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Sequential Separation of Essential Oil Components during Hydrodistillation of Azorean *Cryptomeria japonica* Foliage: Effects on Yield, Physical Properties, and Chemical Composition

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Abstract: The hydrodistillation (HD) process is used to obtain and fractionate essential oils (EOs). In this study, we aimed to evaluate, for the first time, the effects of six different HD timeframes (HDTs: 0–2, 2–10, 10–30, 30–60, 60–120, and 120–240 min) on the yield, physical properties, and chemical profile of Azorean *Cryptomeria japonica* foliage (Az–CJF) EO. An Az–CJF EO obtained by a typical HD over 4 h was used as a control sample, yielding 0.82%, *w*/fresh weight (f.w.), and containing eighty-nine components, as determined by GC–MS. The EO fraction yield revealed a narrow range (0.06–0.18%, *w*/f.w.), with ca. 50% obtained within the first hour. Monoterpene hydrocarbons dominated in Fr1 and Fr2 (92 and 45%, respectively, mainly α-pinene) while oxygen-containing sesquiterpenes prevailed in Frs. 3–6 (42–62%, mainly elemol and eudesmol isomers). Furthermore, Fr2 and Fr3 were the richest in oxygen-containing monoterpenes (9 and 7%, respectively, mainly bornyl acetate) and in sesquiterpene hydrocarbons (6 and 5%, respectively, mainly δ-cadinene), while Fr4 and Fr5 had higher amounts of diterpene hydrocarbons (ca. 22% both, mainly phyllocladene) and Fr6 exhibited the highest oxygen-containing diterpenes content (4%, mainly nezukol). In addition, regression models were established to predict EO yield, HD rate, and composition (major components) for a given HDT. As a result of this study, specific EO fractions can now be targeted in Az–CJF EO by adjusting the HDT. Hence, these findings can help reduce distillation time and, thus, operating costs associated with the HD process. It can also meet specific market demands due to the differential composition of the obtained EO fractions. In turn, this contributes to increasing the commercial potential of *C. japonica* EO.

Keywords: Azores; *Cryptomeria japonica*; biomass waste valorisation; hydrodistillation; essential oil fractionation; high-added value products; GC–MS analysis; regression models

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1. Introduction

Cryptomeria japonica (Thunb. ex L. f.) D. Don (Cupressaceae), commonly known as Japanese cedar or *sugi* in Japanese, is a coniferous forest tree native to Japan. Due to its timber quality, including natural decay-resistant properties, *C. japonica* is extensively cultivated in plantation forests in Japan, China, Korea and Taiwan, as well as in the Azores archipelago (Portugal), where this species (called "criptoméria" in Portuguese) covers 60%

of the total wood-producing forest area. In addition, this beautiful and aromatic evergreen tree is widely grown as an ornamental tree in other temperate zones, including Britain, North America, Nepal, and India [1,2]. Thus, a considerable amount of biomass residues is generated from the wood processing industry and forest management, such as *C. japonica* foliage (CJF), a rich source of valuable essential oils (EOs) that can be recovered in an economical and environmentally sustainable manner without competing for land areas. However, *C. japonica* biomass residues (CJBR) remain a relatively underutilized resource, compared to its potential application in integrated pest management programs or use in developing natural health and cosmetic-related products, as highlighted in our previous critical reviews on the phytochemistry and biological properties of the organic extracts [3] and EOs [4] of *C. japonica* from different geographical origins.

EOs play a key role in plant protection [5] and have been used therapeutically since ancient times [6] due to their high effectiveness. Currently, there has been renewed interest in their usage [7]. Global EO production has increased substantially in the last few decades, which can be attributed to several key factors, including (i) the wide range of biological and pharmacological (including neuroprotective) activities exhibited by EOs and, hence, its extensive use in several industries, namely food, agrochemical, textile, perfumery, cosmetics and hygiene, traditional medicine, and complementary therapies (such as aromatherapy); (ii) increasing consumer demand for safe, eco-friendly, and effective natural products; and (iii) great interest from the scientific community in the discovery of novel drugs or botanical pesticides from natural resources, such as EOs [4,5,8–12]. Nevertheless, only a few EOs and their components (EOCs) or derivatives thereof have been approved as drugs. Thus, the therapeutic potential of EOs is still under scrutiny [13]. Particularly, the fractions and/or individual EOCs of crude EOs can be valuable sources of antimicrobial agents for fighting infectious human and animal diseases or in green plant protection and food preservation [13–18].

