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Chemical characterization of *Echium plantagineum* seed oil obtained by three methods of extraction

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Abstract: Echium seed oil has been considered an important alternative source of omega 3 fatty acids (n-3 FA) for human consumption. Considering the oxidative instability of n-3 FA richer oils, the objective of this study was to determine the chemical and sensory parameters of the oil obtained from *Echium plantagineum* seeds obtained by three extraction methods (hydraulic press: HYD; continuous screw press: PRESS; and solvent technique: SOLV). Stearidonic acid (C18:4, n3), the most important n-3 FA present in the oil, changed from 12.5% to 12.7%. Regarding the minor compounds, PRESS sample showed the highest concentration of gamma-tocopherol (782.24 mg/kg oil), while SOLV samples presented the highest amount of β-sitosterol (73.46 mg/100 g) with no difference of campesterol concentration (159.56 mg/100 g) among the samples. Higher values of total phenolics (19.65 mg GAE/kg oil) and β-carotene (34.83 mg/kg oil) were also found in the SOLV samples, suggesting the influence of hexane in the extraction of these bioactive compounds. High resolution mass spectrometry identified caffeic acid and its derivatives as the main phenolic compounds present in the echium oil. PRESS sample showed the best oxidative stability as measured by PV (0.61 mmol/kg oil) and malondialdehyde (173.13 μmol), probably due to faster time of processing compared to HYD and SOLV samples. Our data showed that the extraction method changed the chemical composition of the minor compounds in the echium oil, but these alterations did not reduce its nutritional quality or sensory acceptability.

KEYWORDS
echium, extraction, oil, phenolic, sensory, sterol

Practical Application: Echium oil represents a great potential source of omega 3 fatty acids, but there is not enough information about its oxidative stability and chemical composition, especially toward minor compounds. Our study characterizes echium oil composition obtained from three extraction methods, contributing to amplify the technical information about this important alternative oil for human consumption.

1 | INTRODUCTION

The consumption of omega-3 fatty acids (n-3 FA), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has been associated with some health benefits, including modulation of immune and inflammatory responses, supporting neurological development and improvement of cardiovascular health (Bilinsk et al., 2019). Fish oil is the most used source of n-3 FA (Adarme-Vega et al., 2014). Nonetheless, the increase in the global demand for n-3 FA supplementation promotes immense pressure on marine stocks. Moreover, fish oil consumption is restrained by lifestyle and personal choices (Ryan & Symington, 2015). For these reasons, the food industry has searched for new alternatives sources to fish oil as n-3 FA supplementation (Adarme-Vega et al., 2014).

Echium seed oil (*Echium plantagineum*, a weed from the Boraginaceae family), contains about 70% polyunsaturated fatty acids (PUFAs), being about 14%–16% stearidonic acid (SDA) and 30% α-linolenic acid (ALA) (Castejón et al., 2018). SDA is the first product of ALA desaturation by Δ6-desaturase in the pathway of EPA and DHA biosynthesis. Δ6-Desaturase is a limiting enzyme that also catalyzes the conversion of linoleic acid (LNA) into arachidonic acid (ARA). The studies have reported in vivo conversion rate of 14%–16% from SDA to EPA, and only 5%–6% from ALA to EPA (Baker et al., 2016; Prasad et al., 2020). In a study carried out by Greupner et al. (2019) in humans, it was observed that DHA, EPA, and docosapentaenoic acid (DPA) concentrations increased after 72 h (21% to DHA and 47% to EPA compared to baseline values) after a single-dose ingestion (26 g) of echium seed oil.

The commercialization of echium oil is approved by Food and Drug Administration and European Commission and is classified as a novel food ingredient (Castejón et al., 2018). However, in Brazil, echium oil has not been approved by the Regulatory Agency (ANVISA) based on insufficient data and information (ANVISA, 2018, 2020). Therefore, this study can also contribute to further the approval of this important product in our country.

The chemical composition of the oil depends on several factors, including genetic background, agricultural practices, the method applied in the extraction, and the storage conditions (Moghaddam & Mehdizadeh, 2017). For example, it is known that the extraction conditions may affect the fatty acid profile, phytosterol, tocopherol, and phenolic compounds concentration, which will influence the sensory and oxidative stability of the oil (Catalán et al., 2017; Tasan et al., 2011).

