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Fatty acid profile, sterols, and squalene content comparison between two conventional (olive oil and linseed oil) and three non-conventional vegetable oils (echium oil, hempseed oil, and moringa oil)

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Abstract: New sources of bioactive compounds are constantly explored for reformulating healthier foods. This work aimed to explore and characterize the fatty acid profile and sterol content of three non-conventional oils used in functional food products (hempseed oil, moringa oil, and echium oil) and to compare them with two conventional ones (extra virgin olive oil [EVOO] and linseed oil). Oxidative stability was assessed by determining their acidity value and peroxide content. All oils showed adequate values for acidity and oxidation status. Echium and hempseed oils showed a high content of polyunsaturated fatty acids (>70%), especially omega-3 fatty acids, while moringa oil was rich in oleic acid. Echium oil, hempseed oil, and moringa oil presented higher sterol content than EVOO, but lower than that of linseed oil. Sitosterol was the most abundant sterol in all samples (97.88–275.36 mg/100 g oil), except in echium oil, where campesterol (170.62 mg/100 g oil) was the major sterol. Squalene was only found in significant amounts in EVOO. In conclusion, non-conventional oils seem to be interesting sources of bioactive compounds and have great potential for the food industry.

KEYWORDS
bioactive compounds, echium oil, hempseed oil, moringa oil, omega-3, plant sterols

Practical Application: Non-conventional vegetable oils can be used as alternative sources of lipids in a variety of food products. Additionally, these oils have great potential to be included in the formulation of functional ingredients for the delivery of omega-3 fatty acids, antioxidants, fiber, among others.

1 | INTRODUCTION

The demand of new sources of edible oils for direct consumption and for food processing purposes has significantly increased in the last decade (Green & Wang, 2020; T. Zhang et al., 2020). This demand is related to specific fatty acids (FAs) or health/nutritional properties attributed to certain compounds present in different vegetable oils and their functionality once they are incorporated to a food matrix. In fact, the beneficial effects of oils can be related to their FA composition or to other minor components such as squalene and plant sterols, among others (Kyçyk et al., 2016). Public health guidelines recommend keeping the dietary intake of saturated fatty acids (SFAs) as low as possible or ≤10% (of total energy intake) according to the European Food Safety Authority and the World Health Organization, respectively, and also advice to substitute them in the diet with unsaturated fats (EFSA, 2010a; U.S. Department of Health and Human Services & U.S. Department of Agriculture, 2015; WHO, 2018). Consequently, the use of highly unsaturated FA sources has been encouraged. In this sense, extra virgin olive oil (EVOO), widely consumed in the Mediterranean area, and linseed oil have been extensively studied for their application to food reformulation as sources of monounsaturated fatty acids (MUFA) and omega-3 FAs, respectively (Alejandre et al., 2016; Gutiérrez-Luna et al., 2020; Skiada et al., 2019, 2020; Zając & Świątek, 2018). Moreover, from the scientific perspective, these two oils have received wide attention, as shown by the high number of papers published during the last years describing their composition, behavior, and health properties.

In addition to their FA profile, these vegetable oils contain other substantial amounts of health beneficial compounds, such as phytosterols, that are known to help maintaining normal blood cholesterol levels (Fattore & Massa, 2018; Gaforio et al., 2015; Skiada et al., 2019). For food labeling purposes, in order to bear this claim, a food should provide at least 0.8 g per day of plant sterols/stanols in one or more servings (EFSA, 2010b).

Other not so conventional or less explored oils such as echium oil (obtained from *Echium plantagineum* L.), hempseed oil (obtained from *Cannabis sativa* L.), and moringa oil (obtained from *Moringa oleifera* L.), with promising potential as healthier sources of FAs have been of increasing interest for the food industry. Among the potential applications for these oils are their use as ingredients in food reformulation strategies and the development of functional ingredients in emulsions (Gutiérrez-Luna et al., 2020; Mikulcová et al., 2017), edible films (Mihaly Cozmuta et al., 2015), and microcapsules (Comunian & Favaro-Trindade, 2016) to improve the nutritional profile of a variety of food products such as oil blends (Dollah et al., 2014), meat products (Alves Pires et al., 2019, 2020), or bakery products (Gutiérrez-Luna et al., 2020).

In particular, echium oil is obtained by extraction from *E. plantagineum* seeds and subjected to further refining. FDA approved its use as dietary ingredient in 2002 (FDA, 2021a), whereas the EU classified it as novel food in 2008 (Decision 2008/558/EC) (European Commission, 2008), allowing its addition into different types of foods. It is becoming a relevant alternative source of omega-3 FAs, but its oxidative stability is yet to be explored, as well as its chemical composition in terms of the presence of minor compounds (Carlini et al., 2021).