An EO is defined by the International Standardization Organization (ISO) as an odorous product (usually a complex mixture) obtained from raw vegetable material (a plant or any of its parts) by hydrodistillation (HD), steam distillation (SD) or dry distillation, or from the epicarp of *Citrus* fruits by a mechanical process without heating [19,20]. Our continuous study on CJBR EOs adheres to the ISO definition since it permits a clear distinction between EOs and EO-like products such as the volatiles isolated by several innovative green techniques (e.g., supercritical fluid extraction) [13]. It is noteworthy that the yield and chemical composition of EOs and, consequently, their quality, specific commercial applications, and price are significantly affected by several factors besides the plant species, such as the geographical location of the plants, environmental parameters; the plant part, age, and developmental stage; post-harvest drying and storage, and the extraction method/protocol used. Previous works on *C. japonica* EOs have reported the variability of its chemical composition according to the different geographical origins of the plants [4]. In addition, our recent research on Azorean *C. japonica* EO revealed that their composition and bioactivity are also influenced by the different distillation methods used [1,21], as well as by the different plant parts analysed [15].

Given this context and considering that the HD process can be used to fractionate EOs and that increasing the concentration of desirable EOCs will improve the product's commercial value, the present comparative study examined, for the first time, the chemical profile, yield, and physical properties (density and colour) of six different EO fractions collected during HD of Azorean CJF (Az–CJF) in sequential timeframes (0–2, 2–10, 10–30, 30–60, 60–120 and 120–240 min). In addition, we obtained Az–CJF EO by HD over 4 h to be used as a control sample. Furthermore, regression models were developed to predict EO yield, HD rate (HDR), and composition (major EOCs) for a given HD timeframe (HDT). Overall, the results of this study can help reduce the distillation time and operating costs associated with the HD process, as well as produce EO fractions with unique compositions with different commercial applications, which increases the value of the *C. japonica* EO industry.

2. Materials and Methods

2.1. Plant Material

Aerial parts of *C. japonica* at the pollination stage were collected in early March 2023 (winter season) from a tree population located on Lomba da Maia (latitude 37°48′32.7″ N, longitude 25°20′6.5″ W, altitude 440 m) in the northeast region of São Miguel Island (Azores archipelago, Portugal). A voucher specimen (number AZB 4581) was deposited in the Herbarium AZB—Ruy Telles Palhinha of the University of the Azores. The plant material cut off from healthy plants was immediately brought to a laboratory at the same university. Then, the strobili attached to the foliage were removed. The fresh foliage sample was immediately stored at −20 °C until further usage in the HD process. A portion of the fresh foliage sample was air-dried until a constant weight for moisture content determination, which was 56% (*w*/*w*). Before the HD process, the foliage sample was cut into small pieces (2–3 cm in length).

2.2. Essential Oil Extraction and Fractionation by Hydrodistillation Method

A fresh CJF sample (300 g) was placed in a 5-L round bottom flask containing 3 L of tap water and connected to a Clevenger-type apparatus. HD was performed over a period of 4 h, starting with the first distillate drop. EO fractions were collected in the following HDTs: 0–2, 2–10, 10–30, 30–60, 60–120, and 120–240 min (Frs. 1–6), captured sequentially without interrupting the HD process. In addition, a control EO sample was collected from a non-fractionated HD (0–240 min) for comparison purposes. The collected fractions and control were dehydrated with anhydrous sodium sulphate, filtered, measured on an analytical scale, and stored in sealed amber vials at 4 °C until further chemical analyses. Each HD process was performed in triplicate. The EO yield (%) was calculated as the EO mass (g) per 100 g of fresh weight (f.w.) of CJF and HDR as the EO mass accumulated per min (EO mg/min).

