



Influence of environmental factors on α -tocopherol levels in *Moringa oleifera* extracts

Júlia C. Kessler^{a,b,c,*}, Yaidelin A. Manrique^{a,b}, Isabel M. Martins^{a,b}, António Ribeiro^c,
Alírio E. Rodrigues^{a,b}, Maria Filomena Barreiro^{c,**}, Madalena M. Dias^{a,b}

^a LSRE-LCM - Laboratory of Separation and Reaction Engineering–Laboratory of Catalysis and Materials Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, Porto 4200-465, Portugal

^b ALiCE - Associate Laboratory in Chemical Engineering Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, Porto 4200-465, Portugal

^c CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança 5300-253, Portugal

ARTICLE INFO

Keywords:

Moringa oleifera

Supercritical fluid extraction (SFE-CO₂)

Extract

Bioactive compounds

Environmental effects

ABSTRACT

Moringa oleifera Lam. (Mo) seed and leaf extracts have shown promise as innovative cosmetic ingredients. First, this study examines extraction yield and chemical composition, using four techniques: supercritical carbon dioxide fluid extraction (SFE-CO₂), SFE-CO₂ with ethanol (EtOH), Soxhlet (SOX), and maceration (MAC). Among these, SFE-CO₂-EtOH achieved the highest yields, while SFE-CO₂ was most effective for extracting α -tocopherol from leaves and oleic and behenic acids from seeds, compounds valued for their antioxidant and emollient properties in cosmetics. Additionally, to assess the environmental impact on the plants and its influence on extraction outcomes, SFE-CO₂ leaf extracts were analysed across three harvest years. The α -tocopherol content (expressed relative to the mass of extract) showed considerable variability: approximately 148 mg•g⁻¹ in 2021, 30.2 mg•g⁻¹ in 2022, and 232.5 mg•g⁻¹ in 2023. These fluctuations correlate with precipitation levels, as excessive rainfall in 2022 lowered yields, while low rainfall, combined with high humidity and mild temperatures in 2021 and 2023 promoted higher yields. Principal Component Analysis (PCA) identified precipitation and humidity as major factors influencing extraction yield and compound selectivity, while average temperatures remained stable. Overall, this study highlights the significant influence of environmental factors on the quality of Mo extracts and underscores the necessity of standardisation to achieve consistent levels of bioactive compounds. Implementing strategies such as controlled cultivation practices or use of standardised compounds is essential to ensure the production of extracts that meet the safety and efficacy standards required for cosmetic and pharmaceutical applications.

1. Introduction

The growing commercial competitiveness of cosmetic products has driven industrial interest in bioactives derived from natural sources. A key focus has been the replacement of synthetic ingredients with natural alternatives, including antioxidants, anti-inflammatories, antimicrobials, as well as anti-ageing agents (Zorić et al., 2022). Among these, α -tocopherol is recognised as a powerful antioxidant that reduces oxidative stress in the skin, helping to counteract the effects of photoaging (Athikomkulchai et al., 2021a). Recent studies have identified α -tocopherol in the leaves of *Moringa oleifera* Lam. (Mo) leaves (Kessler

et al., 2023). Known as the “Tree of life”, Mo presents a rich phytochemical composition and bioactive potential, extensively studied by the scientific community and increasingly used as an ingredient in cosmetic formulations (Nizioł-Lukaszewska et al., 2020; Zeitoun et al., 2020; Athikomkulchai et al., 2021b; Isnaini et al., 2023). Additionally, Mo seeds are a source of behenic and oleic acids, fatty acids that enhance spreadability in cosmetic creams and offer skin-hydrating benefits and activity against photostressor agents (Athikomkulchai et al., 2021a).

The extraction of bioactive compounds from natural matrices must be conducted to maximise efficiency, improve selectivity, and preserve the compounds’ natural properties. Supercritical fluid extraction by

* Corresponding author at: LSRE-LCM - Laboratory of Separation and Reaction Engineering–Laboratory of Catalysis and Materials Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, Porto 4200-465, Portugal.

** Corresponding author.

E-mail addresses: juliakessler@fe.up.pt (J.C. Kessler), barreiro@ipb.pt (M.F. Barreiro).

<https://doi.org/10.1016/j.indcrop.2025.120906>

Received 17 November 2024; Received in revised form 3 March 2025; Accepted 19 March 2025

Available online 25 March 2025

0926-6690/© 2025 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

carbon dioxide (SFE-CO₂) is recognised as a technology that complies with these requirements and is environmentally friendly. A recent review on Mo SFE-CO₂ extraction has emphasised its advantages, particularly its potential for selective extraction of target compounds for cosmetic applications (Kessler et al., 2024). The supercritical solvent, CO₂, is non-toxic, non-flammable, odourless, and leaves no residue in the final product (Zhao and Zhang, 2013). The extract's selectivity is adjustable by tuning the pressure and temperature, and the total extraction yield can be increased by adding a co-solvent, such as ethanol (EtOH). Ethanol enhances the affinity towards the polar compounds and facilitates CO₂ penetration by swelling the cellular structure (Nguyen et al., 2011). Products obtained through SFE-CO₂ have shown favourable results compared to extracts obtained by conventional methodologies such as maceration (MAC) and Soxhlet (SOX) (Nguyen et al., 2011; Da Porto et al., 2016; Ruttarattanamongkol et al., 2014).

In addition to the extraction methodology, the inherent composition of the plant can be influenced by climate and geographic conditions (Vázquez-León et al., 2017). The biological synthesis of bioactive compounds follows a complex pathway that can impact their commercial availability. Plants require antioxidative protection when facing environmental stressors, including intense light, extreme temperatures, salinity, metal toxicity, and, most notably, water deficit (Muñoz and Munné-Bosch, 2019; Niu et al., 2022). For instance, α -tocopherol and unsaturated fatty acids play a crucial role in plant growth and development, particularly in providing photo-oxidative protection (De Filipis, 2016; Iqbal and Bhangar, 2006).

Building upon previous optimisations of Mo leaf and seed extractions using SFE-CO₂ without a co-solvent (Kessler et al., 2023), this study aims to determine the optimal extraction methodology for enhancing the extraction of α -tocopherol from Mo leaves and behenic and oleic acids from Mo seeds. The research highlights the importance and need to identify plant materials rich in α -tocopherol, behenic, and oleic acids that can be exploited at an industrial scale. The extractions of α -tocopherol are used as a case study to assess the viability of incorporating natural ingredients into commercial cosmetic formulations. This work underscores the potential of Mo as a sustainable source of bioactive compounds for cosmetic applications and examines how environmental factors over three years influence the chemical composition of leaf extracts. Understanding these dynamics is crucial for developing effective and sustainable cosmetic formulations. By prioritising natural ingredients, this study aligns with the increasing consumer demand for safer, eco-friendly alternatives while ensuring high standards of efficacy and performance in cosmetic products.

2. Material and methods

2.1. Chemicals

Carbon dioxide (CAS 124–38–9, food grade, <99 %) was acquired from Air Liquid (Paris, France); *n*-hexane (CAS 110–54–3, 99 %), ethanol absolute (EtOH, CAS 64–578–6, \geq 99 %), methanol (200–659–6), and potassium hydroxide (KOH, CAS 1310–58–3) were obtained from Supelco (Madrid, Spain). FAME C_{34:0} (CAS 97–66–3) analytical standard and α -tocopherol (CAS 10191–41–0, 99 %) were purchased from Sigma Aldrich (Madrid, Spain); *n*-heptane (CAS 142–82–5, 99 %) and sulphate anhydrous (CAS 7757–82–6) were acquired from Carlo Erba Reagents (Milan, Italy), and methyl heptadecanoate (CAS 1731–92–6, 97 %) was obtained from Tokyo Chemical Industry (Tokyo, Japan).

