%), germacrene D (10.8%), and β-caryophyllene (8.2%) [72]
S. farfarifolius	Turkey	Aerial parts	0.29	77, 95.00	α-Pinene (48.3%), 1,8-cineole (10.3%), germacrene D (3.4%), and sabinene (3.4%) [73]
S. squalidus	Serbia	Aerial parts	0.025	58, 94.10	p-Cymene (29.3%), α-phellandrene (24.7%), and α-pinene (8.0%) [74]
S. graveolens	Argentina	Leaves	0.57	14, NM	α-Terpinene (60.0%), p-cymene (14.0%), terpinen-4-ol (5.5%), and α-phellandrene (4.0%) [75]
S. chysanthemides	India	Aerial parts	0.02	19, 95.59	β-Thujone (84.17%) and α-terpineol(2.53%) [76]
S. aegyptius	Egypt	Flowers	0.30	19, NM	1,10-Epoxyfuranoeremophilane (55.3%), 1-nonane (17.0%), and myrcene (8.9%) [77]
		Leaves	0.40	17, NM	1,10-Epoxyfuranoeremophilane (66.3%),1-nonane (22.0%), and myrcene (3.4%) [77]
		Stems	0.10	18, NM	1,10-Epoxyfuranoeremophilane (46.4%),1-nonane (19.0%), and myrcene (10.6%) [77]
		Roots	0.05	6, NM	1,10-Epoxyfuranoeremophilane (69.0%), drima-7,9-(11)-diene (19.0%), and β-elemene (4.2%) [77]

Table I- continue

S. ambavilla	India	Aerial parts	0.02	62, 97.00	*allo*-Aromadendrene (40%), α-pinene (14%), α-himachalene (6.2%), and β-himachalene (5.8%) [78]
S. anteuphorbuim	Morocco	Aerial parts	0.40	41, 78.94	Selina-4,11-diene (8.07%), β-gurjunene (7.68%), γ-cadinene (6.86%), and γ-muurolene (5.65%) [79]
S. massaicus	Algeria	Aerial parts	0.18	22, 97.41	m-Cymene (30.58%), n-hexadecanoic acid (14.88%), and docosane-11-decyl (10.43%) [80]
S. pterophorus	South Africa	Leaves	NM	38, 98.00	Limonene (10.3-32.3%), myrcene (14.4-19.7%), sabinene (13.0-18.0%), α-phellandrene (3.4-16.9%), and p-cymene (15.6-16.7%) [81]
S. erucifolius	China	Aerial parts	0.18	37, 85.70	Dibutyl phthalate (16.2%), isoledene (7.7%), β-*cis*-ocimene (5.5%), butylcyclopentane (4.9%), and germacrene D (4.8%) [82]
S. argunensis	Russia	Aerial parts	0.10	17, 99.80	Germacrene D (46.5%), caryophyllene oxide (9.9%), *trans*-caryophyllene (7.6%), and β-thujene (7.5%) [83]

NM – not mentioned

Table II - Antimicrobial activities of *Senecio* essential oils

Essential oils	Description
S. graveolens	Bacterial effects on *M. luteus*, *S. aureus*, and *C. albicans* with MIC values of 8.73, 10.91, and 0.02 mg/mL, respectively [86]
S. othonnae	Potent activity (zone of growth inhibition) against *B. cereus* (15 mm), *S. aureus* (10 mm), *E. faecalis* (15 mm), and *C. tropicalis* (9 mm) [87]
S. nemorensis	Potent activity (zone of growth inhibition) against *B. cereus* (20 mm), *S. aureus* (17 mm), *E. faecalis* (18 mm), and *C. tropicalis* (15 mm) [87]
S. atacamensis	Moderate inhibition against *K. pneumoniae* with an inhibition zone of 18 mm [64]
S. rufinervis	Exhibited significant activity against *B. subtilis* with an inhibition zone of 11 mm [61]
S. pandurifolius	Strong activity against *M. smegmatis* with an inhibition zone of 30 mm [60]
S. belgaumensis	Effective against *S. faecalis*, *A. fumigatus*, and *A. niger* with MIC values of 0.015, 0.045, and 0.104 mg/mL, respectively [88]
S. selloi	Weak activity against *Bacillus subtilis* with MIC and MBC value of 4400 µg/mL [54]
S. leucanthemifolius	Considerable activity (zone of growth inhibition) against two *E. coli* genetically modified strains MG (12 mm) and TG1 (12 mm), *Rhizobium* RP8 (12 mm) and *B. subtilis* (16 mm) [89]
S. giganteus	Moderate activity against *E. coli* and *Shigella sp*. with a diameter of inhibition of 12-14 mm. Besides, the EO was found inactive against *S. aureus*, *K. pneumonia*, and *P. aeruginosa* with an inhibition diameter of 7.5-11 mm [46]
S. nutans	Exhibited an important activity with a diameter of inhibition zone growth of 22 mm and the MIC value of 0.4 mg/mL against pathogenic bacteria *V. cholera* [30]
S. nudicaulis	Inhibited by *Aeromonas hydrophilla* (MIC value 25 µL/mL), followed by *P. aeruginosa*, *E. coli*, and *S. candidus* (each MIC value of 30 µL/mL) [90]
S. acaulis	Exhibited activity against *Cryptococcus neoformans* and *Staphylococcus aureus* with IC50 values of 36.02 and 197.98 µg/mL, respectively [42]
S. anteuphorbium	Demonstrated moderate activities with MIC values in the range of 0.51-1.02 mg/mL for yeasts and between 2.3-4.6 mg/mL for bacteria [25]
S. glaucus	Exhibited the activity against *B. subtilis* and *C. tropicalis* with MIC value of 3.1 µL/mL [34]
S. pedunculatus	Effectiveness against *Bacillus subtilis* (MIC value 11.65 µL/mL) and *A. flavus* (MIC value 8.75 µL/mL) [28]
S. aegyptius	Significant level of activity against *C. albicans* (16-20 mm), *S. aureus* (8-10 mm), *A. flavus* (6-8 mm), *B. subtilis* (7-9 mm), and *E. coli* (7-8 mm) [77]
S. crassiflorus	Active against *B. cereus* with MIC value of 1025 µg/mL [71]
S. pogonias	Best activity against *E. coli* (MIC value 2000 µg/mL), whereas *S. oreophyton* EO was active against *S. coagulase negative 968* (MIC value 1000 µg/mL) [43]
S. anteuphorbuim	Exhibited activity against *S. aureus* and *E. coli* with MIC values of 40.8 and 6.72 mg/mL, respectively [79]