As for hempseed oil is concerned, it also contains a high amount of the omega-3 linolenic acid as compared with most of vegetable oils, and is obtained from cold pressing of *C. sativa* L. It is considered GRAS (Generally recognized as Safe) by the FDA since 2018 (FDA, 2021b) and does not require novel food approval by EFSA due to its long history of consumption (European Commission, 2022). It has received increasing attention due to its content in cannabinoids (Cardenia et al., 2018), but the sterol fraction has hardly been studied.

On the other hand, moringa oil is obtained from *M. Oleifera* seeds, characterized by their high oil content (up to 40%), mainly monounsaturated (oleic acid). The tree is indigenous to Northern India and can be also found in African and Asian countries (Anwar et al., 2005; Tsaknis et al., 1999). Nowadays, due to its adaptability, it is being cultivated in other areas, especially tropical and subtropical lands (Leone et al., 2016), and oils produced under this alternate environmental and agro-climatic conditions have not been investigated yet.

Besides the application in the food industry and their nutritional value (Liu et al., 2018), these oils have demonstrated health benefits related to lipid metabolism, cardiovascular health, and immunomodulatory diseases, among others (Aly et al., 2016; Comunian et al., 2016). Moreover, recent studies have pointed out the versatility of these oils to be used as sources of bioactive compounds for the pharmaceutical and cosmetic industry (Berti et al., 2007; Bouayoun et al., 2018; Oomah et al., 2002; Rincón-Cervera et al., 2020). Even though there is literature available about the composition of the saponifiable fraction of these oils (Ayerza(h), 2019; Bouayoun et al., 2018; Lalas & Tsaknis, 2002), the unsaponifiable fraction has not received the same attention and remains unexplored.

The aim of this work was to explore and characterize the FA profile and sterol content of three non-conventional oils used in functional food products (hempseed oil, moringa oil, and echium oil) and to compare them with two conventional ones (EVOO and linseed oil). Moreover, the

oxidative stability was checked by determining their acidity value and peroxide content.

2 | MATERIAL AND METHODS

2.1 | Oils

Different edible commercialized oils were analyzed: EVOO (*Olea europaea* L.) (variety Empeltre, Spain), linseed oil (*Linum usitatissimum* L.) (First cold pressed. Mandolé, Castellón, Spain), hempseed oil (*C. sativa* L.) (BIO, Bioener, Barcelona, Spain), moringa oil (*M. oleifera* L.) (ACEISUR S.A., Nicaragua) were purchased in local specialized supermarkets (Pamplona, Navarre, Spain and León, Nicaragua in the case of moringa oil). Echium oil (*E. plantagineum* L.) was kindly donated by NEWmega™ Echium Oil De Wit Specialty Oils (De Waal, Tescel, The Netherlands). As regards to EVOO variety Empletre, it was chosen because their olives are known to provide oils with a soft taste, and not excessive bitterness as compared to other varieties (Abenoza et al., 2019), making it more adequate for reformulation purposes.

For each type of oil, two bottles of three different production batches were purchased (received as donation in the case of echium oil). Analytically, two aliquots of each bottle were processed ($n = 12$), and the results were expressed as an average per type of oil.

All samples were kept under the same conditions until analysis: refrigeration temperature, no light exposition, and in their original bottles (amber plastic bottles for echium oil, amber glass bottles for hempseed oil and linseed oil, and plastic transparent bottles for EVOO and moringa oil).

2.2 | Quality parameters

Peroxide value (PV) determination was performed as described by Shantha and Decker (1994). Free acidity index was carried out following the analytical method described in Regulation EEC/2568/91 of the European Commission (Commission Regulation (EEC) No. 2568/91, 1991). Measurements were done in quadruplicate for each batch of oil. PV was given as milliequivalents of active oxygen per kilogram of oil (meq O_2/kg), and free acidity was expressed as the percentage of oleic acid.

2.3 | FA profile

The FA profile was determined by gas chromatography. The methylation for obtaining the fatty acid methyl esters (FAME) was done according to the official method AOAC 969.33 (AOAC, 2002) using Boron trifluoride/methanol. A Perkin-Elmer Autosystem XL gas chromatograph fitted with a capillary column SPTM-2560 (100 m × 0.25 mm × 0.2 μm) and flame ionization detection were used. The temperature of the injection port was 250°C and of the detector was 260°C. The oven temperature was programmed at 175°C during 10 min and increased to 200°C at a rate of 10°C/min, then increased to 220°C at a rate of 4°C/min, which was kept for 15 min. The carrier gas was hydrogen and the pressure was 20.5 psi. Split flow was 120 cm/s. The identification of FAME was done by comparison of the retention times of the peaks in each sample with those of standard pure compounds. Individual methylated standards from Sigma Aldrich Co. (St. Louis, MO, USA) were used for the saturated, monounsaturated, *cis* polyunsaturated FAs, and the *trans t*-Palmitoleic C16:1 Δ9*t*, Elaidic C18:1 Δ9*t*, Brassidic C20:1 Δ13*t*. For linoleic acid isomers, the mixture of linoleic acid *cis/trans* isomers (50% of C18:2Δ9*t*,12*t*; 20% of C18:2Δ9*c*,12*t* and C18:2Δ9*t*,12*c*; 10% of C18:2Δ9*c*,12*c*) also from Sigma was used (Ansorena et al., 2013).