The commercial production of vegetable oils has been based on mechanical crushing followed by chemical solvent extraction (SOLV). Pressing can be carried by utilizing automated systems, such as a continuous screw press (PRESS), or manual devices like hydraulic press (HYD). The former involves an increase in temperature during the process (≈60°C) compared to the HYD (≈40°C). However, the exposition of the seeds to higher temperatures in the PRESS used to be faster (50–150 kg/h) than using HYD (40–100 kg/h) (Akinoso et al., 2009; Fu et al., 2014; Marques et al., 2015; Willems et al., 2008). Both press extraction methods have been deemed healthy because these techniques maintain the naturally present bioactive compounds and are environmentally sustainable (Gaber et al., 2019; Ma et al., 2019). The crushing method application is simple, presents low operation steps, the phytochemicals are preserved, and produces a good quality and oxidative stable oil (Delfan-Hosseini et al., 2017; Nie et al., 2020).

However, the press techniques do not extract all the oil content from the seeds, resulting in a more expensive final product. Thus, to increase the oil yield extraction, solvents can be applied to remove the residual oil still present in the cake after pressing (Tasan et al., 2011). In some countries, the most applied method in industrial production of echium oil is the SOLV with hexane followed by vacuum distillation (Castejón et al., 2018). Although this method is more efficient, it has some limitations regarding high solvent consumption, more expensive operation costs, a relatively high number of processing steps, and lower quality of oil due to the undesired components extracted from the cell walls, solvent residue, presence of free fatty acids, and low oxidative stability (Delfan-Hosseini et al., 2017; Ixtaina et al., 2011; Nie et al., 2020; Willems et al., 2008).

Taking into account the health claim associated with the echium oil toward cardiovascular diseases (Botelho et al., 2015; Shen & Ge, 2018), our objective was to evaluate the influence of three extraction methods on chemical composition, sensory acceptability, and oxidative stability of echium seeds oil.

2 | MATERIALS AND METHODS

2.1 | Materials

Echium plantagineum seeds were purchased from De Wit Speciality oils (ED De Wall, the Netherlands), and kept under refrigeration (8°C) in the dark until extraction. Hexane used for oil extraction was obtained from Labsynth Produtos para Laboratórios Ltda (Diadema, SP, Brazil). Isooctane, isopropanol, methanol, hexane, and butanol were obtained from Merck & Co. (Whitehouse Station, NJ, USA). The organic solvents were

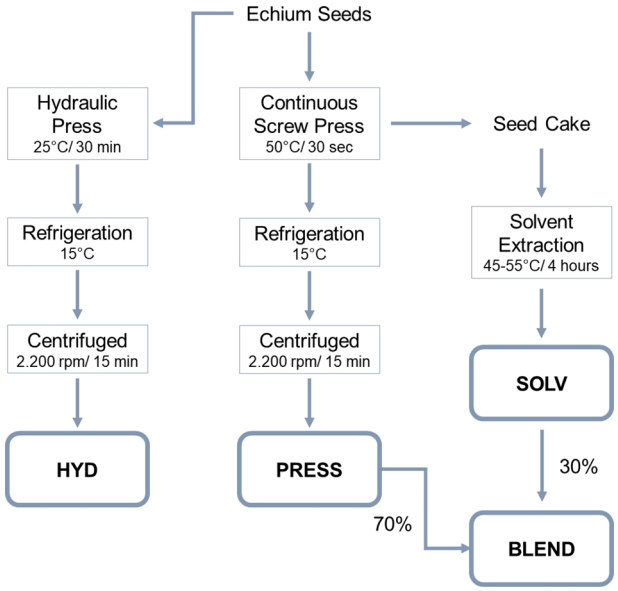

FIGURE 1 Echium oil extraction processes

high-performance liquid chromatography grade. Tocopherol, fatty acids, plant sterols, cumene hydroperoxide (CHP), 1,1,3,3-tetraetoxipropano (TEP), gallic acid, and β-carotene standards analyzed in this study were purchased from Sigma-Aldrich (Sigma Chemical, St. Louis, MO, USA).