2.2. Plant material

Mo aerial parts were provided by Moringa del Sur (Malaga, Spain, moringadelsur.com) and collected in May 2021, 2022, and 2023. To ensure comparability of results, harvesting was conducted in the same area and during the same period each year over the three-year study. All

results refer to samples obtained in 2021, except when the year is otherwise specified.

Leaf and seed samples were prepared following the protocol previously described (Kessler et al., 2023). Briefly, Mo plant materials were acquired already dried at room temperature, ground for 20 seconds (Hr7762/90 Mini Chopper, Philips Walita, Amsterdam, The Netherlands) and sieved (D-42781, Retsch, Haan, Germany) to achieve particle size between 0.50 and 0.70 mm.

2.3. Obtaining Mo extracts

Mo samples were submitted to three different extraction methodologies to evaluate their efficiency in obtaining α -tocopherol from leaves and behenic and oleic acids from seeds.

All the obtained extracts were dried at 40 °C, first using a rotary vacuum evaporator (RE100B, Bibby Scientific Ltd., Stone, UK), and then removing the residual solvent in an air-drying process (Venticell, MMM Medcenter, Planegg, Germany) until reaching a constant weight. The extraction yield was calculated in dry weight, given by the mass ratio of extract obtained and plant material used in each extraction, expressed in percentage (Kessler et al., 2022). The seed and leaf extracts were stored at –20 °C after being dissolved in 25 mL of *n*-hexane and ethanol, respectively, until further analyses.

2.3.1. SFE-CO₂+EtOH of Mo seeds and leaves

The bioactive compounds of Mo seeds and leaves were obtained using SFE-CO₂ with EtOH as a co-solvent to improve the extraction performance. A previously described bench-scale equipment (Kessler et al., 2023) operated individually under optimised pressure and temperature conditions for each sample. The extractions were performed in triplicate at 250 bar and 45 °C for seeds and 195 bar and 55 °C for leaves, using 10 g of sample per extraction. Both EtOH co-solvent and supercritical CO₂ were introduced simultaneously at a continuous flow rate of 0.8 mL•min⁻¹ and 3.2 mL•min⁻¹, respectively, over a 2-hour period. The extract was recovered by removing the extraction cell and flushing the apparatus with pressurised EtOH and CO₂ for 15 minutes.

2.3.2. Soxhlet extraction of Mo seeds

Mo seeds were submitted to intermittent extraction in a 250 mL Soxhlet apparatus (SOX) using solvents with different polarities: EtOH (logP=0.8) and *n*-hexane (logP=3.76). Each extraction was performed in duplicate with a ratio of 1:5 raw material:solvent (m/v), for 4 hours, resulting in 14 cycles for EtOH and 34 for *n*-hexane.

2.3.3. Maceration extraction of Mo leaves

Mo leaves were extracted by maceration (MAC) using a 250 mL stock flask filled with EtOH or *n*-hexane with a ratio of 1:5 (m/v). The extractions were performed in duplicate for 4 hours, at room temperature, under magnetic stirring (Agimatic-N, J.P. Selecta, Barcelona) at 240 rpm.

2.4. Chemical composition of Mo extracts

The chemical composition of the obtained extracts and the quantification of their respective target compounds were assessed using a gas chromatography-flame ionization detector (GC-FID) (Nexis GC-2030, Shimadzu, Japan) and a gas chromatography-mass spectrometer (GC-MS) (TQ8040 NX Triple Quadrupole, Shimadzu, Japan) for seed and leaf extracts, respectively.

2.4.1. FAME analysis of Mo seed

The lipidic fraction of Mo seed extracts was transesterified using the methodology outlined by Cristofori et al (Cristofori et al., 2008). Briefly, 0.1 mL of seed extract diluted at 5 mg•mL⁻¹ in *n*-hexane (0.005 mg of extract) was converted in methyl esters by mixing 0.1 mL of KOH at 1 M in methanol, submitted to vortex stirring for 3 min, and added to 0.3 mL

of *n*-hexane to dilute and stop the reaction plus 0.1 g of anhydrous sodium sulphate to remove any moisture from the samples.

The transesterified samples were analysed based on the European Standard EN14103/2020 procedure (European Committee for Standardization, 2020); 90 μL of the supernatant was added to 100 μL of a 0.05 $\text{mg}\cdot\text{mL}^{-1}$ methyl heptadecanoate (C_{17}) solution previously prepared in heptane. Finally, 1 μL of the mixture was analysed using a GC-FID equipped with a splitless injector, an autoinjector (AOC-20i), and a capillary column (30 $\text{m}\times 0.25$ mm, 0.23 μm film thickness) for FAMES (OPTIMA BioDiesel F, Macherey-Nagel, Düren, Germany).

The injector and detector temperatures were set at 250 $^{\circ}\text{C}$, using a split injector 1:20 at 100 kPa for 1 min. The oven was initially placed at 50 $^{\circ}\text{C}$ for 1 min, increased from 50 to 200 $^{\circ}\text{C}$ at 25 $^{\circ}\text{C}\cdot\text{min}^{-1}$, and then established at 230 $^{\circ}\text{C}$ for 18 min at 3 $^{\circ}\text{C}\cdot\text{min}^{-1}$.

The retention time of the compounds was compared to those obtained from the FAME standard and analysed under similar conditions.

2.4.2. Volatile composition of Mo leaves

Mo leaves volatile compounds were assessed using a GC-MS equipped with a splitless injector, an automatic sampler (AOC-20s+i), and a cross-bonded fused column (30 $\text{m}\times 0.25$ mm, 0.25 μm film thickness) for low-polarity phases (Rxi-5Sil MS, Restek, Bellefonte, USA). The analysis procedure was previously optimised (Kessler et al., 2023), in which 1 μL of a known concentration of each sample diluted in EtOH was injected at 280 $^{\circ}\text{C}$. The equipment operated in linear velocity flow control mode using ultrapure helium at 1 $\text{mL}\cdot\text{min}^{-1}$. The mass scanning was established at m/z 40–500, and the ion and interface temperatures were set at 250 $^{\circ}\text{C}$ and 260 $^{\circ}\text{C}$, respectively.

The oven worked isothermally at 40 $^{\circ}\text{C}$ for 1 min, raised to 200 $^{\circ}\text{C}$ for 2 min at a rate of 7 $^{\circ}\text{C}\cdot\text{min}^{-1}$, then increased to 250 $^{\circ}\text{C}$ for 2 min at 15 $^{\circ}\text{C}\cdot\text{min}^{-1}$, and finally kept at 280 $^{\circ}\text{C}$ for 1 min at 20 $^{\circ}\text{C}\cdot\text{min}^{-1}$.

The volatiles were identified by comparing the mass spectra of the individual molecule with those available in the database software from the National Institute of Standards and Technology (NIST 21, 27, 107, 147). The respective linear retention time (LRI) was calculated as previously described (Kessler et al., 2022), while the mass content of α -tocopherol was obtained through its calibration curve ($y = 1.04 \times 10^{10}x - 7.87 \times 10^6$, $R^2 = 0.9996$).

2.5. Statistical analysis

The statistical significance of the extraction yield, relative concentration, and weather forecast data over the harvest seasons were calculated using ANOVA and Tukey tests, with $\alpha = 0.05$ (significance level). The relationship between the selectivity (relative concentration, %), mass content of α -tocopherol (mg of α -tocopherol $\cdot\text{g}$ of extract $^{-1}$), season, temperature ($^{\circ}\text{C}$), humidity (%), and precipitation ($\text{L}\cdot\text{m}^{-2}$) were evaluated through the Principal Component Analysis (PCA) using the multivariate exploratory technique (Statistica StatSoft, version 14, USA) after data range standardising.