2.4 | Sterol analysis

Sterols were extracted following the method described by Berasategi et al. (2012) and determined by gas chromatography and mass selective detection.

Three grams (±0.02 g) of oil sample were added to 1 ml of internal standard (5α-Cholestane from Sigma-Aldrich Co. [St. Louis, MO, USA]), solved in hexane:isopropanol at a concentration of 2 mg/ml. They were subjected to saponification and further extraction of the unsaponifiable fraction. For that purpose, ethanol (20 ml) and KOH (50%) (5 ml) were added to the sample and subjected to warm agitation for 1 h (<50°C). Thirteen milliliters of distillated water were added and six extractions with 20–25 ml of hexane were done, collecting the organic phase of each extraction, which were all merged. The solvent was rotavaporated, and the sample was further dried under nitrogen flow. This unsaponifiable fraction was derivatized with 400 μl of Tri-Sil, in a hot water bath (60°C for 45 min), to form the trimethyl silyl ether (TMS) derivatives. The excess of Tri-Sil was evaporated under nitrogen flow and the sample was diluted in 10 ml of hexane. The TMS derivatives of sterols were analyzed in an Agilent Technologies 6890N Gas chromatographer (GC) coupled to a 5975 Mass Selective Detector (Agilent Technologies). GC was equipped with a capillary column (50.0 m × 250 μm × 0.25 μm nominal—WCOT fused silica UF5ms). The carrier gas was He (1 ml/min), and the chromatographic conditions were as follows: initial oven temperature was maintained during

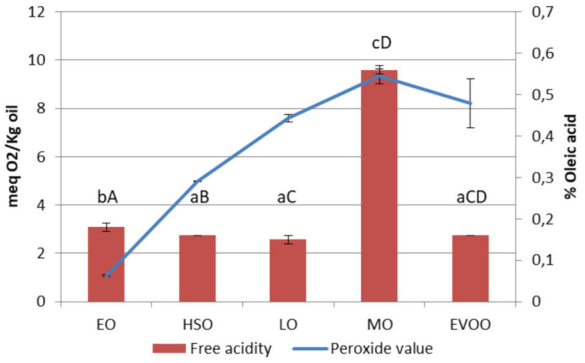

FIGURE 1 Peroxide value (meq O_2/kg oil) and free acidity (% oleic acid) of oil samples. Data correspond to mean values of the quadruplicates per oil type. Different lower case letters represent significant differences in free acidity and different upper case letters represent significant differences in peroxide value ($p < 0.05$) based on post hoc Bonferroni test. Error bars denote standard deviation of the mean. Abbreviations: EO, echium oil; EVOO, extra virgin olive oil; HSO, hempseed oil; LO, linseed oil; MO, moringa oil.

0.5 min at 250°C and subsequently programmed from 250 to 290°C at a rate of 50°C/min, and at a rate of 0.5°C/min from 290 to 291°C. The inlet pressure used was 28.47 psi. The injector temperature was 280°C, and the samples were injected (1 μl) in a splitless mode. The identification of the peaks was based on comparison of their mass spectra with the spectra of the Wiley library (HPCHEM, Wiley, 275, 6th ed.), with standard compounds if available and also with those obtained from the literature. Quantification was based on an internal standard method, and results were expressed in mg/100 g oil.

2.5 | Statistical analysis

The statistical analysis of data was done using STATA/IC 12.1 program. A one-way ANOVA with post hoc Bonferroni test was used in order to evaluate the significant differences among types of oil. A significance level of $p \leq 0.05$ was used for all evaluations. Mean and standard deviation are shown in tables.

3 | RESULTS AND DISCUSSION

3.1 | Oxidative stability

The five edible oils showed adequate values for PV and free acidity (FFA) (Figure 1). The results for EVOO (8.2 meq O_2/kg and 0.15%) were acceptable according to the legislation (Commission regulation (EC) No. 182/2009, 2009; Commission Regulation (EEC) No, 2568/91, 1991; FAO, 1999) and comparable to those reported by Skiada et al. (2019, 2020). In spite of the absence of specific values of PV for novel oils such as hempseed oil, echium oil, and moringa oil, the values obtained in our samples did fall into the legal limits for virgin oils (<20 meq O_2/kg) and for cold pressed oils and other edible oils (<10 meq O_2/kg) (Casal et al., 2010; FAO, 1999). For free acidity, values in the non-conventional oils were low, moringa oil being the highest with 0.56%. The results portray absence of oxidation problems, and hence, the preservation of the nutritional quality of the oils.