2.2 | Oil extraction

Three oil extraction processes were carried out in this study: PRESS, SOLV, and HYD. In the first extraction, seeds (70 kg) were screw pressed (Scott Tech, ERT 50, Vinhedo, São Paulo, Brazil), yielding 14.2 kg of oil. During the process, the oil temperature was kept below 50°C, followed by fast refrigeration (15°C). The oil was centrifuged (IEC, Centra GP8R, London, UK) at 2200 rpm/15 min at room temperature. This oil was identified as PRESS. The echium seed cake (55 kg) resulting from the screw press was submitted to SOLV in a pilot-scale extractor (Ecirtec Ltd., Bauru, Brazil), using hexane for 5 h, giving 3.85 kg of oil. During extraction, the temperature was kept between 45 and 55°C. This oil was identified as SOLV. Then, the SOLV oil was blended with PRESS oil (30:70 w/w) resulting in the BLEND oil.

In the third process, echium seeds (1 kg) were manually pressed using a HYD (Carver Inc., Wabash, IN, USA), yielding 0.2 kg of oil. The temperature was kept at 25°C. The oil was immediately refrigerated to 15°C and centrifuged at 2200 rpm/15 min. This oil was identified as HYD. The oils were stored in amber flasks in the dark at 8°C. Figure 1 summarizes the three processes applied in this study to extract the oil.

2.3 | Fatty acid profile

Oil samples were esterified according to Shirai et al. (2005). Fatty acid quantification was carried out using gas chromatography equipped with a mass spectrometry (GC–MS; Agilent 7890A GC, Santa Clara, CA, USA). A fused silica capillary column (J&W DB-23 Agilent 122−236; 60 m × 0.25 mm inner diameter) was used to separate the fatty acids compounds. The injection volume was 1 µl. High-purity helium was used as the carrier gas at a flow rate of 1.3 ml/min with a split injection of 50:1. The oven temperature was programmed from 80 to 175°C at a rate of 5°C/min, followed by another gradient of 3°C/min to 230°C, which was maintained for 5 min. The GC inlet and transfer line temperatures at 250 and 280°C, respectively. GC-MS was performed using 70 eV EI in scan acquisition and recorded as TIC. All mass spectra were acquired over an m/z range of 40−500. Fatty acids in the samples were tentatively identified by National Institute of Standards and Technology (NIST) mass library (Gaithersburg, MD, USA) and by comparing the retention times with those of a standard mixture of fatty acid methyl esters (FAME 37 - Sigma-Aldrich). Results were expressed as % of fatty acids and carried out in triplicate.

2.4 | Analysis of the minor compounds

Tocopherol content was determined by HPLC (Agilent 1200) using a modified method proposed by Gliszczynska-Swiglo and Sikorska (2004) using a Zorbax Eclipse Reverse-Phase C18 column (150 mm × 4,6 mm; 5 µm) with pre-column LC8-D8 (Phenomenex AJ0-1287, Torrance, CA, USA). Tocopherol was identified by comparing their retention times with those of corresponding standards. Samples were analyzed in duplicate, and results expressed as mg/kg oil.

Plant sterols were determined according to Laakso (2005). Echium oil was weighed (300 mg), mixed with 0.25 mg of IS (5α-cholestan-3β-ol) diluted in 25 µl of hexane: isopropanol (3:2) and evaporated to dryness through a nitrogen stream. Derivatives of phytosterols were separated on a fused silica capillary column coated with 5% phenyl/95% dimethylpolysiloxane (J&W HP-5 30 m × 0.32 mm × 0.25 µm; Agilent Technologies Inc.), using gas chromatography equipped with a mass spectrometry (GC–MS, Agilent 7890A GC). Phytosterols samples were tentatively identified by NIST mass library. Reference standards were purchased from Sigma and used to identify the peaks. Quantification was calculated based on a standard curve prepared with stigmasterol, using the area ratio sterol/IS. The results were expressed mg/100 g oil and carried out in triplicate.

The analysis of total carotenoids was determined according to Tura et al. (2007). Echium oil (1 g) was weighed and then dissolved in isooctane up to 10 ml of final volume. The absorbance was read in a microplate at 475 nm using a Synergy HTX Multi-Detection Microplate Reader (BioTek Instruments Inc., Winooski, VT, USA). The method proposed by Lichtenthaler (1987) was used for the extraction of total chlorophyll.

2.5 | Phenolic compounds

Phenolic compounds were extracted according to Ballus et al. (2015). The absorbance at 760 nm was measured using a Synergy HTX Multi-Detection Microplate Reader (BioTek Instruments Inc.). The quantitative results were calculated using an analytical curve of gallic acid and expressed as milligram of gallic acid equivalents per kilogram of oil (mg GAE/kg oil). The oil extracts were analyzed in triplicate.