6. CONCLUSION AND FUTURE OUTLOOK

In the present review, the medicinal uses, the composition of EO and the pharmacological properties of different species of *Senecio* have been underlined. The genus *Senecio* belongs to the Senecioneae tribe of the family Asteraceae, which is distributed in Europe, western and Central Asia, and northern Africa. It has been widely used in popular medicine in several countries due to its interesting pharmacological properties. As of December 2022, 55 *Senecio* species had been studied for their composition of EOs of various parts and evaluated for pharmacological potential. Among these, the most studied is *S. nutans* from four countries. In terms of the EO, *Senecio* EOs contain a variety of major components, in particular monoterpenes. According to the reports published in this regard, α-pinene has been identified as the main component of a large number of EOs. However, the frequency of other groups of natural components like sesquiterpenes (hydrocarbons and oxygenated) was considerably lower than that of monoterpenes. In addition, the obtained EOs have been tested and evaluated for their biological - mainly antimicrobial and antioxidant - activities. However, most scientific studies have focused on testing EOs through in vitro studies, while biological media available in vivo and biochemical investigations relating to the mechanism of action of the tested EOs are lacking. Several in vitro evaluations might be developed in vivo in order to evaluate their abilities to address numerous diseases. Since the probable toxicological impacts of the *Senecio* species have not so far been evaluated, these characteristics should be taken into consideration in future reports.

Disclosure statement

The authors declare no conflict of interest in this article.

Acknowledgement

This research was supported by the Geran Penyelidikan Universiti (Kecemerlangan@UPSI) under grant number 2025-0012-103-01, funded by Universiti Pendidikan Sultan Idris.

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Short note

Changes in Fatty acids and 4-Desmethylsterols Content During walnut (*Juglans regia L.*) fruit Development

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Received: November 22, 2023
Accepted: July 15, 2024

The evolution of fatty acids and 4-desmethylsterols composition during the maturation of two fruit of walnut varieties (Franquette and Local gd) was investigated. Fruit samples were collected at regular intervals period from 7 to 35 days after fruiting date (DAFD). Qualitative and quantitative analyses were made by GC-MS. Oil content showed increasing trends. During fruit development, unsaturated fatty acids are the major component, among which Linoleic acid (C18:2) was the most predominant, followed by Linolenic acid (C18:3), and Oleic acid (C18:1). The evolution of the 4-desmethylsterols content was marked by the predominance of β-sitosterol during all ripening stages, with a maximum value at 7 DAFD (269.56mg / 100g of oil) in Franquette variety and at 21 DAFD (122.17mg / 100g of oil) in Local gd variety.

Keywords: *Fatty acids; 4-Desmethylsterols; Walnut; Development; β-sitosterol*

1. INTRODUCTION

Walnut (*Juglans regia* L.) is a crop of high economic interest to the food industry. The edible part of the fruit (the seed or kernel) is globally popular and valued for its nutritional, health and sensory attributes. The high oil and essential fatty acid contents of the walnut kernel make it a good source for the commercial production of edible oil. Oil contents as high as 740 g/kg kernel (Soxhlet extraction, *n*-hexane) have been reported for some commercial walnut varieties [1].

Walnut oil (WO) can be extracted easily by screw pressing [2]. Employing a pilot plant screw-press, the highest oil recovery (660 g/kg kernel) was achieved at 7.5 g/100 g kernel moisture and 50°C pressing temperature. Fresh WO is very low in free fatty acid concentration, peroxides and phosphatides [3] because of which it may be consumed directly, without refining. Walnut oil is composed mainly of triglycerides, in which monounsaturated (oleic acid mainly) and polyunsaturated fatty acids (PUFAs, linoleic and ?-linolenic acids) are present in high amounts [4]. According to [5] WO has a perfect balance of *n*-6: *n*-3 PUFAs, a ratio of 4:1, which was showed to decrease the incidence of cardiovascular risk [6].