3. Results

3.1. Extraction yield of Mo seed and leaf extracts

Fig. 1 shows the extraction yields of Mo seed (1a) and leaf (1b) obtained by supercritical extraction (SFE- CO_2 and SFE- CO_2 +EtOH), Soxhlet extraction (SOX-EtOH and SOX-Hex), and maceration (MAC-EtOH and MAC-Hex).

Extraction using SFE- CO_2 +EtOH demonstrated significantly higher efficiencies compared to SFE- CO_2 without a co-solvent ($\alpha = 0.05$). For seeds, SFE- CO_2 +EtOH reached a yield of 21.10 ± 0.33 %, approximately six times higher than the 3.38 ± 0.05 % achieved with SFE- CO_2 alone. Similarly, leaf extracts using SFE- CO_2 +EtOH yielded 9.59 ± 0.17 %, about seven times greater than the 1.30 ± 0.04 % obtained with SFE- CO_2 alone.

The trends observed in these results are consistent with previous studies, which reported yields of 31.90 ± 0.51 % for seeds and 2.9 ± 0.7 % for leaves using SFE- CO_2 +EtOH, compared to 28.71 ± 0.67 % for seeds and 1.6 ± 0.4 % for leaves with SFE- CO_2 alone (Nguyen et al., 2011; da Silva et al., 2022). While the overall trends align, the variation in the reported extraction yields values can be attributed to differences in operational parameters, such as pressure, temperature, and extraction time, which significantly influence the solubility and mass transfer rates of bioactive compounds. Additionally, geographic and climatic factors, including soil composition, precipitation, and temperature, impact the phytochemical composition and abundance in Mo. Discrepancies in plant material preparation, including drying methods, particle size, and storage conditions, can also contribute to the variability in the reported extraction results.

The addition of 20 % EtOH as a co-solvent to supercritical CO_2 significantly enhanced extraction efficiency by increasing the solubility of polar compounds, explaining the marked improvement in seed and leaf yields compared to SFE- CO_2 alone. The co-solvent increases the polar affinity of compounds obtained from the raw material, often boosting the total extraction yield and modifying the selectivity of target compounds (Zorić et al., 2022). These effects reflect the polar and nonpolar characteristics of the solvents, similar to conventional extractions. These effects align with the solvent characteristics observed in conventional extractions, where polar and nonpolar properties determine compound solubility and extraction efficiency.

For SOX and MAC methodologies, the extraction yields also showed statistically significant differences depending on the solvents used. Seed extracts showed no significant differences in yields between EtOH (36.98 ± 0.51 %) or *n*-hexane (37.60 ± 1.15 %), both of which were significantly higher than those achieved with SFE- CO_2 ($\alpha = 0.05$). Previous studies reported a yield of 36.44 ± 2.97 % using SOX-Hex extraction for 8 hours (Da Porto et al., 2016), twice the time used in this study.

In contrast, leaf extraction presented a higher affinity to MAC-EtOH ($\alpha = 0.05$), with 13.80 ± 0.22 % extraction yield compared to 3.75 ± 0.09 % obtained with MAC-Hex. However, the yields obtained with MAC-EtOH were statistically lower than those achieved with SFE-

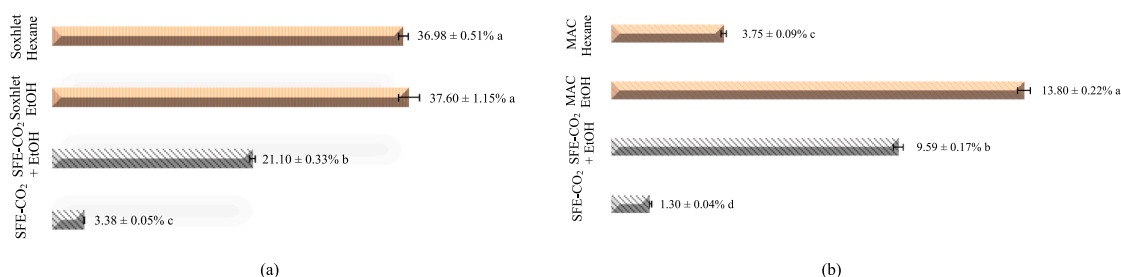


Fig. 1. Extraction yield of Mo (a) seed and (b) leaf obtained by supercritical extraction (SFE- CO_2 and SFE- CO_2 +EtOH); Soxhlet extraction (SOX-EtOH and SOX-Hex) and maceration (MAC-EtOH and MAC-Hex). Averages with different letters in the same column indicate significant differences with $\alpha = 0.05$.

CO₂+EtOH.

3.2. FAME composition of Mo seed extracts

Extracts from Mo seeds obtained through SFE-CO₂ and Soxhlet extractions underwent fatty acid methyl ester (FAME) identification. Table 1 shows the relative concentrations of the identified compounds, reflecting the selectivity achieved in each extraction method. Oleic acid is the predominant compound regardless of the extraction method, with selectivity reaching up to 84 % for SOX-Hex, SOX-EtOH, and SFE-CO₂, higher than the 82 % observed for SFE-CO₂+EtOH. This result highlights the impact of solvent polarity and extraction method on compound selectivity. The apolar nature of oleic acid aligns well with SOX-Hex and SFE-CO₂, resulting in high selectivity. However, the inclusion of ethanol in SFE-CO₂+EtOH reduced selectivity due to its mixed polarity, which facilitates interactions with both polar and nonpolar compounds, thereby decreasing the preferential extraction of oleic acid. In contrast, the extended solvent contact time in SOX extractions enhanced ethanol's ability to extract oleic acid. This effect is attributed to ethanol's amphiphilic nature, enabling interactions with both the hydrophilic carboxyl group and the hydrophobic tail of oleic acid, thereby improving its extraction efficiency.

Behenic acid was identified as the second most abundant compound, with a significantly higher relative concentration in the SFE-CO₂+EtOH extract (7.76 ± 0.21 %) compared to conventional methods, specifically SOX-EtOH (5.99 ± 0.22 %) and SOX-hex (5.16 ± 0.19 %). SFE-CO₂ alone yielded 6.90 ± 0.09 %, a selectivity statistically equal to all other samples. These values align with those reported in the literature (Bhutada et al., 2016; Kleiman et al., 2008).

Oleic and behenic acids are recognised as signature compounds of Mo seed extracts, previously reported at 77.63 % and 5.25 % using SOX-Hex, and 77.06 % and 5.33 % using SFE-CO₂ alone at 300 bar and 40 °C (Da Porto et al., 2016). These fatty acids exhibit emollient properties and high oxidative stability, highly desirable in cosmetic formulations (Kleiman et al., 2008).

In addition to oleic and behenic acids, Mo seed extracts contained minor amounts of lignoceric acid (4.51–6.41 %), arachidic acid (3.02–3.60 %), palmitic acid (none to 0.70 %), and myristic acid (0.12–0.16 %), contributing to their overall chemical profile.

3.3. Volatile profile of Mo leaf extracts

The volatile profile of Mo leaf extracts was assessed by GC-MS and revealed the presence of eight different chemical groups, shown in Table 2. The most significant compounds and their activities on cosmetic formulations were described in the previous study on screening of Mo plant parts (Kessler et al., 2023).

Table 3 compares the selectivity and mass content of α-tocopherol for the SFE-CO₂, SFE-CO₂+EtOH, and MAC extractions from samples collected in 2021, and the mass content of α-tocopherol obtained using SFE-CO₂ in 2022 and 2023.