2.3. Essential Oil Composition Analysis

Gas chromatography–mass spectroscopy (GC–MS) analyses were conducted with a Shimadzu GCMS–QP2010 Ultra gas chromatograph–mass spectrometer (Shimadzu Corp., Tokyo, Japan), using a ZB–5MSPlus (5% phenyl, 95% methyl siloxane) capillary column (60 m length × 0.25 mm i.d., with a film thickness of 0.25 µm) from Phenomenex Inc. (Torrance, CA, USA). Helium was the carrier gas at a flow rate of 36.3 cm/s. The EO was dissolved in methylene chloride (0.1 g/mL), and the injection volume was 0.1 µL at a split ratio of 24.4:1. The injector and detector temperatures were adjusted to 260 °C. The oven temperature program was set between 50 °C and 260 °C at 2 °C/min and then held at 260 °C for 5 min. The transfer line and ion source temperatures were set at 300 °C and 260 °C, respectively. The MS was operated in electron impact (EI) mode with an ionization energy of 70 eV and a mass scan range of 40–400 amu with a scan time of 0.3 s. The *C. japonica* EOCs' identity was established using two methods described in Lima et al. [15]. Briefly, one of the methods involved comparing the EOCs retention indices (RI), calculated as ISO 7609 [22], relative to *n*-alkane standard indices, whereas the other one compared their GC–MS spectra to two MS databases: (i) a lab-made library with commercially available standards and components of reference EOs, and (ii) other libraries (NIST11, Wiley10, and FFNSC4.0). For quantification, EOCs' raw percentage was calculated by integrating total ion current (TIC) chromatogram peaks without correction factors, as in our previous report [23].

2.4. Statistical Analysis

All determinations were performed in triplicate, and the results are expressed as means ± standard deviations. The influence of HDTs (0–2, 2–10, 10–30, 30–60, 60–120, 120–240, and 0–240 min) on the following parameters: EO yield, EO HDR, EO density, and chemical composition was determined by a one-way analysis of variance (ANOVA) test. For each response variable, the validity of model assumptions was checked by examining resid-

uals, as described in Montgomery [24]. Since the influence of HDTs was significant ($p < 0.05$) for all response variables, Duncan's multiple range test was conducted at a significance level of 5% for multiple means comparison and subsequent letter grouping generation.

The relationships between the HDTs (excluding the 0–240 min) and EO yield, the EO HDR, and the individual concentration of selected EOCs (α-pinene, camphene, sabinene, myrcene, limonene, terpinen-4-ol, bornyl acetate, δ-cadinene, elemol, germacrene-D-4-ol, γ-eudesmol, α+β-eudesmol, rosa-5,15-diene, phyllocladene, and nezukol) were adequately modelled by the Power, exponential decay, logarithmic or linear models shown in Equations (1)–(4), respectively. Equation (4) represents a linear regression model. The other three models (Equations (1)–(3)) are nonlinear (NLIN), and their parameters were estimated iteratively using the NLIN Regression Procedure of IBM SPSS Statistics (software version 28.0.1.0), and the fitted models met all the adequacy requirements of NLIN models described by Bates and Watts [25].

$$Y = \theta_1 X^{\theta_2} + \varepsilon \quad (1)$$

$$Y = Exp\left(\theta_1 - \frac{\theta_2}{X}\right) + \varepsilon \quad (2)$$

$$Y = \theta_1 Log(X) + \theta_2 + \varepsilon \quad (3)$$

$$Y = \beta_0 + \beta_1 X + \varepsilon \quad (4)$$

In the four regression equations, Y is the dependent (response) variable, X is the independent (HDT, excluding the 0–240 min) variable, and ε is the error term assumed to have a normal distribution with constant variance. The validity of the normality, constant variance, and independence assumptions on the error terms was checked by examining residuals [25].

3. Results and Discussion

3.1. Az–CJF EO Extraction and Fractionation by HD Method

The HD process can be used both to obtain and fractionate EOs. However, to the best of our knowledge, no studies on using HD to fractionate EOs from *C. japonica* have been reported so far. In this study, sequential separation of EOCs during HD of Azorean *C. japonica* fresh foliage was performed. Six distinct HDTs were selected based on several preliminary experiments. To determine whether collecting EO at different HDTs would generate fractions with varying EO characteristics, we compared the resulting EO fractions with control EO (Figure 1).

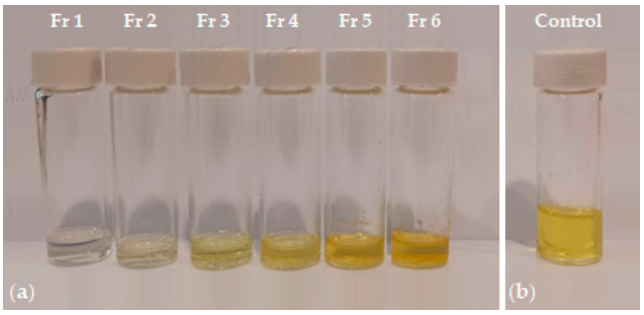

Figure 1. Azorean *Cryptomeria japonica* foliage essential oils obtained by the hydrodistillation process (**a**) with fractionation at specific timeframes and (**b**) without fractionation.