Walnut fruit is also rich in phytosterols [7]. Several epidemiologic and experimental studies report that dietary phytosterols may offer protection from cancers such as colon, breast, and prostate cancers. Phytosterols are the major fraction of unsaponifiable in vegetable oil, being the most relevant the 4-desmethylsterols [8]. The most frequent 4-desmethylsterols are ?-sitosterol, campesterol, and stigmasterol [8].

The objective of this work was to study, fatty acids and 4-desmethylsterols composition of two walnuts varieties (Franquette and Local gd) during fruit ripening, to determine their optimal period of harvest.

2. MATERIAL AND METHODS

2.1. SAMPLES

Juglans regia L. fruits were obtained from the National Institute for Research in Rural Engineering, Water and Forest (*INRGREF*) of Tunisia. Walnut trees were grown on the Agronomy farm in the north of Tunisia (Mateur, Bizerte), in a wet zone (rainfall average of 600 mm per year). Two varieties of walnuts, Local gd and Franquette were studied during seed development. The harvest period was stretched from 7 to 35 days after fruiting date (DAFD, 1-week intervals), from the middle of August 2011 until the end of September 2011, when the fruit reached complete maturity.

2.2. REAGENTS AND STANDARDS

Acetone, chloroform, diethyl ether and petroleum ether were purchased from Fisher Scientific SA. (Loughborough, UK). Ethanol was obtained from Scientific Limited (Northampton, UK). Fatty acid methyl ester (FAME) standards were purchased from Nu-Chek-Prep (Elysian, MN, USA). Sterols including 5-α-cholestanol (I.S) were purchased from Sigma (St. Louis, MO, USA), The individual phytosterols standards used for peak identification: cholesterol, fucosterol, stigmasterol, sitosterol, and campesterol were purchased from Sigma (Sigma–Aldrich Corporation, St. Louis, MO). *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA), from Pierce (Rockford, USA), was used as derivatisation reagent. TLC silica plates (silica gel 60 G, F254, 20 - 20 cm, 0.25 thicknesses), potassium hydroxide pellets, pyridine and anhydrous sodium sulphate were obtained from Merck (Darmstadt, Germany).

2.3. DETERMINATION OF OIL CONTENT

Walnut kernels (about 30 g) from each variety were ground into a fine powder using a hand mortar. Then the oil was extracted using a Soxhlet extractor with petroleum ether as a solvent. After 6 h of extraction, samples were evaporated under vacuum, weighted and their oil yield was determined

2.4. DETERMINATION OF FATTY ACIDS BY GC-MS

Fatty acids were converted to their corresponding methyl esters according to the method described by Carreau and Dubacq (1978). The fatty acid methyl esters analysis of samples was performed using a Hewlett Packard HP 6890 Series Gas Chromatographer (GC) coupled with a Hewlett Packard 5975 Mass Selective Detector mass spectrometer (MS). For the quantitative determination using a calibration factor of 1.0 (external standard Dodecanese). Separation was done in a HP-5 MS 30 m length, inner diameter 0.25 µm, 0.25 µm film thickness; oven temperature, isotherm at 180°C for 1 min, from 180 to 200°C at a rate of 15°C/min, from 200 to 220°C at a rate of 3ºC/min and isotherm at 300°C for 2 min. Injector and detector temperatures were 250 and 230°C, respectively. Injection volume: 2 µL; split ratio 1:100. Helium was used as carrier gas at 0.9 mL /min. For MS detector source temperature at 230°C, scan range 50-550 U and the spectra obtained were compared with the NIST/EPA/NIH Mass Spectral Library W9N11.L database.

2.5. SAPONIFICATION

Unsaponifiable lipids were determined by the saponifying 5 g of lipid extracts with 50 mL ethanolic KOH 12% (w/v) mixed with both 500 ml 5-α-cholestanol solution (internal standard: 0.2% (w/v)) and heating at 60°C for 1.30 h. After cooling, 50 mL of H_2O was added and the unsaponifiable matter was extracted four times with 50 mL petroleum ether. The combined ether extract was washed with 50 mL of ethanol – H_2O (1:1). The ether extracted was dried over anhydrous Na_2SO_4 and evaporated. The dry residues were dissolved in chloroform for TLC analysis.

2.6. THIN-LAYER CHROMATOGRAPHY

The unsaponifiable matter was separated into subfractions on preparative silica gel thin-layer plates, using 1-dimensional TLC with hexane -diethyl ether (65:35 v:v) as the developing solvent. The unsaponifiable (4 mg in l00 mL $CHCl_3$) was applied on the silica gel plates in 3 cm bands. To correctly identify the bands 5-α-cholestanol was applied on the left and the right sides of the TLC plates. After development, the plate was sprayed with 2, 7-dichlorofluorescein and viewed under UV light. The sterol bands were identified based on the reference spots. Those bands corresponding to 4-desmethylsterols were scraped off separately and extracted three times with chloroform - diethyl ether (1:1), filtered to remove the residual silica, dried in a rotary evaporator and stored at 10°C for further analysis.