3.2 | Saponifiable fraction

The saponifiable fraction of oils is of great interest, not only from the nutritional and healthy point of view but also because it can affect the oxidation rate of the unsaponifiable compounds present in a food matrix (Barriuso et al., 2017; Z. S. Zhang et al., 2020). The analysis of the FA profile (Table 1) revealed that the five oils presented a similar percentage of unsaturated fraction (72–74%), being polyunsaturated fatty acids (PUFA) the major one in echium oil, hempseed oil, and linseed oil, whereas MUFA was the major one in moringa oil and EVOO. Regarding the PUFA-rich oils, SFA fraction was lower ($p < 0.05$) for linseed oil, and MUFA fraction was lower ($p < 0.05$) in hempseed oil as compared to the others, whereas no statistically significant differences were noticed for the PUFA fraction among them. Nevertheless, certain particular features can be highlighted for each oil.

Echium oil was characterized by the presence of stearidonic acid (12.09 g/100 g oil). This FA is found in the seeds and leaves of the boragenase plant family, among them, echium, borage, corn gromwell, evening primrose, and blackcurrant (Guil-Guerrero, 2007; Walker et al., 2013). Stearidonic acid is the metabolic intermediate between α-linolenic acid and EPA or DHA, and interestingly, it has been observed that SDA displays similar biological functions as EPA and DHA (Y. Li et al., 2017). Additionally, its consumption has been associated with health benefits and preventive roles in inflammation, dyslipidemia, cancer, and cardiovascular disease (Comunian et al., 2016; Y. Li et al., 2017; Prasad et al., 2021).

Moringa oil can be considered an oil rich in MUFAs, quite similar in its saponifiable fraction to EVOO. Table 1 shows that both oils had comparable amounts of oleic acid (72.08 and 70.22 g/100 g oil, respectively). However, some differences can be distinguished in the SFA and PUFA fraction. Whereas higher palmitic acid was observed in EVOO as compared to moringa oil, moringa oil showed significantly higher values for stearic, arachidic, eicosenoic and, above all, behenic acid. The distribution of FAs presented

TABLE 1 Fatty acid profile of analyzed oils (g/100 g of oil)