A significantly higher relative concentration was obtained using SFE-CO₂ (21.51 ± 0.79 %), followed by MAC-EtOH (10.64 ± 0.22 %), MAC-

Hex (8.43 ± 0.52 %), and finally SFE-CO₂+EtOH (5.67 ± 0.09 %). The mass content of α-tocopherol in the total extract composition, measured in mg of α-tocopherol per g of extract and hereby abbreviated to mg•g⁻¹, showed a similar trend. SFE-CO₂ achieved the highest value of 148.0 ± 4.9 mg•g⁻¹, significantly surpassing all other extraction methods. In comparison, MAC-EtOH yielded 80.4 ± 4.3 mg•g⁻¹, MAC-Hex 34.1 ± 0.5 mg•g⁻¹, and SFE-CO₂+EtOH 26.5 ± 0.3 mg•g⁻¹. These results focus on optimising the selectivity of the extraction towards target compounds for cosmetic applications, without considering the extraction yield.

However, additional information can be obtained by analysing the balance between selectivity and extraction yield. Despite its lower yield, SFE-CO₂ extraction is shown to be the most effective method for obtaining extracts with higher relative concentration in α-tocopherol. In contrast, while the conventional method utilising the polar solvent (MAC-EtOH) resulted in the highest yield, MAC-EtOH product represents 15 % less selectivity in terms of α-tocopherol compared to SFE-CO₂ extraction alone. These differences can be explained by the extraction of different compounds with higher affinity to EtOH (da Silva et al., 2022), as well as the increased surface contact and time between the sample structure and the solvent due to the solid-liquid interaction, improving solvent accessibility and mass diffusion (Kessler et al., 2024; da Silva et al., 2022).

The main factor influencing the compounds' solubility in the CO₂ solvent may be related to its density. At the extraction conditions used in this study, 195 bar and 55 °C, the supercritical CO₂ presents 747 kg•m⁻³ (National Institute of Standards and Technology NIST, 2022), easily increased by adding EtOH. The co-solvent enhances the solvation power of the mixture due to its higher density (789 kg•m⁻³) and, therefore, improves the solubility of more compounds, reducing the extract's selectivity towards α-tocopherol.

3.4. Optimised leaves extraction over three years

Table 3 shows the mass content of α-tocopherol extracted by SFE-CO₂ from Mo leaves obtained during the harvest season (May) of 2021, 2022, and 2023. The respective extracts' chromatograms are presented in Fig. 2, where apparent differences in the peak intensity of each compound can be observed, namely in the α-tocopherol peaks.

Significant differences were observed across the three years, despite the raw material originating from the same cultivar and undergoing identical drying and extraction processes. The quantity of α-tocopherol obtained per gram of extract was 148.0 ± 4.9 mg•g⁻¹, 30.2 ± 0.4 mg•g⁻¹, and 232.5 ± 2.3 mg•g⁻¹ in the years 2021, 2022, and 2023, respectively, with relative concentrations ranging from 21.51 ± 0.79 % in 2021–2.76 ± 0.08 % in 2022.

The lower values of α-tocopherol in the samples collected in 2022 may be attributed to environmental factors. The plants were harvested in Malaga, the southeastern region of Spain, typically known for its subtropical Mediterranean climate characterised by mild winters and hot summers with low precipitation. Weather forecast data from the Spanish state meteorological agency, AEMET - Agencia Estatal de Meteorología (Malaga weather station, 2024), show the historical

Table 1

Relative concentration of transesterified compounds from Mo seed extracts obtained using different methodologies and analysed by GC-FID.

Compound (order of detection)	RT FAME (min)	RT (min)	Relative concentration (%)			
			SFE-CO ₂	SFE-CO ₂ +EtOH	SOX-EtOH	SOX-Hex
Myristic acid methyl ester (1)	11.258	11.270	0.159 ± 0.002 ^a	0.150 ± 0.002 ^{ab}	-	0.12 ± 0.03 ^b
Palmitic acid methyl ester (2)	11.788	11.771	0.70 ± 0.03 ^a	0.64 ± 0.02 ^a	-	0.63 ± 0.08 ^a
Oleic acid methyl ester, Elaidic acid methyl ester (3)	16.697	16.997	84.70 ± 0.20 ^a	81.71 ± 0.07 ^b	84.58 ± 0.84 ^a	84.09 ± 6.41 ^a
Arachidic acid methyl ester (4)	20.712	20.755	3.02 ± 0.10 ^b	3.50 ± 0.06 ^a	3.37 ± 0.08 ^{ab}	3.60 ± 0.15 ^a
Behenic acid methyl ester (5)	22.494	22.872	6.90 ± 0.09 ^{ab}	7.76 ± 0.21 ^a	5.99 ± 0.22 ^{bc}	5.16 ± 0.19 ^c
Lignoceric acid methyl ester (6)	27.199	27.281	4.51 ± 0.04 ^b	6.23 ± 0.01 ^a	6.065 ± 0.004 ^a	6.41 ± 0.10 ^a

Averages with different letters in the same line indicate significant differences with α = 0.05.

Table 2

Relative concentration of compounds from Mo leaf extracts obtained using different methodologies and analysed by GC-MS.