The characterization of phenolic composition was carried out by high resolution mass spectrometry (LC-ESI – QTOF-MS) according to Ferro et al. (2019). Analysis by liquid chromatography was performed using a Shimadzu chromatograph (Shimadzu Co., Tokyo) equipped with an LC-30AD quaternary pump, SPD-20, a photodiode array detector (PDA) and a SIL-30AC autoinjector. The chromatographic separation was performed on a Phenomenex Luna C18 column (4.6 × 250 mm × 5 µm). The electrospray ionization (ESI) of the high resolution mass spectrometer MAXIS 3G - Bruker Daltonics (Bruker Daltonics, Bremen, Germany) was operated in negative mode with a resolution of 30,000 to 400 m/z. The operating conditions were: Nebulizer: 2 Bar, Dry gas: 8 L /min, Temp: 200°C, HV: 4500 V. The mobile phase consisted of a mixture of two solvents: (A) water and 0.25% formic acid and (B) acetonitrile 80%, 0.25% formic acid, and 19.75% water. The mobile phase flow rate was 1.0 ml/min, and the gradient started at 10% solvent B, rose to 20% B in 10 min, 30% B at 20 min, 50% B at 30 min, 50% B at 32 min, 90% B at 38 min, 10% B at 45 min and finished at 55 min. Before the analyses, an external calibration performed to verify the accuracy of the accurate masses. Data analysis was performed using MAXIS 3G software—Bruker Daltonics 4.3. The compounds were identified by comparing the exact masses, MS/MS mass spectrum, and molecular formulas found with those available in the literature.

2.6 | Oxidative quality

The IUPAC Official Method (1997) was used to determine the oil sample acidity. The peroxide value (PV) was determined according to Shanta and Decker (1994), while malondialdehyde (MDA) concentration was carried out according to McDonald and Hultin (1987) and Hong et al. (2000) methods, with some modifications, using HPLC (Agilent 1200).

2.7 | Surface color analysis

Color was measured using a Hunter Color Lab colorimeter, model Color Quest XE (Hunter Associates Laboratory Inc., Reston, VA, USA). Universal Software 4.10 was used for calibration against black and white reference tiles with an illumination of D65 and a 10° angle.

2.8 | Sensory analysis

Sensory analysis was applied to evaluate color, odor, taste, and general appearance of the oil samples. The acceptability of each type of oil was assessed through a structured hedonic scale of nine (9) points, with the maximum acceptability "9 - I liked it very much" and the minimum acceptability "1 - I really disliked it," according to Meilgaard et al. (2015). The panel was formed by 50 untrained tasters who signed the Informed Consent Form (ICF), approved by the Research Ethics Committee of the Faculty of Pharmaceutical Sciences of the University of São Paulo. Each taster received 5 ml each oil in a transparent plastic container encoded with three random digits, and samples were offered in a random order.

2.9 | Statistical analysis

Results were compared using one-way analysis of variance followed by Tukey HSD. Values were expressed as mean ± SD. A p-value of 0.05 was adopted to reject the null hypothesis. Calculations were performed using software Statistica v.13 (TIBCO Software Inc., Palo Alto, USA), and graphs were made using GraphPad Software (San Diego, CA, USA), and GeoGebra 3D Software (Orlando, FL, USA).

3 | RESULTS AND DISCUSSION

3.1 | Chemical composition of the oils

Table 1 presents the chemical composition of the oil samples obtained from the three extraction methods. Echium oil can be characterized as a good source of non-marine n-3 FA, due to its high proportion of α-linolenic (≈28%) and stearidonic (≈13%) acids (Table 1), also observed in other

TABLE 1 Chemical composition of the echium oil samples according to the extraction methods