Fatty acid	EO	HSO	LO	MO	EVOO
Caprilic C8:0	ND	0.21 (0.25)^{a}	ND	ND	ND
Capric C10:0	ND	ND	ND	ND	ND
Lauric C12:0	ND	ND	ND	0.02 (0.00)^{a}	ND
Myristic C14:0	ND	0.03 (0.00)^{a}	0.02 (0.00)^{a}	0.13 (0.00)^{b}	ND
Palmitic C16:0	6.95 (0.19)^{b}	6.65 (0.06)^{b}	4.88 (0.08)^{a}	6.21 (0.02)^{b}	13.88 (0.03)^{c}
t-Palmitoleic C16:1	ND	ND	ND	ND	0.02 (0.05)^{a}
Palmitoleic C16:1n-7	0.07 (0.06)^{a}	0.10 (0.01)^{a}	0.05 (0.00)^{a}	1.49 (0.01)^{c}	1.16 (0.03)^{b}
Stearic C18:0	3.89 (0.09)^{c}	3.01 (0.05)^{b}	3.59 (0.04)^{c}	6.24 (0.02)^{d}	2.57 (0.03)^{a}
ΣTrans isomers C18:1	0.04 (0.02)^{a}	ND	0.08 (0.03)^{a}	0.26 (0.08)^{b}	0.20 (0.19)^{b}
Oleic C18:1 n-9	15.21 (0.15)^{b}	13.78 (0.06)^{a}	16.45 (0.09)^{c}	70.22 (0.22)^{d}	72.08 (0.23)^{e}
c-Vaccenic C18:1 n-7	0.51 (0.12)^{a}	0.75 (0.01)^{b}	0.64 (0.01)^{a}	ND	ND
t-Linoleic C18:2	ND	0.03 (0.03)^{a}	0.02 (0.01)^{a}	ND	ND
c-t Linoleic C18:2	0.32 (0.01)^{b}	0.25 (0.01)^{a}	0.26 (0.01)^{a}	ND	0.24 (0.00)^{a}
t-c Linoleic C18:2	ND	ND	ND	ND	ND
Linoleic C18:2 n-6	16.45 (0.04)^{c}	57.35 (0.28)^{e}	16.89 (0.02)^{d}	0.99 (0.00)^{a}	7.78 (0.08)^{b}
Arachidic C20:0	0.13 (0.01)^{a}	0.70 (0.03)^{c}	0.12 (0.01)^{a}	3.95 (0.00)^{d}	0.42 (0.00)^{b}
γ-Linolenic C18:3 n-6	10.78 (0.04)^{c}	0.59 (0.01)^{b}	0.06 (0.01)^{a}	0.06 (0.00)^{a}	ND
Eicosenoic C20:1 n-9	0.56 (0.01)^{d}	0.29 (0.00)^{b}	0.01 (0.00)^{a}	1.87 (0.02)^{e}	0.21 (0.00)^{c}
α-Linolenic C18:3 n-3	32.82 (0.25)^{d}	15.77 (0.18)^{c}	56.42 (0.35)^{e}	1.08 (0.02)^{b}	0.67 (0.03)^{a}
Stearidonic C18:4 n-3	12.09 (0.24)^{a}	ND	ND	ND	ND
Eicosadienoic C20:2 n-6	ND	0.23 (0.02)^{b}	0.07 (0.01)^{a}	0.17 (0.06)^{b}	0.15 (0.09)^{ab}
Behenic C22:0	0.02 (0.01)^{a}	0.19 (0.02)^{c}	0.17 (0.10)^{bc}	6.20 (0.03)^{d}	0.08 (0.00)^{b}
t-Brassidic C20:1	0.04 (0.00)^{a}	ND	0.10 (0.00)^{b}	0.12 (0.00)^{c}	ND
Erucic C22:1 n-9	0.01 (0.01)^{a}	ND	0.10 (0.00)^{b}	ND	ND
Eicosatrienoic C20:3 n-3	ND	ND	ND	ND	ND
Arachidonic C20:4 n-6	ND	0.08 (0.05)^{a}	ND	ND	0.52 (0.05)^{b}
Eicopentaenoic C20:5 n-3	ND	ND	ND	ND	ND
Nervonic C24:1 n-9	0.10 (0.09)^{a}	ND	ND	ND	ND
Docosatrienoic C22:3 n-9	ND	ND	ND	ND	ND
Docosapentaenoic C22:5 n-6	ND	ND	ND	ND	ND
Lignoceric C24:0	0.01 (0.01)^{a}	ND	0.08 (0.01)^{b}	1.01 (0.06)^{c}	ND
Docosapentaenoic C22:5 n-3	ND	ND	ND	ND	ND
Docosahexaenoic C22:6 n-3	ND	ND	ND	ND	ND
ΣSFA	10.98 (0.28)^{b}	10.79 (0.34)^{b}	8.78 (0.20)^{a}	22.74 (0.07)^{d}	16.96 (0.04)^{c}
ΣMUFA	16.46 (0.19)^{b}	14.92 (0.06)^{a}	17.25 (0.09)^{b}	73.58 (0.24)^{c}	73.46 (0.21)^{c}
ΣPUFA	72.15 (0.44)^{c}	74.02 (0.42)^{c}	73.43 (0.33)^{c}	2.29 (0.03)^{a}	9.12 (0.06)^{b}
ΣTrans	0.40 (0.01)^{b}	0.27 (0.04)^{a}	0.46 (0.05)^{b}	0.38 (0.08)^{ab}	0.47 (0.23)^{b}
ΣN3	44.91 (0.25)^{d}	15.77 (0.18)^{c}	56.42 (0.35)^{e}	1.08 (0.02)^{b}	0.67 (0.03)^{a}
ΣN6	27.24 (0.08)^{d}	58.02 (0.25)^{e}	16.95 (0.02)^{c}	1.04 (0.01)^{a}	8.30 (0.12)^{b}
N6/N3	0.83 (0.01)^{b}	3.68 (0.03)^{d}	0.30 (0.00)^{a}	0.96 (0.01)^{c}	12.45 (0.36)^{e}
PUFA/SFA	6.57 (0.20)^{c}	6.87 (0.25)^{c}	8.37 (0.23)^{d}	0.10 (0.00)^{a}	0.54 (0.00)^{b}
PUFA+MUFA/SFA	8.07 (0.22)^{c}	8.25 (0.29)^{c}	10.33 (0.27)^{d}	3.34 (0.02)^{a}	4.87 (0.03)^{b}

Note: Data correspond to mean values of the quadruplicates per oil type. Standard deviations appear in parentheses. Values with different letters within rows are significantly different ($p < 0.05$) based on post hoc Bonferroni test. ND indicates that the fatty acid was not detected in the sample.

Abbreviations: EO, echium oil; EVOO, extra virgin olive oil; HSO, hempseed oil; LO, linseed oil; MO, moringa oil.

in this study was comparable with the results obtained by Ayerza(h) (2019), who studied the oil extracted from moringa seeds from three different locations in Ecuador. EVOO FA composition has been extensively studied and our results are comparable to the literature (Berasategi et al., 2012; Kyçyk et al., 2016; Maestri et al., 2019), even though great variation among varieties, manipulation, and storage conditions of this oil can be expected.

Regarding the omega-6/omega-3 ratio, echium oil and moringa oil showed, as it occurred with linseed oil, values <1, whereas hempseed oil reached a value of 3.68. EVOO showed the highest value (12.45).

3.3 | Unsaponifiable fraction

3.3.1 | Sterols

The characterization of the unsaponifiable fraction of all oils showed the presence of sterols and, additionally, in MUFA-rich oils, squalene was also noticed. The sterol composition of the five analyzed oils revealed a very different relative distribution of compounds among them, being identified between 86.1% and 99.4% of the quantified compounds obtained in the chromatographic analysis (Figure 2). Table 2 shows the detailed profile for each type of oil, and the ions used for characterization purposes. All identified plant sterols, except for cycloartenol, were 4-desmethylsterols (campesterol, stigmasterol, sitosterol, Δ5 and Δ7-avenasterol), to which efficient cholesterol-lowering capacity has been widely described. As regards to cycloartenol, it is a 4,4-dimethylsterol that has been recently associated to anticancer, anti-inflammatory, and antinociceptive activities (T. Zhang, Xie, et al., 2020).