2.7. QUANTITATIVE DETERMINATION OF STEROLS BY GC-MS

GC-MS analysis of sterol TMS derivatives was performed on an Agilent 6890 N Network GC system equipped with a capillary column Agilent Ultra 1 column (length 16. 5 m, i.d. 0. 2 mm, film thickness 0.11 µm) and coupled to an Agilent 5973 Network mass selective detector. Helium was used as a carrier gas at 1 mL /min. The injector temperature was set at 280°C. The oven temperature was kept at 200°C, 2 min, increased at 5°C/min until 270°C and at 20°C/min until 300°C. The sterol TMS derivatives were immediately injected separately into a GC (Hewlett Packard 7683) performed in the split mode. The electron impact (EI) mass spectra were recorded at ionisation energy of 70 eV and the ion source temperature was set at 280°C.

2.8. STATISTICAL ANALYSIS

All extractions and determinations were conducted in triplicate. The data were analysed using the analysis of variance (Anova). Comparisons of means were achieved using the Statistical Analysis System XLSTAT (version 2013). Differences between varieties were assessed using Duncan test. Differences at $p < 0.05$ were significant.

3. RESULTS AND DISCUSSION

3.1. CHANGES IN TOTAL LIPID CONTENT

The evolution of oil content of two walnuts varieties during maturation stages (expressed as % of dry weight) showed that oil accumulation followed a similar pattern in all varieties, increasing the amount of oil between the 7th and the 14th DAFD and reached 47.2% and 42.4% for Franquette and Local gd varieties, respectively (Fig. I). This increase can be explained by the fact that between 7th and 14th DAFD the lipids synthesized by immature walnuts are used for the development of new fruit tissues. Then, between 14th and 28th DAFD the lipid biosynthesis was much slower, there was a stationary phase where the rate of lipid accumulation remained constant. In the last stage, the oil content was maximal 49.70% for Local gd and 56.91% for Franquette. The quantitative characterisation of the oil content at complete maturity of our samples agreed with that reported by [4]. It was reported that the total lipid content increased with maturity [9]. The study of the lipids accumulation during linseed development is important to decide the best moment to harvest walnut fruits.

3.2. DYNAMIC ACCUMULATION OF FATTY ACIDS CONTENT DURING MATURATION

The proximate composition of fatty acids of two walnut varieties through the maturation stages are summarised in Table I. In all stages of maturity, five major fatty acids were identified. Their variation in content followed the same pattern. Results showing that linoleic and linolenic acids, major fatty acids detected tend to decrease their percentages during fruit ripening. These results match the ones obtained by[10]. This reduction of linolenic acid concentration during ripening has been produced by genetic modifications in the desaturation step from linoleic acid to linolenic acid controlled by linoleate or omega-3 fatty acid desaturases [11]. The fall in the linoleic acid percentage content was accompanied by a concomitant reduction in that α-linolenic acid. As expected, total PUFA was the main group of fatty acids ranging from 87.63 to 95.54% in Franquette and from 86,36 to 90,86% in Local gd through the maturation stages. Similar results in the fatty acid composition were obtained for different walnut selections and varieties [12]. In fact, as ripening advances certain metabolic processes, which involve changes in the profile of certain compounds such as fatty acids and phytosterols, occur. These variations are reflected in the oxidative stability and the nutritional value of the final product.

3.3. DYNAMIC ACCUMULATION OF 4-DESMETHYLSTEROLS

The accumulation of 4-desmethylsterols during maturation of Walnuts is shown in Table II. β- Sitosterol is the major compound at every stage of maturity that accounted for over 90% of the total of 4-desmethylsterols, with a maximum value (269.56 mg / 100g of

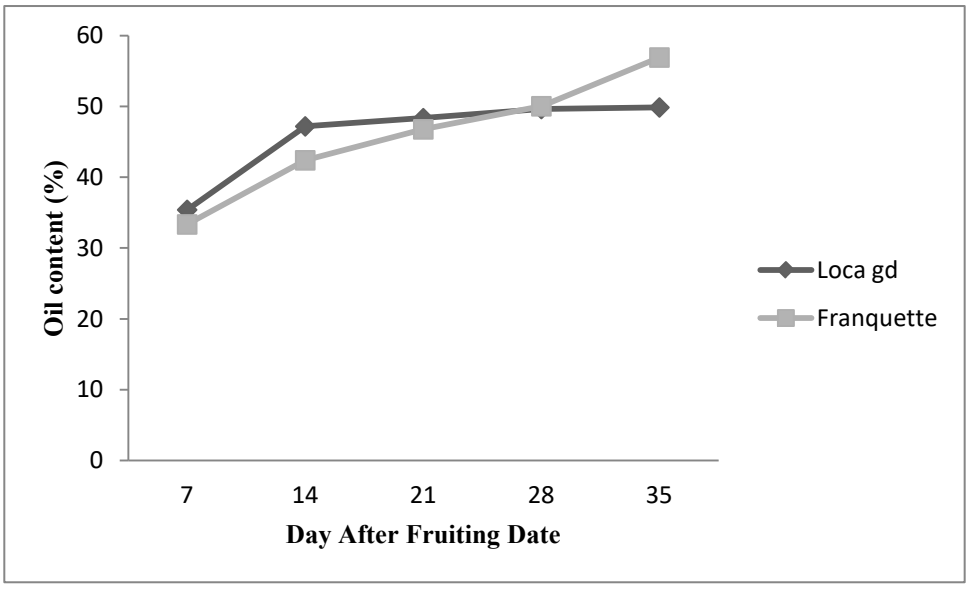

Fig1. Changes in oil content (% of dry weight) during walnut maturation.