Chemical group	Compound (order of detection)	Base peak	LRI	RT (min)	Relative concentration (%)			
					SFE-CO ₂ (Kessler et al., 2023)	SFE-CO ₂ +EtOH	MAC-EtOH	MAC-Hex
Alcohol	1-Tetracosanol (33)	57, 97, 83	3300	38.071	-	15.02 ± 0.62	7.92 ± 1.84	18.07 ± 0.61
Aldehydes	2,4-Nonadienal (1)	81, 138, 41	1004	7.101	0.13 ± 0.01	-	-	-
	Octanal (2)	43, 44, 56	1016	7.344	0.74 ± 0.06	-	-	-
	Nonanal (6)	57, 41, 43	1107	9.285	1.29 ± 0.22	-	-	-
	<i>cis,cis,cis</i> -7,10,13-Hexadecatrienal (20)	79, 67, 41	2426	29.837	0.71 ± 0.04	-	-	-
	Pentadecanal (27)	82, 57, 43	2838	33.436	0.50 ± 0.02	0.45 ± 0.04	-	-
Fatty acids	Palmitic acid (14)	88, 101, 43	1992	24.560	5.28 ± 0.26	5.28 ± 0.08	10.01 ± 1.93	4.29 ± 0.11
	Linoleic acid (16)	67, 81, 95	2161	27.246	3.28 ± 0.05	tr	tr	tr
	Linolenic acid (17)	79, 95, 67	2167	27.324	18.85 ± 0.05	8.13 ± 0.03	20.76 ± 4.43	9.32 ± 0.22
	Ethyl linolenate (18)	79, 67, 95	2167	27.324	-	-	0.34 ± 0.08	-
	Ethyl pentadecanoate (19)	88, 101, 43	2194	27.669	2.25 ± 0.18	0.71 ± 0.07	0.23 ± 0.02	0.63 ± 0.10
	Arachidic acid (25)	88, 43,101	2795	33.108	0.65 ± 0.03	-	-	-
Flavonoids	5-O-acetyl-thio-octyl-β-L-rhamnofuranoside (23)	43, 71, 189	2552	31.070	-	-	1.26 ± 0.50	-
Hydrocarbons	Dodecane (7)	57, 43, 71	1196	11.172	1.15 ± 0.09	-	-	-
	Tetradecane (8)	57, 43, 71	1395	15.100	1.46 ± 0.11	-	-	-
	8-Hexylpentadecane (22)	57, 71, 43	2500	30.525	3.55 ± 0.14	1.78 ± 0.02	1.27 ± 0.22	1.95 ± 0.30
	Heptacosane (24)	57, 71, 43	2701	32.436	10.04 ± 0.19	7.35 ± 0.40	5.43 ± 0.97	7.59 ± 1.55
	Squalane (26)	57, 71, 85	2800	33.141	0.518 ± 0.003	0.45 ± 0.05	0.22 ± 0.03	0.53 ± 0.18
	Nonacosane (28)	57, 71, 43	2901	33.926	17.74 ± 0.74	15.89 ± 0.56	9.33 ± 0.72	16.65 ± 3.84
Sterols	γ-sitosterol (34)	43, 55, 57	3305	38.145	tr	12.54 ± 0.62	6.44 ± 0.32	15.49 ± 0.02
	Fucosterol (35)	314, 55, 69	3320	38.346	tr	1.40 ± 0.04	2.84 ± 0.46	1.95 ± 0.10
Terpenes	α-pinene (3)	93, 91, 92	1020	7.445	0.136 ± 0.003	-	-	-
	<i>p</i> -cymene (4)	119, 134, 91	1034	7.736	tr	-	-	-
	<i>D</i> -limonene (5)	68, 93, 67	1038	7.824	0.74 ± 0.08	0.19 ± 0.01	0.36 ± 0.04	0.35 ± 0.04
	2-Phytene (9)	70, 71, 57	1824	22.127	-	0.24 ± 0.03	0.63 ± 0.12	0.12 ± 0.01
	<i>cis</i> -Phytol (10)	70, 69, 57	1835	22.290	1.82 ± 0.24	4.48 ± 0.12	10.96 ± 1.75	2.74 ± 0.01
	<i>trans</i> -Phytol (15)	71, 43, 57	2106	26.522	-	1.17 ± 0.11	2.69 ± 0.49	0.63 ± 0.06
	β-amyrone (36)	218, 203, 55	3352	38.765	1.87 ± 0.01	0.82 ± 0.12	1.49 ± 0.23	1.37 ± 0.08
Tocopherols	γ-tocopherol (30)	151, 416, 191	3051	35.289	0.36 ± 0.01	0.28 ± 0.01	0.73 ± 0.16	0.55 ± 0.05
	Vitamin E (α-Tocopherol) (31)	165, 430, 164	3129	36.081	21.51 ± 0.79	5.67 ± 0.09	10.64 ± 0.22	8.43 ± 0.52
Unknown	Unknown (11)		1837	22.312	-	0.59 ± 0.08	1.21 ± 0.25	0.32 ± 0.02
	Unknown (12)		1858	22.617	tr	0.68 ± 0.07	1.71 ± 0.30	0.43 ± 0.07
	Unknown (13)		1876	22.878	0.74 ± 0.05	1.28 ± 0.11	3.08 ± 0.60	0.73 ± 0.20
	Unknown (21)		2450	30.058	-	-	0.44 ± 0.05	-
	Unknown (29)		3042	35.202	1.17 ± 0.08	2.87 ± 0.28	-	-
	Unknown (32)		3245	37.409	3.52 ± 0.07	13.91 ± 1.33	-	7.87 ± 1.54

LRI: linear retention indices calculated through Kovats retention index equation for series of alkanes C8-C10 using a cross-bonded fused column in GC-MS, RT: retention time.

Table 3Selectivity (relative concentration) and mass content of α -tocopherol in Mo leaves obtained by SFE-CO₂ and maceration.

α -tocopherol	SFE-CO ₂			SFE-CO ₂ +EtOH	MAC EtOH	MAC-Hex
	2021	2022	2023		2021	
Selectivity (%)	21.51 \pm 0.79 ^a	2.76 \pm 0.08 ^e	8.75 \pm 0.09 ^{cd}	5.67 \pm 0.09 ^d	10.64 \pm 0.22 ^b	8.43 \pm 0.52 ^{bc}
Mass content (mg _{compound} •g _{extract} ⁻¹)	148.0 \pm 4.9 ^b	30.2 \pm 0.4 ^d	232.5 \pm 2.3 ^a	26.5 \pm 0.3 ^d	34.1 \pm 0.5 ^d	80.4 \pm 4.3 ^c

Calibration curve: α -tocopherol ($y = 1.04 \times 10^{10}x - 7.87 \times 10^6$; $R^2 = 0.9996$; $LOD = 6.18 \times 10^{-4} \text{ g}\cdot\text{L}^{-1}$; $LOQ = 1.87 \times 10^{-3} \text{ g}\cdot\text{L}^{-1}$). Averages with different letters in the same column indicate significant differences with $\alpha = 0.05$.

averages for temperature, precipitation, and humidity from February to May 2021, 2022, and 2023 (Fig. 3). The temperature remained without significant differences over the years, and humidity has shown statistically higher percentages in 2021. However, the most noticeable variation was in precipitation data, where the season of 2022 reached 64.11 L·m⁻², double that of the 2021 and 2023 seasons. This elevated precipitation is likely the primary factor contributing to the lower yield of α -tocopherol in the samples collected in 2022.

α -Tocopherol, the primary form of vitamin E in plants, is predominantly located in leaf chloroplasts, where it is distributed across the chloroplast envelope, thylakoid membranes, and plastoglobuli. A close relationship has been established between changes in plant metabolism, such as the absence of watering, and the production of tocopherol, with multiple studies demonstrating a strong correlation between chlorophyll loss (a key photosynthetic pigment) and increased tocopherol synthesis during these physiological processes (Mesa and Munné-Bosch, 2023; Lushchak and Semchuk, 2012; Sadiq et al., 2019; Munné-Bosch, 2005). α -Tocopherol plays a vital role in photoprotection, stabilising thylakoid membranes in response to environmental stressors like water deficit, high light exposure, extreme temperatures, and oxidative stress (Muñoz and Munné-Bosch, 2019). This compound is particularly significant in alleviating photo-oxidative stress, which is driven by excess excitation energy in chloroplasts, and is regulated by the altered expression of genes involved in relevant pathways (Munné-Bosch, 2005). Beyond protecting plant membranes from lipid peroxidation and reactive oxygen species (ROS), α -tocopherol also serves as a signalling molecule, facilitating the plant's adaptive response to environmental stressors such as drought, one of the leading agricultural challenges (Muñoz and Munné-Bosch, 2019; Niu et al., 2022).

Excessive water intake during the 2022 harvest may have reduced levels of α -tocopherol in the plants, subsequently diminishing the quality of the extracts. Conversely, the lower levels of precipitation recorded in 2021 and 2023 led to higher mass content of α -tocopherol in the extracts, likely improving their overall quality.

Principal Component Analysis (PCA) (Fig. 4) provide insights into the relationship between leaf extract characteristics (selectivity and mass content of α -tocopherol in the total extract composition) and averaged historical weather data, including season, temperature, precipitation, and humidity. Factor 1 and Factor 2 together explain 77.72 % of the total variance, capturing significant correlations. The average temperature, which remained relatively stable from 2021 to 2023, shows little influence on the selectivity for α -tocopherol or its mass content, consistent with the non-significant findings in Fig. 3. A strong correlation exists between precipitation and mass content of α -tocopherol in the third quadrant of both Factor 1 and Factor 2. This observation aligns with the trends shown in Table 3 and Fig. 3, indicating that increased precipitation negatively affects extract composition. Meanwhile, humidity plays a role in affecting selectivity, especially in Factor 2, indicating that atmospheric moisture significantly influences the selectivity of α -tocopherol in the extracts. Overall, the analysis highlights the importance of humidity and precipitation over selectivity and mass content of α -tocopherol, respectively, with minimal effect from the variances in temperature.