Sterol content of the three PUFA-rich oils ranged between 360 and 516 mg/100 g oil, all of them were phytosterols, except traces of cholesterol identified in linseed oil. Given the potential variability linked to different cultivars, geography, and processing conditions, among other factors, these data can be comparable from the quantitative stand point. However, statistically significant differences in the quantitative distribution of the individual compounds were observed, and hempseed oil being the one with the lowest value.

Sterol profile in echium oil was characterized by the highest content of campesterol among the studied oils (170.62 mg/100 g oil), accounting for a 34% of total sterols (Figure 2). Important amounts of sitosterol (153.41 mg/100 g oil) and Δ5-avenasterol (73.55 mg) were also found. Due to the scarce literature available about the sterol composition of echium oil, the results obtained in this study could be directly compared with two studies where different extraction methods were applied to *E. plantagineum* seeds (Carlini et al., 2021; Rincón-Cervera et al., 2020). Rincón-Cervera et al. (2020) showed a total value of 437.23 mg/100 g oil, similar to that found in our oil samples. In that work, only three sterols were quantified, with campesterol reported as the most abundant as well, except when the method of extraction was with ethanol in Randall mode, with which sitosterol showed similar values than campesterol. The third sterol identified was stigmasterol in lower concentrations. Carlini et al. (2021) found only campesterol and sitosterol in their samples, with campesterol as the most abundant one. More data from other different species of echium (*Echium vulgare* L.) reported by Nogala-Kalucka et al. (2010) and Minkowski et al. (2010) suggest important amounts of phytosterols ranging from 424.9 to 720 mg/100 g oil, and also identifying campesterol as the predominant compound. This is a rather unusual finding, as sitosterol is normally the largest sterol in seed oils (Fernández-Cuesta et al., 2012; Zarrouk et al., 2019; T. Zhang et al., 2020).

In hempseed oil, sitosterol was the major component comprising up to 76% of the total sterol content, higher than that found in echium oil and linseed oil, where it represented just a third of their sterol concentration. The rest of hempseed oil sterol's profile was composed by campesterol, Δ5-avenasterol, cycloartenol, and <5 mg/100 g oil of stigmasterol and other unknown compounds (Table 2). These results are comparable to those described by previous studies (Liang et al., 2015; Montserrat-De La Paz et al., 2014) who also found sitosterol as the major sterol. Total sterols in hempseed oil quantified in this study was similar to that described by Montserrat-De La Paz et al. (2014) whose results ranged from 270 to 380 mg/100 g oil.

For linseed oil, >50% of its sterol distribution was composed by sitosterol (182 mg/100 g oil) and Δ7-avenasterol (154.64 mg/100 g oil), followed by important amounts of campesterol and Δ5-avenasterol. The total amount of sterols found in our work was within the range reported by Tańska et al. (2016) (409–538.83 mg/100 g oil).

In the case of MUFA characterized oils, moringa oil and EVOO's sterol concentration was significantly different, being 2.9 folds higher in moringa oil (439.24 mg/100 g oil) than in EVOO (147.18 mg/100 g oil). Although there are literature data to compare relative percentage of sterols in moringa oil, no absolute amounts data (mg/100 g oil) have been found to compare our data with other papers. Sitosterol was found to be the major sterol in both types of oil, but in significantly higher amounts in moringa oil. In the same line, higher concentrations of stigmasterol, campesterol, Δ5-avenasterol, and Δ7-avenasterol were also found in moringa oil (Table 2). Cycloartenol was the only sterol found to be higher (five folds) in EVOO than in moringa oil. Moringa oil had the highest content of stigmasterol among the studied oils, representing up to 19%