Table I - Changes in Major fatty acids composition (g/100g of oil) during walnut maturation.

Fatty acid composition g/100g of oil	Franquette						Local gd				
	DAFD						DAFD				
	7	14	21	28	35		7	14	21	28	35
C16:0	0.05±0.01	6.60±0.28	6.95±0.00	7.03±0.01	8.38±0.01		6.16±0.03	6.62±0.03	5.65±0.00	5.63±0.28	6.60±0.28
C18:0	4.90±0.07	2.94±0.00	2.89±0.14	2.92±0.03	3.66±0.00		3.70±0.14	3.85±0.07	3.44±0.00	3.63±0.04	3.65±0.01
C18:1	21.21±0.14	24.04±0.06	25.06±0.04	23.92±0.01	22.72±0.14		23.00±0.24	27.20±0.14	27.65±0.07	26.16±0.01	24.78±0.03
C18:2	63.35±0.14	66.27±0.14	64.99±0.00	65.95±1.41	60.90±0.00		63.00±1.41	62.01±0.01	64.04±0.03	64.41±0.00	61.37±0.01
C18:3	30.14±0.01	29.27±0.03	29.03±0.04	27.62±0.03	26.73±0.03		26.95±0.01	26.93±0.00	26.82±0.03	25.04±0.03	24.99±0.00
SFA	4.95±0.08	9.54±0.28	9.84±0.14	9.95±0.04	12.04±0.01		9.86±0.17	10.47±0.10	9.09±0.01	9.26±0.32	10.25±0.29
MUFA	21.21±0.28	24.04±0.06	25.06±0.04	23.92±0.01	22.72±0.14		23.00±0.24	27.20±0.14	27.65±0.07	26.16±0.01	24.78±0.03
PUFA	93.49±0.15	95.54±0.17	94.02±0.04	93.57±1.44	87.63±0.03		89,95±1.42	88,94±0.01	90,86±0.06	89,45±0.03	86,36±0.01
UFA	114.7±0.43	119.58±0.23	119.62±0.08	117.49±1.45	110.35±0.17		112.95±0.66	116.14±0.15	118.51±0.13	115.61±0.04	111.14±0.04
MUFA/SFA	4.28	2.52	2.54	2.40	1.88		2.33	2.60	3.04	2.82	2.41
PUFA/SFA	18.90	10.01	9.55	9.40	7.28		9.12	8.50	10.00	9.66	8.42
UFA/SFA	23.17	12.53	12.15	11.80	9.16		11.45	11.10	13.03	12.48	10.84

DAFD: Days after Fruiting date
Results were given as means ± SD from triplicate estimations.
SFA: saturated fatty acid, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, UFA: unsaturated fatty acids.

Table II - Changes in 4-desmethylsterols composition during walnut maturation

Phytosterol composition mg/100g of oil	Franquette						Local gd					
	DAFD						DAFD					
	7	14	21	28	35		7	14	21	28	35	
Cholesterol	0.76±0.03	0.30±0.01	0.87±0.00	0.42±0.01	0.90±0.07		0.79±0.01	0.78±0.00	0.67±0.03	0.96±0.44	0.90±0.07	
Fucosterol	2.78±0.01	9.00±0.00	7.27±0.04	1.86±0.01	1.58±0.01		19.74±0.01	26.91±0.03	1.03±0.03	2.27±0.01	0.09±0.03	
β-Sitosterol	269.56±0.03	146.55±0.14	163.17±0.14	171.30±0.00	152.80±0.28		102.10±0.00	105.43±0.03	122.17±0.01	117.35±0.00	95.15±0.14	
Campesterol	11.04±0.03	15.09±0.07	23.29±0.00	5.76±0.03	5.59±0.01		4.07±0.03	4.15±0.00	0.58±0.42	4.59±0.01	1.70±0.07	
Total (mg/100g of oil)	284.14±0.1	170.94±0.22	194.60±0.18	179.34±0.05	160.87±0.37		126.70±0.05	137.27±0.06	124.45±0.49	125.17±0.46	97.84±0.31	

DAFD: days after Fruiting date
Results were given as means ± SD from triplicate estimations.

oil) at 7 DAFD in the Franquette variety and (122.17 mg / 100g of oil) at 21 DAFD in the Local gd variety. At complete maturity, Franquette and Local gd had β-sitosterol contents of 152.80mg /100 g of oil and 95.15mg /100 g of oil, respectively followed by the campesterol and the fucosterol, the cholesterol represents the minor compound of 4-desmethylsterols. Generally, the sterol composition of walnut cultivars has been indicated to depend on the ripening stage of the fruit. The qualitative characterisation of our samples agreed with those listed in the literature [13]. The differences in individual contents of phytosterol when compared to literature, may be due to the cultivars used and to cultivation and/or environmental factors. Table II show that the change in profiles of campesterol, fucosterol and cholesterol was very similar during ripening of walnut. This result could be explained by the fact that these compounds had the same biosynthetic precursor [14].