	HYD	PRESS	SOLV	*p*-value
Fatty acids (%)				
Palmitic acid (C16:0)	15.33 ± 0.23	16.01 ± 0.84	15.60 ± 0.39	*0.367*
Heptadecanoic acid (C17:0)	0.14 ± 0.01	0.14 ± 0.00	0.14 ± 0.01	*0.860*
Stearic acid (C18:0)	9.64 ± 0.13	10.09 ± 0.52	9.74 ± 0.29	*0.329*
Oleic acid (C18:1 n9)	13.40 ± 0.33^{b}	12.86 ± 0.22^{a}	13.59 ± 0.17^{b}	*0.026*
Linoleic acid (C18:2 n6)	11.09 ± 0.08	11.05 ± 0.20	11.37 ± 0.12	*0.066*
γ-linolenic acid (C18:3 n6)	8.73 ± 0.05	8.64 ± 0.16	8.83 ± 0.08	*0.195*
α-linolenic acid (C18:3 n3)	28.20 ± 0.14	28.05 ± 0.51	27.48 ± 0.22	*0.079*
Stearidonic acid (C18:4 n3)	12.73 ± 0.04	12.46 ± 0.26	12.49 ± 0.09	*0.154*
Arachidic acid (C20:0)	0.13 ± 0.01^{ab}	0.11 ± 0.00^{b}	0.13 ± 0.01^{a}	*0.032*
Eicosenoic acid (C20:1 n9)	0.61 ± 0.01	0.59 ± 0.03	0.63 ± 0.03	*0.100*
Tocopherol (mg/kg oil)				
Gamma-tocopherol	741.69 ± 5.10^{a}	782.24 ± 6.35^{b}	741.46 ± 2.54^{a}	*0.006*
Plant sterol (mg/100 g oil)				
β-sitosterol	53.89 ± 2.86^{a}	59.91 ± 11.02^{ab}	73.47 ± 3.43^{b}	*0.031*
Campesterol	137.22 ± 3.33	149.79 ± 10.09	159.56 ± 11.62	*0.062*
Total phenolic compounds (mg GAE/kg oil)	4.77 ± 0.00^{a}	15.67 ± 0.28^{b}	19.65 ± 0.27^{c}	*<0.001*
Beta-carotene (mg/kg oil)	4.08 ± 0.00^{a}	10.54 ± 0.07^{b}	34.83 ± 0.19^{c}	*<0.001*
Chlorophyll (mg/kg oil)	0.99 ± 0.02^{a}	1.02 ± 0.03^{b}	1.01 ± 0.02^{b}	*0.008*

Note: Values are expressed as the mean ± standard deviation. Means followed by the same letter are not different ($p < 0.05$).

studies (Gray et al., 2010; Nogueira et al., 2019). This type of extraction process does not promote changes in the fatty acid composition, since the level of oxidation during the process can be controlled by antioxidant compounds naturally present in the oil (Shahidi & Zhong, 2010), protecting PUFA from oxidation. Thus, the few changes observed in Table 1 have no practical relevance. It has been suggested that the extraction method could affect the fatty acid composition, mainly when organic solvents are compared with other extraction techniques, due to the solubility of the different lipid-type solutes in the solvent (Qin & Zhong, 2016; Vasquez et al., 2021). However, while most of the studies have not observed relevant differences in fatty acids profile when extraction methods are compared, significant changes in minor compounds have been reported using vegetable oils (Tai & Brunner, 2019; Uquiche et al., 2012; Vasquez et al., 2021).

The PRESS oil showed a higher concentration of gamma-tocopherol than HYD and SOLV samples, while no alpha- or delta-tocopherol were found in the samples (Table 1). Gamma-tocopherol has been observed as the major isomer present in the echium oil, corresponding to 92.6% of total tocopherols, according to Gray et al. (2010). The reduction of gamma-tocopherol found in HYD and SOLV samples can be a consequence of oxidation because the seeds were processed for 30 min and 4 h, respectively, while the screw continuous pressing took only 30 s. Degradation of tocopherol during oil oxidation has also been shown in other studies (Fernández-Cuesta et al., 2014; Wang et al., 2010; Zajdenwerg et al., 2011), confirming its antioxidant action.

In terms of plant sterols, beta-sitosterol was found in a higher amount ($p = 0.034$), while campesterol also showed a trend ($p = 0.062$) of being higher in the SOLV sample compared to HYD samples. Other authors have also reported that oils extracted with solvents presented a higher concentration of plant sterols than oils obtained by crushing due to a greater interaction when solvents are used in place of oils (Aguirre et al., 2014; Fernández-Cuesta et al., 2014).