3.2. Yield, Hydrodistillation Rate (HDR), Density, and Colour of the Az–CJF EO and Its Fractions

Table 1 presents the yield, HDR, density, and colour of the Az–CJF control EO and fractions collected at different HDTs.

Table 1. Yield, hydrodistillation rate (HDR), density, and colour of the fractions and control from Azorean *Cryptomeria japonica* foliage essential oil obtained by hydrodistillation.

HDTs (min)	Essential Oil				
	Samples	Yield (%, *w*/f.w.)	HDR (mg/min)	Density (g/cm^3)	Colour
0–2	Fr1	0.139 ± 0.031 ^{c}	208.5 ± 47.2 ^{a}	0.847 ± 0.013 ^{b}	incolour
2–10	Fr2	0.061 ± 0.014 ^{e}	22.8 ± 5.1 ^{b}	0.878 ± 0.014 ^{ab}	pale yellow
10–30	Fr3	0.074 ± 0.008 ^{de}	11.1 ± 1.2 ^{c}	0.888 ± 0.025 ^{a}	yellowish
30–60	Fr4	0.090 ± 0.012 ^{d}	9.0 ± 1.2 ^{c}	0.887 ± 0.029 ^{a}	yellowish
60–120	Fr5	0.143 ± 0.011 ^{c}	7.1 ± 0.6 ^{d}	0.891 ± 0.019 ^{a}	yellow
120–240	Fr6	0.180 ± 0.003 ^{b}	4.5 ± 0.1 ^{e}	0.879 ± 0.034 ^{ab}	yellow
0–240	Control	0.820 ± 0.078 ^{a}	10.0 ± 1.0 ^{c}	0.889 ± 0.006 ^{a}	yellowish

Results are shown as means ± standard deviations (n = 3). Within each column, values followed by the same letter are not significantly different ($p < 0.05$). HDTs—hydrodistillation timeframes; f.w.—fresh weight.

Az–CJF EO fractions and control presented yield values of 0.06–0.18% (∑ 0.69%) and 0.82%, respectively, revealing no significant losses in the EO fractionation by the HD method. The control EO yield value was in accordance with previous research (0.5–0.8%, *w*/f.w.) [26], using similar extraction protocols and units of measurement. In general, we observed the significant effects of HDTs on EO fraction yield, with decreasing values as follows: Fr6 > Fr5 ≈ Fr1 > Fr4 > Fr3 ≈ Fr2. The EO fractions and control also had significantly different HDR, presenting values of 4.5–208.5 and 10 mg/min, respectively, with EO HDR values decreasing as follows: Fr1 >> Fr2 > Fr3 > Fr4 > Fr5 > Fr6. Thus, a significantly higher EO HDR was observed in the first 2 min of the HD. The quick decrease in the following fractions was most likely due to the simultaneous distillation of monoterpene hydrocarbons (MHs), EOCs with the lowest boiling points. We achieved the maximum EO fraction yield at 120–240 min HDT (Fr6), which, despite being the fraction that showed the lowest HDR, had the longest collection interval (2 h). On the other hand, when analysing the density of EO fractions, there was minimal variation among the fractions, except for Fr1, which exhibited a lower density (0.847 g/cm^3) compared to other fractions (0.878–0.891 g/cm^3), as well as to control EO (0.889 g/cm^3). Thus, all the studied EO samples had a density lower than water. Finally, a colour gradient was easily observed from Fr1 to Fr6 (Figure 1), which could be attributed to the progressive accumulation of sesquiterpenes and diterpenes during the HD process. This phenomenon becomes evident as we progress from Fr1 (primarily comprising MHs with no colour) to subsequent fractions.

Overall, the EO was extracted very quickly at the start of HD (0–2 min), rapidly decreasing and stabilizing as HD progressed. During the first hour, 53% of the EO had already been extracted (20% in Fr1 and 33% in Frs. 2–4), with the remaining 47% extracted over the next 3 h (21% in Fr5 and 26% in Fr6).

Considering the available literature, we could only compare our findings to those from previous studies on the influence of HDTs on EO yield in other Cupressaceae family members (*Juniperus virginiana*, *J. excelsa*, *J. sabina*, and *J. communis*) [27,28] and *Pinus* species (*P. heldreichii*, *P. peuce*, and *P. mugo*) [29]. These studies demonstrated a significantly higher EO yield during the first few minutes of the HD fractionation process, as observed in the present study.