β-Sitosterol was the major sterol to accumulate, campesterol and fucosterol were also present at low amounts. The essential roles that sterols perform in plant tissues as structural components of membranes ensure that they must be present during all stages of the fruit development [15]. There was a link between sterol accumulation and enzyme activities in developing fruit. However, during the fruit development there are periods of increased sterol accumulation that correlated with the increased activity of key enzymes in the sterol biosynthetic pathway [16].

In the literature, there are no available studies on the changes in the sterol composition of walnut during fruit development. Total sterol content was reported to decrease gradually during linseed development as well [14].

A decline in the total sterol content during fruit development may be related to four reasons: (1) The biosynthesis of sterols occurs in the early stage of fruit ripening [17]. (2) The downregulation of enzymatic synthesis results in a decline in the sterol accumulation at the end of ripening [16]. (3) Sterols become more diluted as the oil content of the fruit increases with ripening [17]. (4) Existed sterols may be converted to steroidal hormones, and vitamins, which regulate the growth and development of immature tissues [16].

4. CONCLUSION

Based on these results, it can be concluded that variety and ripening impact on the fatty acid and phytosterol composition of Walnut (Juglans *regia* L.). The higher level of fatty acid was detected at the 14th DAFD for Franquette and 21st DAFD for Local gd. 4-desmethylsterols, the higher level was detected at the 7th DAFD for Franquette and 14th DAFD for

Local gd. an immature walnut was a good source of 4-desmethylsterols just as a mature walnut. Therefore, immature walnut may be proposed as a source of phytosterols and the essential linoleic fatty acid for functional food and nutraceutical applications.

Acknowledgements
This work is part of a National Research Project. We thank the Ministry of Higher Education and Scientific Research of Tunisia for financially supporting this investigation. Part of this work was carried out at "Hospital del Mar Research Institute", Human Pharmacology and Clinical Neurosciences Research Group, 08003 Barcelona, Spain.

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Enterprise Europe Network (EEN)

Anno 2025

(aggiornato al 31 marzo 2025)

A German research institute specialized in nutrition food technology and bioeconomy offers opportunities for product and process development
Ref. TODE20241004015
The german research institute is specialized in the development, analytics and production in the field's nutrition, food science and biotechnology. It wants to support and help companies that have a need for product development or process optimization, but do not have the necessary technical, analytical or human resources. In addition to participating in research projects, collaboration is also sought in the form of commercial agreements with technical support.
Deadline 16 Oct 2025

French company seeks solution for eliminating bacteria from high-quality vanilla (beans or powder)
Ref. TRFR20241007025
A French SME specialises in importing vanilla and spices from Madagascar. It is looking for equipment to guarantee products 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 a partner capable of providing food processing equipment dedicated to eliminating pathogenic microflora and with a sterile outlet for vacuum packaging
Deadline 7 Oct 2025

Portuguese biotech company that developed an EU-approved mitochondria-targeted antioxidant active ingredient for cosmetics is seeking licensing or B2B supply partnership with established B2C cosmetic companies
Ref. BOPT20240930016
A Portuguese based biotech company launched an EU-approved mitochondria-targeted antioxidant active ingredient for cosmetics. The company is actively seeking licensing agreements or B2B supply partnerships with well-established B2C cosmetic companies focused in the European, USA and Brazilian markets. The company's unique mitochondrial targeting enhances skin protection against oxidative stress from internal and external sources.
Deadline 7 Oct 2025

Chilean granel shop located in Ñuñoa is looking for technologies in europe to introduce Circular Economy in their process.
Ref. TRCL20241018053
Chilean granel shop located in Ñuñoa is looking for technologies in europe to introduce Circular Economy in their process. They participate in a pilot project with the Municipality and the Chamber of Tourism of the neighborhood and anothers restaurants and bars with the aim to implement the management of organic wastes.
In this framework they are looking for technologies in Europe to use and implement in their process
18 October 2025

Transforming Resilient Grains into Nutritious Foods through Precision Fermentation
Ref. RDRTR20241204013
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.
Deadline 4 Dec 2025

Danish food company seeks suppliers of alternative proteins and ingredients and food production facilities to optimise their healthy food products
Ref. BRDK20241220015
A fast-growing women-led food company from Denmark sells their healthy alternative protein products to the catering and food service sector across the country.
To consolidate their market position, they are looking for suppliers of alternative proteins and ingredients, which can be integrated into new recipes and products, as well as contract production facilities, to scale up their production volumes.
Deadline 20 December 2025

Swiss company seeks food-side-streams suppliers to turn waste into innovative biopolymers
Ref. TRCH20250123005
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.
Deadline 23 Jan 2026

A Polish company that produces plant-based alternatives to meat and fish is looking for wholesalers and distributors.
Ref. BOPL20250129015
The Polish company is looking for wholesalers and distributors who will help us introduce our products to their Real and Food Service markets.
It also has white label products, and it has a wide product portfolio.
Deadline 29 January 2026

Caffeine alternative ingredient for Food & Beverage and Nutraceutical products
Ref. TOIT20250113016
A food-tech startup on a mission to awaken the planet one ingredient at a time. Koncentra is a healthy and sustainable caffeine alternative for food and beverage and nutraceutical products.
It offers similar beneficial effects of caffeine, like energy and focus, but avoids the typical side effects such as tachycardia, sleep disruption, cardiovascular and gastrointestinal pathologies.
It is looking for commercial partnerships with technical assistance, Investment Agreements and R&D cooperation.
Deadline 13 January 2026