Total phenolic compounds, β-carotene, and chlorophyll were also higher in the SOLV samples probably due to the use of an organic solvent (hexane) in the oil extraction and the solubility of these compounds in this solvent (Bobbio & Bobbio, 1995). Uquiche et al. (2012), Ghazani et al. (2014), and Norshazila et al. (2017) observed that phenolics, among other minor compounds, were better co-extracted from rapeseed cake using hexane as solvent compared with supercritical CO_2 extraction. However, hexane causes air pollution, toxicity, and harmfulness (Kumar et al., 2017; Qin & Zhong, 2016), making it necessary to investigate other green alternatives. Although

TABLE 2 Chemical composition of echium oil compared with other vegetable oils

	Echium[a]	Canola	Soy	Flaxseed	Perilla	Hemp	Chia	Walnut	Sunflower
Fatty acids (%)									
Total n-3 FA	40.51	9.20	9.40	61.08	61.93	16.70	63.64	9.78	0.30
Total PUFA	60.20	28.70	64.60	73.46	76.25	75.46	83.48	71.71	54.60
Tocopherol (mg/kg oil)									
Alpha-tocopherol	—	154.10	179.30	4.35	33.52	32.20	5.10	19.25	206.50
Gamma-tocopherol	782.24	338.40	636.80	432.95	453.88	733.80	70.38	364.53	23.90
Delta-tocopherol	—	—	330.30	3.40	10.85	28.70	1.48	350.14	19.40
Plant sterol (mg/kg oil)									
Campesterol	1497.90	3244.00	1191.40	651.73	186.58	505.69	387.77	26.53	266.50
β-sitosterol	599.10	4919.00	2060.80	1376.33	3186.12	1905.07	2433.56	998.95	2300.10
Stigmasterol	—	106.00	772.80	249.73	105.25	100.23	177.47	2.22	319.80
References	—	Ghazani et al., 2014	Ghazani and Marangoni, 2013	Waszkowiak et al., 2020	Li et al., 2015	Montserrat-de la Paz et al., 2014	Shen et al., 2018	Gao et al., 2018	Ghazani and Marangoni, 2013

[a]Data from PRESS sample.

all the triacylglycerol is miscible in hexane, it has been shown that the extraction of minor compounds can change if they are free or bound to body structures (Baumler et al., 2010). The difference between PRESS and HYD could be a consequence of higher oxidation of these compounds in the hydraulic pressing, as before observed to gamma-tocopherol, since the extraction yield was the same for both methods (20%). However, as commented by Vasquez et al. (2021), when yield is increased, the concentration of bioactive compounds obviously decreases by the triglyceride dilution effect. Thus, results from SOLV samples should take this dilution factor into account, besides the known effect of the hexane increasing minor compounds' extraction.

Compared with other vegetable oils (Table 2), the chemical composition of echium oil, independent of extraction process, suggests that echium oil is an important source of PUFA, in special n-3 FA, being like perilla, flaxseed, and chia oils, but showing SDA concentration as the biggest advantage. Among tocopherol isomers, echium oil shows a concentration of gamma-tocopherol like hemp and soybean oils, while its profile of plant sterols differed from the others, due to its high content of campesterol, low amount of beta-sitosterol, and absence of stigmasterol.

The qualitative analysis of the phenolic compounds in PRESS and HYD samples determined by LC-ESI – QTOF-MS is shown in Table 3. Eight compounds were identified in the samples, including caffeic acid hexoside I [M - H - 341], caffeic acid hexoside II [M - H - 341], caffeoyl-p-coumaroylquinic acid [M - H - 499], caffeic acid hexoside dimer [M - H - 683], caffeic acid hexoside derivative [M - H - 401], rosmarinic acid [M - H - 359], caffeic acid [M - H - 179], and 5,7-dihydroxychromone [M - H - 177]. Rosmarinic acid and caffeic acid have also been reported in *Trichodesma indicum* oil and *Cordia sebestena*, which belong to the Boraginaceae family (Dai et al., 2010; Górnaś et al., 2019). Caffeic acid is also found in chia oil (Ixtaina et al., 2011), caffeoyl-p-coumaroylquinic acid has been found in *Cichorium intybus* and *Inula helenium*, plants of the Asteraceae family (Jaiswal et al., 2011), and 5,7-dihydroxychromone has been reported in peanut hulls (Sheng et al., 2014). The antioxidant activity of these phenolic compounds toward reactive species by hydrogen transfer or transition metal chelation is widely known (Fraga et al., 2010; Jaganath & Crozier, 2010). Thus, the phenolic compounds shown in Table 3 confers to the echium oil a better oxidative stability during storage.