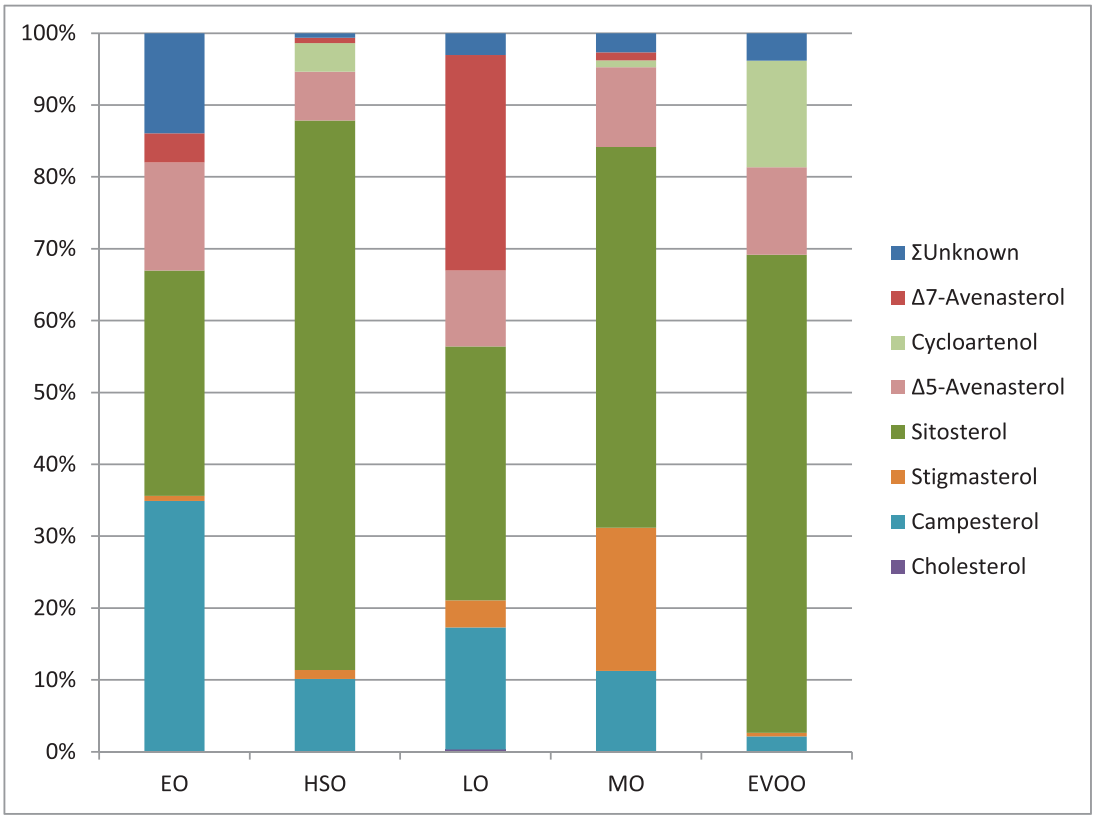

FIGURE 2 Distribution of total area (%) obtained through GS-MS for the sterol analysis of the different analyzed oil samples. Abbreviations: EO, echium oil; EVOO, extra virgin olive oil; HSO, hempseed oil; LO, linseed oil; MO, moringa oil.

of the sterol content. Zhao et al. (2019) studied the composition of unsaponifiable matters of Chinese moringa oil and concluded that >80% of it was composed by phytosterols, in which *β*-sitosterol and stigmasterol were the most predominant, which matches our results. Lalas and Tsaknis (2002) analyzed *M. oleifera* seeds variety Periyakulam 1 and EVOO, and the only resemblance found was that sitosterol was the most predominant sterol in both oils. Leone et al. (2016) pointed out that the sterol composition can be modified depending on origin and environmental conditions and concluded that information about available commercial varieties grown in different environments is scarce.

According to the literature, EVOO phytosterol concentration ranges from 800 to 2600 mg/kg, being predominantly sitosterol, Δ5-avenasterol, and campesterol (Berasategi et al., 2012; Kyçyk et al., 2016). Our results are within these ranges (1471.8 mg/kg oil), but just as in every vegetable oil, the sterol fraction in EVOO can be affected by several factors including growing conditions, cultivar, fruit maturation, oil extraction, refining procedures, and storage conditions (Almeida et al., 2020; Kyçyk et al., 2016; C. Li et al., 2011; Lukić et al., 2013).

3.3.2 | Squalene and other compounds

In addition to the phytosterols found in moringa oil and EVOO, squalene was also detected in both oils and represented ~90% of the total unsaponifiable fraction in EVOO, while it was < 1% in moringa oil.

Squalene is an intermediate hydrocarbon in the biosynthesis of phytosterols and terpenes in plants. It is widely used in the pharmaceutic and cosmetic industry. The common source has been shark liver oil, but restrictions on their use have created a need to find alternative sources (Martínez-Beamonte et al., 2020). Squalene could be partially responsible to the health benefits attributed to EVOO, having some associated beneficial properties such as being a natural antioxidant, decreasing serum cholesterol concentrations, also photoprotective, tumor-protective, cardioprotective, and anticancer properties (Gaforio et al., 2015; Kalogeropoulos, 2010). There is a large variation in squalene content in EVOO (150–747 mg/100 g oil) associated to cultivars and agronomic conditions (Lozano-Grande et al., 2018). In the case of moringa oil, no data available about squalene content was found for *M. oleifera*,

TABLE 2 Squalene and sterol content of vegetable oils (mg/100 g of oil)