Ukrainian enterprise offers deoiled sunflower lecithin powder under distribution services agreement.
Ref. BOUA20250109015
Ukrainian producer offers pure deoiled sunflower lecitin powder (food additive E-322) with the content of phospholipids min.97%.
Product of food and dietary grade is available. In food industry lecitin is used as an emulsifier and antioxidant.
Lecitin as a diet additive is used in food rations of people as an additional source of phospholipids. Producer is looking for long-term sales partners under distribution services agreement. Manufacturing under private label for retail chains is possible.
Deadline 9 January 2026

A Greek biotechnology company offering advanced DNA-based olive oil traceability solutions seeks partners in the extra virgin olive oil (EVOO) market under commercial agreements.
Ref. BOGR20250219021
A Greek biotechnology company specializing in DNA-based authentication and traceability solutions offers innovative services to ensure the integrity and origin of extra virgin olive oil (EVOO).
The company seeks international partners, including olive oil producers, exporters/importers, and supermarket chains producing premium EVOO private labels, under commercial agreements.
Deadline 19 February 2026

Romanian cluster organisation is looking for partners interested to join on a project funded under Promotion of Agricultural Products (AGRIP) – Call for proposals for multi programmes 2025 – Promotion of agricultural products (AGRIP-MULTI-2025)
Ref. TORO20250213013
Romanian cluster organisation is looking for a consortium leader/partners organisations interested to apply with a project funded under the AGRIP-MULTI-2025 call, to increase the awareness of the Union sustainable agriculture practices beneficial for the climate, the environment and animal welfare by the European consumers and to enhance the competitiveness and consumption of sustainably produced agri-food products.
Deadline 13 February 2026

AI-Driven Optical Technology for Enhancing Cereal Quality and Milling Efficiency – Seeking Industry and Research Partners for Horizon Europe Collaboration
Refg. TOPT20250207007
Seedsight, a Germany- and Portugal-based deep-tech startup, specializes in AI-driven optical technology for cereal quality assessment and milling optimization.
Their patented solution integrates artificial intelligence with advanced optics to enhance flour extraction yields, reduce waste, and improve food security, by enabling rapid, cost-effective grain analysis, that help millers, traders, and food processors boost efficiency and sustainabilit,
They seek industry and research partners for a Horizon.
Deadline 7 February 2026

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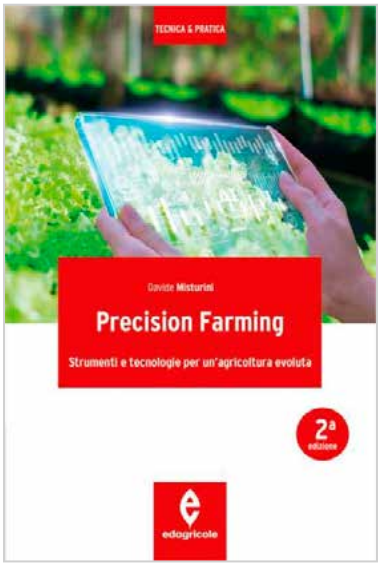

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Pagine 564 - formato 19.5 x 26 cm
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Introduzione - Il quadro applicativo dell'Agricoltura di Precisione: dall'automazione ai sistemi informativi aziendali e alla robotica - La gestione della variabilità spaziale e temporale nell'agricoltura di precisione: introduzione alla geostatistica e cenni di machine learning - Il telerilevamento in agricoltura di precisione - Sensori e metodi per rilievi prossimali delle proprietà del suolo e della coltura - Piattaforme a controllo remoto e robotiche per il monitoraggio e la gestione delle colture - Sistemi di posizionamento globale e sistemi di guida delle macchine agricole - I sistemi di mappatura delle produzioni - Modelli di simulazione in agricoltura di precisione - Machine Learning in Agricoltura di Precisione - Lavorazioni variabili del terreno e semina a dose variabile - La fertilizzazione di precisione - Irrigazione di precisione - Trattamenti fitosanitari in agricoltura di precisione - Applicazioni ai sistemi colturali erbacei ed ortivi di pieno campo - Applicazioni alla viticoltura ed altre colture arboree - Valutazione economica dell'agricoltura di precisione.

CURATORI:
Raffaele Casa, Professore Ordinario di Agronomia presso il Dipartimento di Scienze Agrarie e Forestali (DAFNE) dell'Università degli Studi della Tuscia. Da oltre 20 anni si occupa di ricerca in agricoltura di precisione in progetti nazionali ed internazionali. Ha partecipato alla redazione del "Piano Nazionale per lo sviluppo dell'Agricoltura di Precisione" del Masaf. È stato membro del Mission Advisory Group del satellite Sentinel-2 dell'Agenzia Spaziale
Europea. È stato membro del comitato di supporto tecnico-scientifico all'Agenzia Spaziale Italiana e del Consiglio Direttivo della Società Italiana di Agronomia. È stato direttore del Master in Agricoltura di Precisione organizzato da una rete di atenei (Tuscia, Padova, Teramo, Firenze, Salerno), CNR e CREA.