3.2 | Oxidative stability of the oils

Figure 2 presents the primary (Figure 2a) and secondary (Figure 2b) markers of oxidation, represented by hydroperoxides (PV) and MDA concentrations, respectively, while Figure 2c shows the acidity of the oil samples. The hydroperoxide values were below the legal limits allowed to crude oil (Codex, 1999), suggesting that the degree of oxidation in the three extraction methods was inside the normal range applied in the industrial extraction. As before commented, the time by which seeds were processed (HYD: 30 min and SOLV: 4 h vs. PRESS: 30 s) showed a higher influence than temperature in terms of PV.

TABLE 3 Phenolic compounds found in the echium oil according to the extraction methods

	Retention time (min)	Molecular mass	*m/z* fragments
CONTINUOUS SCREW PRESS			
Caffeic acid hexoside I	2.3	341,1126	101,0250 (100); 113,0270 (81), 179,0583 (43)
Caffeic acid hexoside II	2.5	341,1130	157,0374 (100); 119,0384 (81); 179,0579 (39)
Caffeoyl-p-coumaroylquinic acid	2.5	499,1585	157,0385 (100); 341,1117 (21)
Caffeic acid hexoside dimer	2.5	683,2322	341,1127 (100); 161,0470 (5)
Caffeic acid hexoside derivative	2.5	401,1348	341,1124 (100); 179,0583 (84)
Rosmarinic acid	30.3	359,0811	161,0261 (100); 197,0475 (30); 179,0364 (26)
HYDRAULIC PRESS			
Caffeic acid hexoside	2.4	341,1146	157,0391 (100)
Caffeic acid hexoside derivative	2.4	401,1364	341,1134 (100)
5,7-dihydroxychromone	25.0	177,0128	132,0255 (100); 135,0477 (75)
Caffeic acid	30.3	179,0373	134,0383 (100); 135,0309 (30)

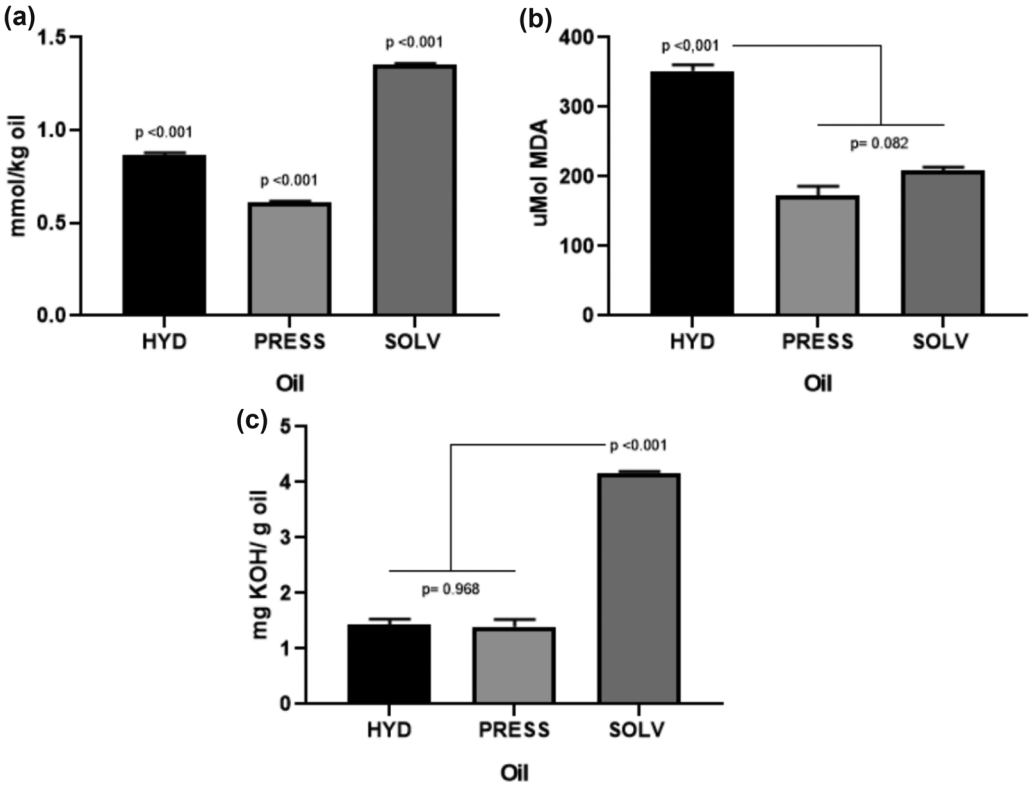

FIGURE 2 Oxidative quality of the oil samples: (a) Peroxide value, (b) MDA, and (c) Acidity. Values were expressed as mean and SEM

However, the HYD sample showed the highest concentration of MDA. This result, associated with the lower concentration of gamma-tocopherol and phenolic compounds, suggests that among the three methods, HYD promoted more oxidation than the two others. Although MDA of the HYD sample was higher, it agreed with the values found in other edible oils (Custodio-Mendoza et al., 2019). The higher oxidative susceptibility of echium oil has been reported in previous studies carried out in our group (Espinosa et al., 2015; Nogueira et al., 2019; Roschel et al., 2021).