Current commercial strategies include the standardisation of plant extracts, a process that ensures consistent quality and efficacy. This approach involves identifying active and marker compounds within the

plant, conducting comprehensive compositional analysis, and employing chemical fingerprinting to characterise the extract (Ong, 2004). Once environmental factors have significantly influenced the availability of the mass content of α -tocopherol in Mo leaves, leading to natural variability in extracts, standardisation adjustments in its concentration could play an important role to a defined and consistent level, thereby enhancing the reliability, safety, and effectiveness of products, particularly for cosmetics and drugs applications. Standardisation improves batch-to-batch consistency, supports compliance with regulatory standards, and improves consumer trust in natural products (Govindaraghavan and Sucher, 2015).

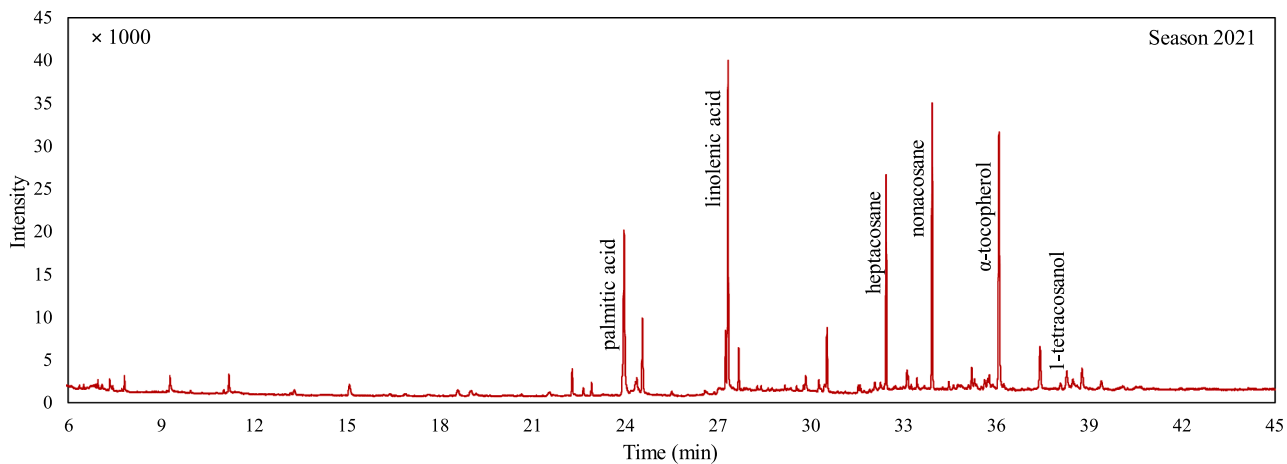
4. Conclusions

This study investigates the significant impact of extraction methodologies and environmental factors on Mo seed and leaf extracts, particularly focusing on α -tocopherol content in Mo leaves. The use of supercritical fluid extraction using carbon dioxide (SFE-CO₂) combined with ethanol (EtOH) proved highly effective for both seed and leaf extractions, significantly enhancing the yield by increasing the affinity for polar compounds. In contrast, Soxhlet (SOX) and maceration (MAC) methods using EtOH and *n*-hexane (Hex) showed distinct extraction behaviors. For seeds, both solvents provided similar yields, indicating their comparable efficiency for seed extraction. However, for leaves, low-polar solvents yielded better results, reflecting the specific chemical composition of the plant material.

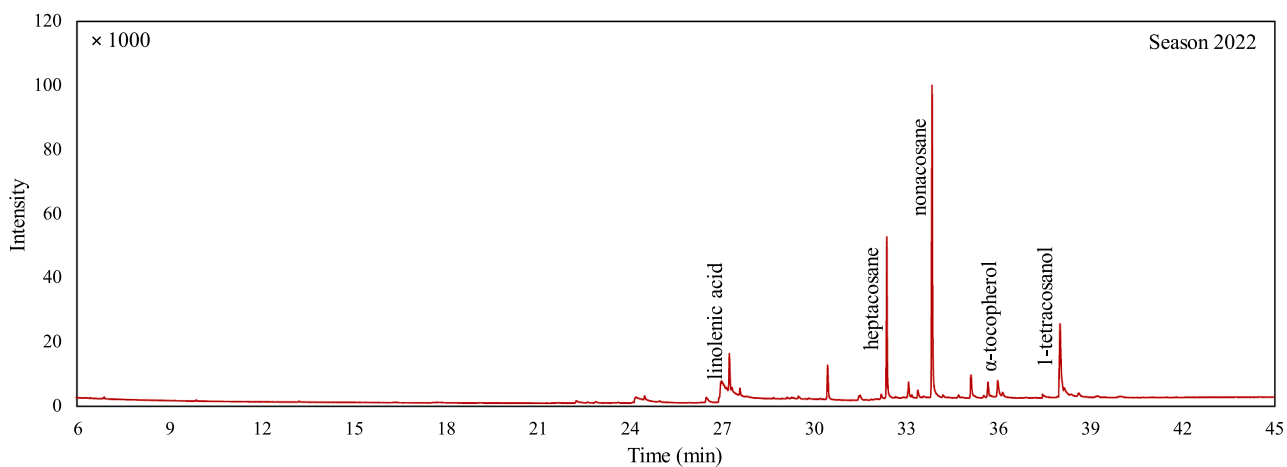
The selectivity for key compounds such as α -tocopherol and fatty acids varied across methods, underscoring the importance of selecting the appropriate extraction technique to target specific compounds for optimal use in cosmetic formulations. Seed extracts were characterised by oleic and behenic acids as the predominant fatty acids, with minor contributions from lignoceric, arachidic, palmitic, and myristic acids. The highest oleic acid selectivity (84 %) was observed with SOX-Hex, SOX-EtOH, and SFE-CO₂ methods, while SFE-CO₂+EtOH produced a more balanced profile between oleic and behenic acids, enhancing its value for cosmetic applications. The volatile analysis of leaf extracts revealed that SFE-CO₂ alone outperformed other methods in α -tocopherol selectivity despite its lower overall yield, making it the preferred method for isolating this compound.

Finally, environmental factors, including precipitation and humidity, were found to play a crucial role in the yield and composition of Mo leaf extracts across three years of harvesting. While temperature remained relatively constant, excessive rainfall in 2022 significantly reduced the mass content of α -tocopherol, as shown by the PCA analysis. These findings suggest that water availability and atmospheric conditions influence the plant's metabolic processes, ultimately affecting the quality of the extracts. As α -tocopherol is essential for photoprotection and plant response to environmental stress, these results highlight the need for alternative solutions to guarantee extract quality for cosmetic applications. Standardisation processes could ensure the α -tocopherol level consistency for potential industrial applications, enabling reliable photoprotective benefits in end products.

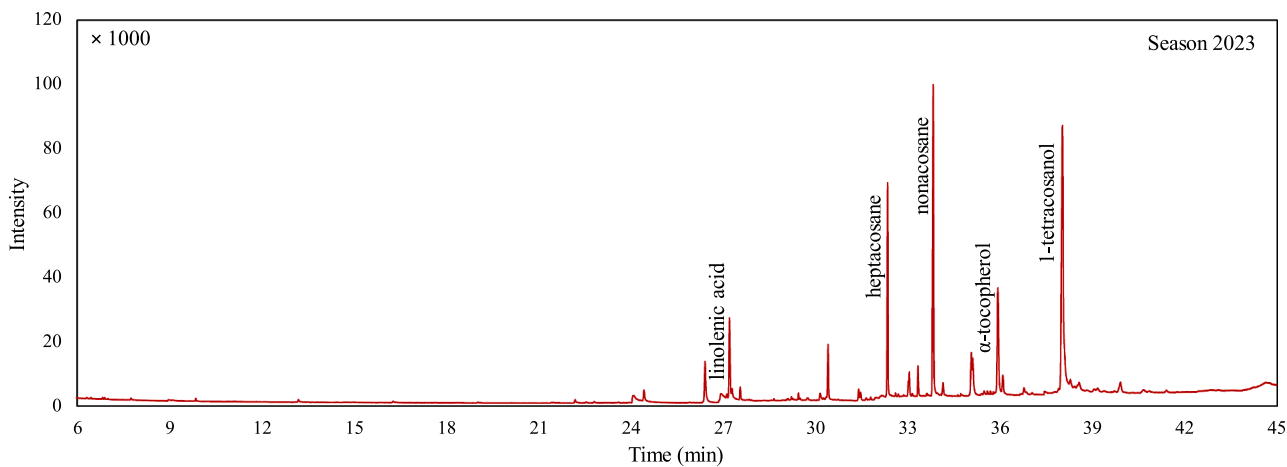
This study lays the groundwork for future research into the stabilisation of Mo leaf extracts through microencapsulation, using SFE-CO₂ as the extraction method. Microencapsulation offers advantages in improving the stability and controlled release of bioactive compounds,