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CONGRESSI

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. The 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/

Palmex Indonesia 2025

14 - 15 May | Jakarta, Indonesia

15th Edition of PALMEX Indonesia 2025 will be held at Jakarta International Expo (JIEXPO), Kemayoran. Their world-class, state-of-the-art facilities has been selected by a host of international organisers as the venue for their international trade exhibitions, world congresses, and a wide variety of MICE events.

This edition promises to be particularly notable, as it will be hosted at a significantly larger venue, providing ample space for exhibitors, attendees, and innovative showcases. This expanded setting will allow for a more comprehensive presentation of cutting-edge technologies, industry advancements, and networking opportunities, making it an unmissable occasion for professionals and enthusiasts in the palm oil sector.

More information: https://palmex-indo.com/

Sustainable Palm Oil Dialogue 2025

21 May | Roissy-en-France, France

Social Sustainability in Global Supply Chains.

What is the role and responsibility of companies in Europe in creating fair, equitable and sustainable conditions in global supply chains?

As businesses operate across borders, the need to address social issues like labour rights, fair wages, safe working conditions, and community impacts becomes essential. Prioritising these elements not only improves the well-being of workers and related communities but also fosters the resilience of supply chains, strengthens brand reputation, boosts consumer trust, and ensures compliance with global standards and legislations. Integrating social responsibility into supply chains is a fundamental aspect of building a resilient and ethical global economy.

At the Sustainable Palm Oil Dialogue 2025, we will look at what companies are doing to prevent negative social impacts in their supply chain and to ensure they have a positive impact on workers, local communities and Indigenous people; we will look at different legislative frameworks that are being developed for human rights due diligence and we will not only highlight the “why” but also the “how” of inclusive procurement and smallholder inclusion. More information on: https://www.spod-europe.eu/

EFPRA Congress 2025

4 - 7 June | Riga, Latvia

Registration for the next EFPRA Congress in Riga is now open. The 23rd EFPRA Congress will take place from the 4–7 June in Radisson Blue Lavija Conference and Spa Hotel. The programme for the Congress itself is still being arranged, until then, guests can start looking forward to visiting Riga.

Among the main speeches: Circular Feed Platform: A Disruptive Promoter of New Feed Potentials; Potential Use of Rendered Proteins in Feed for Farmed Animals and Alternative Protein Sources; The Changing Landscape of Aquafeed

Composition; Status quo and future outlook of the European leather industry.
Moreover, an intensive program of activities is foreseen.
More information on: https://efpra2025riga.eu/

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 explore 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, manufactures, 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/

Japan Olive Oil Prize

13 June | Tokyo, Japan

Japan Olive Oil Prize is an international contest based in Tokyo, Japan that aims at promoting quality extra-virgin olive oils of all origins.
Since its establishment in 2013, JOOP has been strongly committed to helping producers of high-quality EVOO get exposed to potential commercial partners in Japan, while at the same time educating Japanese consumers on how to choose a product of quality versus a commercial product. We are proud that JOOP has grown to be recognized as the most professional EVOO competition in Japan, which is the leading country in Asia for the consumption of EVOO.
JOOP is the best tool to promote your EVOO and brand in the Japanese market.
How to register to japan olive oil prize 2025:

1 Sign in to JOOP's website and create your personal account.
2 Register your EVOO(s) and complete the payment no later than May 30, 2025.
3 After you receive the confirmation email, it's time to send us the oil samples!
The JOOP prize will be awarded on a point-based system between 75 (minimum) and 100 (maximum), in categories including Organic, Blend, Monovarietal, P.D.O., P.G.I., and Flavored.
Oils will be competing in the following categories: Organic, Blend, Monocultivar, P.D.O. and P.G.I. Oils with the highest score from each Country will be awarded "Best of Country" and oils with distinctive qualities in High Polyphenols Content and Flavored will be awarded with Certificate of Excellence.
More information on: https://jooprize.com/

Argus Biofuels & Feedstocks Latin America Conference

25 – 26 June | São Paulo, Brazil

Creating a new commercial environment for biofuels growth across Latin America
The Argus Biofuels & Feedstocks Latin America Conference returns to São Paulo, Brazil on 24-26 June 2025. This is your opportunity to dive into a new regulatory environment, understand what this means for biofuels growth and forge new business relationships with stakeholders in the region.
This Latin American flagship event brings together 300+ senior representatives from 20+ countries across 3 days. Explore feedstock developments, pricing, investments in new builds, infrastructure to aid feedstock and biofuels trade, developments in SAF, linkages with the carbon industry, and much more.
Agenda highlights:

- Explore Fuel of the Future legislation and new incentives that will drive biofuel production
- Learn about new SAF, HVO and ethanol opportunities domestically and globally
- Get to grips of new infrastructure developments, at Ports and storage to aid feedstock and biofuels trade
- The ultimate Latin American networking experience – build your contact list and do business

More information on:
https://www.argusmedia.com/en/events/conferences/latin-america-biofuels

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.
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 Cardiometabolic 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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La
Rivista Italiana
delle
Sostanze Grasse

Via Giuseppe Colombo, 79
20133 MILANO

Editorial office:
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