In our last study, both oils obtained by screw pressing or SOLV were protected from oxidation only by decreasing the storage temperature (Roschel et al., 2021). When the temperature reduction was not possible, antioxidants and a mixture with high oleic oil were applied to protect the

TABLE 4 Sensory analysis of echium seed oil

	HYD	PRESS	SOLV	BLEND	*p*-value
Color	6.12 ± 1.91	6.70 ± 1.69	—	5.94 ± 1.85	*0.103*
Odor	5.10 ± 1.98	5.06 ± 1.73	—	5.54 ± 1.62	*0.316*
Flavor	5.66 ± 2.16	5.68 ± 2.06	—	5.38 ± 2.18	*0.722*
Acceptability	6.12 ± 1.79	6.04 ± 1.74	—	5.68 ± 1.79	*0.411*
Color					
L*	11.32 ± 0.62^{a}	18.00 ± 0.28^{b}	7.24 ± 0.06^{a}	11.08 ± 3.04^{a}	*0.010*
a*	−0.83 ± 0.28^{a}	−0.56 ± 0.20^{ab}	2.97 ± 0.40^{c}	0.96 ± 0.58^{b}	*0.002*
b*	10.31 ± 0.14^{c}	13.23 ± 0.13^{d}	5.10 ± 0.15^{a}	6.02 ± 0.03^{b}	*<0.001*

Note: Values are expressed as the mean ± standard deviation. Means followed by the same letter are not different ($p < 0.05$).

echium oil from oxidation. Better results can be obtained when the oxidation is controlled since the extraction oil step, using a continuous screw instead of the HYD.

Acidity was higher in the SOLV sample, being above the legal limit allowed to crude oil (Codex, 1999) due to the longer time of process, promoting hydrolysis of triglycerides, increasing the free fatty acids, and in consequence, higher acidity usually associated to the loss of nutritional value and unpleasant sensory changes (Hassanzadazar et al., 2018).

3.3 | Sensory analysis and color determination

In industrial practice, the oil obtained from crushing is mixed with the oil obtained after SOLV (Tasan et al., 2011). For this reason, a mixture containing 70% PRESS + 30% SOLV, designed as BLEND, was prepared to be submitted to sensory and instrumental color analysis. The extraction method promoted a change in the color of the samples (Table 4) determined by the CIELab system. PRESS was the lightest sample. Positive a* values observed in the SOLV and BLEND samples could be associated with the higher amount of beta-carotene present in these oils. However, the instrumental color differences did not change the color, odor, flavor, and general sensory acceptability among the samples. The *p*-value of the tasters' judgment ($p = 0.374$) showed that there was a consensus that all samples presented an acceptability score between 5 (neither like nor dislike) and 6 (like slightly), which is good in terms of sensory analysis of pure oils. A detailed descriptive sensory analysis should be performed in further studies to drive the extraction method choice toward the highest oil acceptability.

4 | CONCLUSIONS

Comparing the three extraction methods, our data showed that there was no change in the fatty acids profile, while a higher concentration of minor bioactive compounds was found in the PRESS sample, due to the lower oxidative damage compared to HYD sample. The use of hexane also contributed to increase the minor compounds concentration, but it is important to highlight that other options than hexane should be evaluated looking for non-pollution alternatives.

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AUTHOR CONTRIBUTIONS

Giovanna Calixto Garcia Carlini: Formal analysis; methodology; writing—review and editing. Roseli Aparecida Ferrari: Formal analysis; methodology. Helton Cherubim Ota: Formal analysis; methodology. Tayse Ferreira Ferreira da Silveira: Conceptualization; writing—review and editing. Inar Alves Castro: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; writing—original draft, review, and editing.

CONFLICT OF INTEREST

The authors wish to confirm that there are no known conflicts of interest associated with this work and there has been no significant support for this publication that could have influenced its outcome.

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