(a)



(b)



(c)

Fig. 2. Chromatograms of SFE- CO_2 extracts from *Moringa oleifera* leaves across the (a) 2021, (b) 2022, and (c) 2023 seasons.

which can be critical for cosmetic formulations. Further studies will explore the efficacy of these microencapsulated extracts compared to free α -tocopherol in human skin studies, focusing on their ability to provide long-lasting hydration and photoprotection. These findings will contribute to the development of more efficient and sustainable delivery

systems for cosmetic applications, offering consumers natural, bioactive alternatives for skincare. Additionally, efforts to standardise the production process of Mo extracts will be essential to ensure their quality and consistency in large-scale commercial applications.

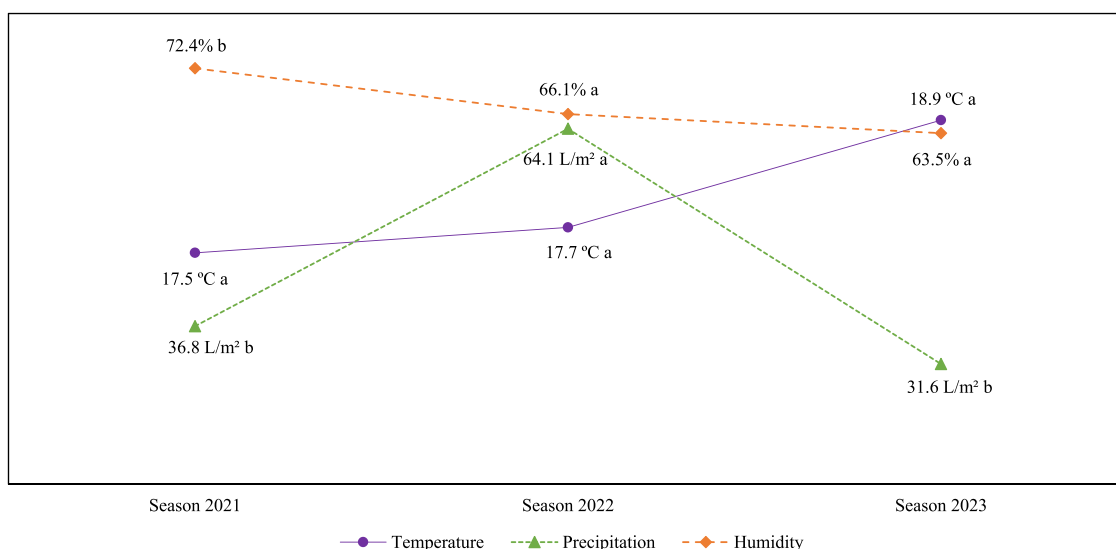


Fig. 3. Historical weather data from Málaga, Spain, including average temperature, precipitation, and humidity for February to May during the years 2021, 2022, and 2023 (Malaga weather station, 2024).

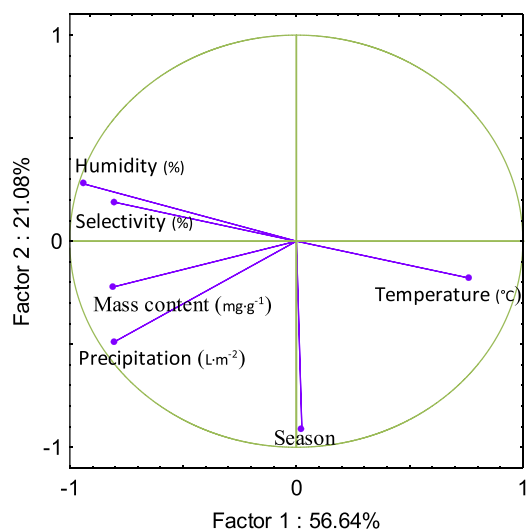


Fig. 4. Correlation of historical weather data with extract selectivity (relative concentration) and mass content of α -tocopherol via PCA. Data from the Agencia Estatal de Meteorología (AEMET) (Malaga weather station, 2024).

CRedit authorship contribution statement

Dias Madalena: Writing – original draft, Supervision, Project administration, Data curation, Conceptualization. **Kessler Júlia:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Martins Isabel:** Writing – review & editing, Methodology, Data curation. **Manrique Yaidelin:** Writing – review & editing, Methodology, Data curation. **Ribeiro António:** Writing – review & editing, Methodology, Formal analysis. **Barreiro Maria:** Writing – review & editing, Supervision, Project administration, Formal analysis. **Rodrigues Alirio:** Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by national funds through FCT/MCTES (PIDDAC): LSRE-LCM, UIDB/50020/2020 (DOI: 10.54499/UIDB/50020/2020) and UIDP/50020/2020 (DOI: 10.54499/UIDP/50020/2020); ALICE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020); CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); and LA Sus-TEC, LA/P/0007/2021 (DOI:10.54499/LA/P/0007/2020). Júlia Cristié Kessler acknowledges her PhD scholarship (ref. 2020.06656.BD, DOI: 10.54499/2020.06656.BD) from FCT.

Data availability

Data will be made available on request.

References

- Athikomkulchai, S., Tunit, P., Tadtong, S., Jantrawut, P., Sommano, S.R., Chittasupho, C., 2021a. *Moringa oleifera* seed oil formulation physical stability and chemical constituents for enhancing skin hydration and antioxidant activity. *Cosmetics* 8, 1–18. <https://doi.org/10.3390/cosmetics8010002>.
- Athikomkulchai, S., Tunit, P., Tadtong, S., Jantrawut, P., Sommano, S.R., Chittasupho, C., 2021b. *Moringa oleifera* seed oil formulation physical stability and chemical constituents for enhancing skin hydration and antioxidant activity. *Cosmetics* 8, 2. <https://doi.org/10.3390/cosmetics8010002>.
- Bhutata, P.R., Jadhav, A.J., Pinjari, D.V., Nemade, P.R., Jain, R.D., 2016. Solvent assisted extraction of oil from *Moringa oleifera* Lam. seeds. *Ind. Crops Prod.* 82, 74–80. <https://doi.org/10.1016/j.indcrop.2015.12.004>.
- Cristofori, V., Ferramondo, S., Bertazza, G., Bignami, C., 2008. Nut and kernel traits and chemical composition of hazelnut (*Corylus avellana* L.) cultivars. *J. Sci. Food Agric.* 88, 1091–1098. <https://doi.org/10.1002/jsfa.3203>.
- Da Porto, C., Decorti, D., Natolino, A., 2016. Microwave pretreatment of *Moringa oleifera* seed: effect on oil obtained by pilot-scale supercritical carbon dioxide extraction and Soxhlet apparatus. *J. Supercrit. Fluids* 107, 38–43. <https://doi.org/10.1016/j.supflu.2015.08.006>.
- L.F. De Filippis. (2016). Plant secondary metabolites. In *Plant-Environment Interaction* (eds M.M. Azooz and P. Ahmad). <https://doi.org/10.1002/9781119081005.ch15>.
- European Committee for Standardization. (2020). EN 14103:2020 Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) – Determination of ester and linolenic acid methyl ester contents.
- Govindaraghavan, S., Sucher, N.J., 2015. Quality assessment of medicinal herbs and their extracts: Criteria and prerequisites for consistent safety and efficacy of herbal medicines. *Epilepsy Behav.* 52, 363–371. <https://doi.org/10.1016/j.yebeh.2015.03.004>.
- Iqbal, S., Bhangar, M., 2006. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *J. Food Compos. Anal.* 19, 544–551. <https://doi.org/10.1016/j.jfca.2005.05.001>.
- Isnaini, N., Prajaputra, V., Maryam, S., 2023. Formulation and evaluation of O/W body cream containing patchouli oil (*Pogostemon cablin* Benth.) and Drumstick oil