RT	Compound	Characteristic ions (m/z)	EO	HSO	LO	MO	EVOO
11,2	*IS Cholestane*[*]	217, 357, 372	–	–	–	–	–
9,39	Squalene	425, 341	ND	ND	ND	3.56 (0.60)[a]	1461.13 (72.35)[b]
	Sterols						
15,9	Unknown 1	237, 502	5.70 (0.47)[b]	ND	0.50 (0.01)[a]	ND	0.66 (0.16)[a]
16,78	Cholesterol—TMS	129, 329, 353, 368, 458	ND	ND	1.62 (0.10)[a]	ND	ND
18,60	Unknown 2	365, 455, 470	ND	ND	2.85 (1.97)[a]	ND	ND
20,5	Campesterol—TMS	129, 343, 382, 472	170.62 (3.53)[d]	36.53 (0.55)[b]	87.71 (5.61)[c]	49.47 (1.03)[b]	3.15 (0.17)[a]
21	Stigmasterol—TMS	129, 255, 394, 484	3.35 (0.17)[a]	4.36 (0.25)[a]	19.47 (0.42)[a]	87.50 (2.87)[b]	0.76 (0.09)[a]
21,3	Unknown 3—TMS	129, 379, 469, 484	ND	ND	3.97 (0.20)[a]	ND	ND
22,2	Unknown 4—TMS	75, 129, 343, 386	19.63 (1.70)[b]	ND	1.79 (1.23)[a]	ND	ND
22,5	Unknown 5—TMS	129, 283, 343, 495	10.90 (1.26)[c]	ND	ND	4.86 (0.57)[b]	0.64 (0.07)[a]
22,7	Unknown 6—TMS	129, 283, 484	6.34 (0.40)[b]	ND	0.82 (0.57)[a]	ND	ND
23,8	Sitosterol—TMS	129, 357, 396, 486	153.41 (4.04)[a]	275.36 (5.70)[b]	182.17 (1.31)[ab]	232.76 (14.04)[b]	97.88 (3.07)[a]
24,04	Δ5-Avenasterol—TMS	129, 296, 386	73.55 (1.86)[c]	24.33 (1.09)[a]	54.59 (1.02)[bc]	48.69 (2.62)[ab]	17.91 (1.25)[a]
24,6	Unknown 7	216, 373, 388, 471	2.73 (0.33)[a]	ND	ND	ND	4.36 (0.27)[b]
25,2	Unknown 8—TMS	129, 281, 296, 386	12.01 (1.27)[b]	2.24 (0.24)[a]	2.01 (0.07)[a]	1.87 (0.09)[a]	ND
25,9	Unknown 9	269, 357, 400, 469, 484	6.52 (0.39)[b]	ND	3.83 (2.78)[ab]	2.69 (0.28)[a]	ND
26,25	Unknown 10	75, 255, 471, 486	4.23 (0.43)[b]	ND	ND	2.29 (0.38)[a]	ND
26,6	Cycloartenol	365, 408, 483, 498	ND	14.41 (0.61)[b]	ND	4.22 (0.38)[a]	21.82 (1.08)[c]
27,03	Δ7-Avenasterol	343, 386	19.83 (1.90)[b]	2.70 (0.34)[a]	154.64 (3.18)[c]	4.88 (0.26)[a]	ND
	Total sterols		488.82 (12.83)[d]	359.93 (7.08)[b]	515.97 (11.65)[d]	439.24 (21.06)[c]	147.18 (4.95)[a]

Note: Data correspond to mean values of the quadruplicates per oil type. Standard deviations appear in parentheses. Values with different letters within rows are significantly different ($p < 0.05$) based on post hoc Bonferroni test.

Abbreviations: EO, echium oil; HSO, hempseed oil; LO, linseed oil; MO, moringa oil; EVOO, extra virgin olive oil; TMS, trimethyl silyl ether derivatives; ND, not detected.

[*]Standard.

but there was for other species such as *M. peregrina* with 0.14 mg/100 g oil (Al-Dabbas et al., 2010).

Compounds that could not be identified were reported as unknown and their characteristic ions are shown in Table 2. Identification was not possible due to lack of standards and literature data to compare mass spectra. For those that showed 129 as fragmentation ion, it is hypothesized that they could be silanized compounds. Even though the unknown fraction was found in all samples, the oil with the highest unknown content was echium oil with almost 14%. For the rest of oils, unknown components were between 0.6 and 3.8% of total area (Figure 2).

4 | CONCLUSION

The five analyzed oils showed adequate values for acidity and oxidation status. Echium and hempseed oils showed a high content of PUFAs, especially omega-3 FAs, while moringa oil was rich in oleic acid. Echium oil, hempseed oil, and moringa oil presented higher sterol content than EVOO, but lower than that of linseed oil. Sitosterol was the most abundant sterol in all samples, except in echium oil, where campesterol was the major sterol. Squalene content in EVOO was a unique aspect, since no other oil in this study presented a similar contribution of this compound.

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AUTHOR CONTRIBUTIONS

Katherine Gutiérrez-Luna: Data curation; formal analysis; methodology; software; writing – original draft; writing – review & editing. Diana Ansorena: Conceptualization; funding acquisition; methodology; resources; supervision; writing – review & editing. Iciar Astiasarán:

Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing

CONFLICT OF INTEREST

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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