- (*Moringa oleifera*) as potential moisturizing agent. JPPIPA 9, 8001–8007. <https://doi.org/10.29303/jppipa.v9i10.4292>.
- Kessler, J.C., Manrique, Y.A., Martins, I.M., Rodrigues, A.E., Barreiro, M.F., Dias, M.M., 2023. *Moringa oleifera* L. screening: SFE-CO₂ optimisation and chemical composition of seed, leaf, and root extracts as potential cosmetic ingredients. Separations 10, 210. (<https://www.mdpi.com/2297-8739/10/3/210>).
- Kessler, J.C., Martins, I.M., Manrique, Y.A., Rodrigues, A.E., Barreiro, M.F., Dias, M.M., 2024. Advancements in conventional and supercritical CO₂ extraction of *Moringa oleifera* bioactives for cosmetic applications: a review. J. Supercrit. Fluids 214, 106388. <https://doi.org/10.1016/j.supflu.2024.106388>.
- Kessler, J.C., Vieira, V.A., Martins, I.M., Manrique, Y.A., Afonso, A., Ferreira, P., Mandim, F., Ferreira, I.C.F.R., Barros, L., Rodrigues, A.E., Dias, M.M., 2022. Obtaining aromatic extracts from portuguese *thymus mastichina* l. by hydrodistillation and supercritical fluid extraction with CO₂ as potential flavouring additives for food applications. Molecules 27, 694. <https://doi.org/10.3390/molecules27030694>.
- Kleiman, R., Ashley, D.A., Brown, J.H., 2008. Comparison of two seed oils used in cosmetics, moringa and marula. Ind. Crops Prod. 28, 361–364. <https://doi.org/10.1016/j.indcrop.2008.04.003>.
- Lushchak, V.I., Semchuk, N.M., 2012. Tocopherol biosynthesis: chemistry, regulation and effects of environmental factors. Acta Physiol. Plant 34, 1607–1628. <https://doi.org/10.1007/s11738-012-0988-9>.
- Malaga weather station. Historical hourly data. 2024. Available from (<https://x-y.es/aemet/est-6155A-malaga-aeropuerto#indiceP1>).
- Mesa, T., Munné-Bosch, S., 2023. α -Tocopherol in chloroplasts: nothing more than an antioxidant? Curr. Opin. Plant Biol. 74, 102400. <https://doi.org/10.1016/j.pbi.2023.102400>.
- Munné-Bosch, S., 2005. The role of α -tocopherol in plant stress tolerance. J. Plant Physiol. 162, 743–748. <https://doi.org/10.1016/j.jplph.2005.04.022>.
- Muñoz, P., Munné-Bosch, S., 2019. Vitamin E in plants: biosynthesis, transport, and function. Trends Plant Sci. 24, 1040–1051. <https://doi.org/10.1016/j.tplants.2019.08.006>.
- National Institute of Standards and Technology (NIST). Isothermal Properties for Carbon Dioxide. 2022. Accessed August 2024. (<https://nist.gov/>).
- Nguyen, H.N., Gaspillo, P.D., Maridable, J.B., Malaluan, R.M., Hinode, H., Salim, C., Huynh, H.K.P., 2011. Extraction of oil from *Moringa oleifera* kernels using supercritical carbon dioxide with ethanol for pretreatment: optimization of the extraction process. Chem. Eng. Process 50, 1207–1213. <https://doi.org/10.1016/j.cep.2011.08.006>.
- Niu, Y., Zhang, Q., Wang, J., Li, Y., Wang, X., Bao, Y., 2022. Vitamin E synthesis and response in plants. Front. Plant Sci. 13, 994058. <https://doi.org/10.3389/fpls.2022.994058>.
- Nizioł-Lukaszewska, Z., Furman-Toczek, D., Bujak, T., Wasilewski, T., Hordyjewicz-Baran, Z., 2020. *Moringa oleifera* L. extracts as bioactive ingredients that increase safety of body wash cosmetics. Dermatol. Res. Pr. 2020, 8197902. <https://doi.org/10.1155/2020/8197902>.
- Ong, E.S., 2004. Extraction methods and chemical standardization of botanicals and herbal preparations. J. Chromatogr. B. 812, 23–33. <https://doi.org/10.1016/j.jchromb.2004.07.041>.
- Ruttaratnamongkol, K., Siebenhandl-Ehn, S., Schreiner, M., Petrasch, A.M., 2014. Pilot-scale supercritical carbon dioxide extraction, physico-chemical properties and profile characterization of *Moringa oleifera* seed oil in comparison with conventional extraction methods. Ind. Crops Prod. 58, 68–77. <https://doi.org/10.1016/j.indcrop.2014.03.020>.
- Sadiq, M., Akram, N.A., Ashraf, M., Al-Qurainy, F., Ahmad, P., 2019. Alpha-tocopherol-induced regulation of growth and metabolism in plants under non-stress and stress conditions. J. Plant Growth Regul. 38, 1325–1340. <https://doi.org/10.1007/s00344-019-09936-7>.
- da Silva, M., Trancoso, J., Tormen, L., Bombardelli, M.M., Corazza, M.L., Bainy, E.M., 2022. Extraction of compounds from *Moringa oleifera* leaves using supercritical CO₂ plus ethanol as a cosolvent. J. Food Process Eng. 45, e13979. <https://doi.org/10.1111/jfpe.13979>.
- Vázquez-León, L.A., Páramo-Calderón, D.E., Robles-Olvera, V.J., Valdés-Rodríguez, O.A., Pérez-Vázquez, A., García-Alvarado, M.A., Rodríguez-Jimenes, G.C., 2017. Variation in bioactive compounds and antiradical activity of *Moringa oleifera* leaves: influence of climatic factors, tree age, and soil parameters. Eur. Food Res. Technol. 243, 1593–1608. <https://doi.org/10.1007/s00217-017-2868-4>.
- Zeitoun, H., Michael-Jubeli, R., El Khoury, R., Baillet-Guffroy, A., Tfayli, A., Salameh, D., Leift, R., 2020. Skin lightening effect of natural extracts coming from Senegal botanical biodiversity. Int. J. Dermatol. 59, 178–183. <https://doi.org/10.1111/ijd.14699>.
- Zhao, S., Zhang, D., 2013. Supercritical fluid extraction and characterisation of *Moringa oleifera* leaves oil. Sep. Purif. Technol. 118, 497–502. <https://doi.org/10.1016/j.seppur.2013.07.046>.
- Zorić, M., Banožić, M., Aladić, K., Vladimir-Knežević, S., Jokić, S., 2022. Supercritical CO₂ extracts in cosmetic industry: current status and future perspectives. Sustain. Chem. Pharm. 27, 100688. <https://doi.org/10.1016/j.scp.2022.100688>.