3.3. Chemical Composition of the Az–CJF EO and Its Fractions

The chemical composition profile of the Az–CJF control EO and fractions collected at different HDTs, is presented in Table S1 of the Supplementary Materials. Figure 2a,b–d show the chromatographic profiles of the control EO and representative fractions (top, middle, and bottom HDTs), respectively.

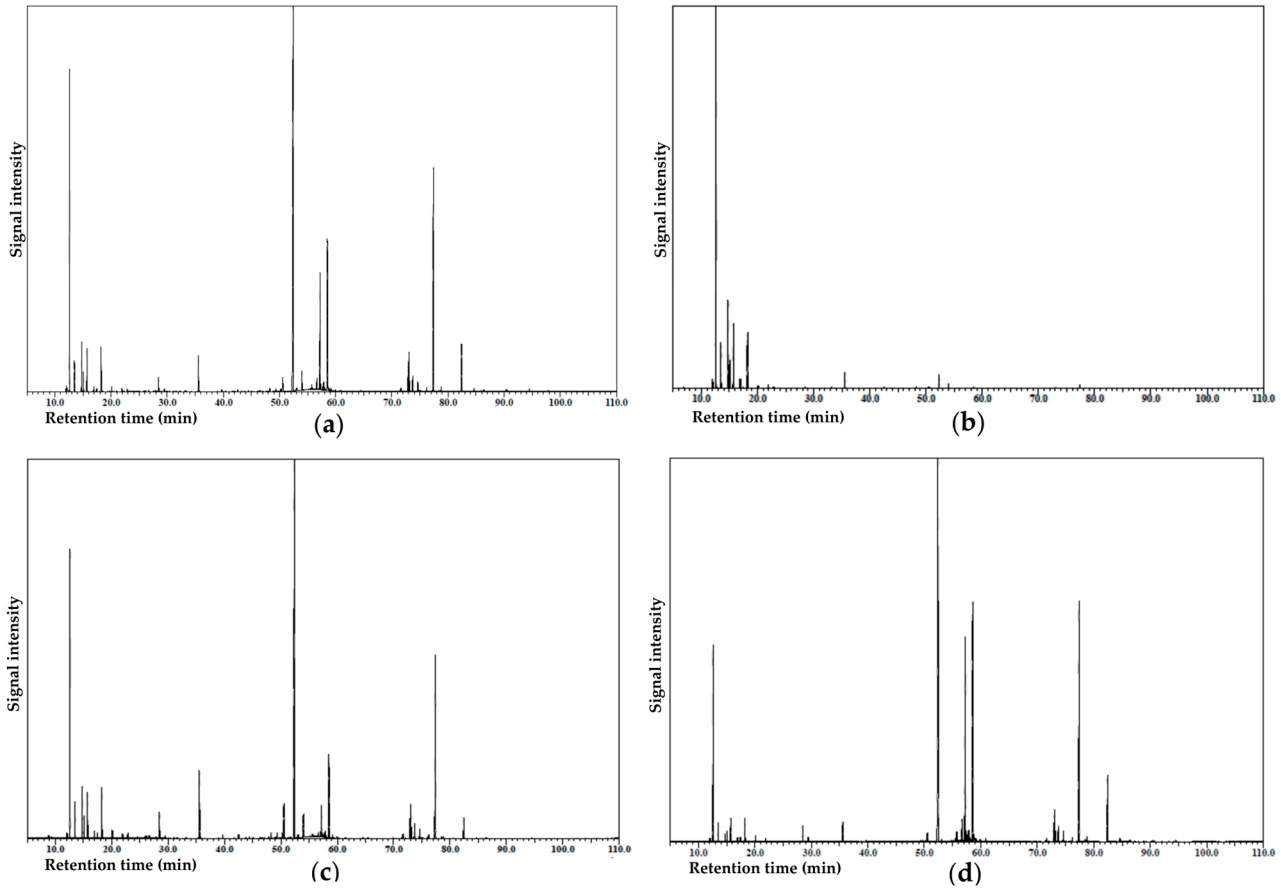

Figure 2. Total ion current (TIC) representative chromatograms on a ZB5MSPlus column of Azorean *Cryptomeria japonica* foliage essential oil (EO) samples obtained by hydrodistillation: (**a**) control EO (0–240 min), (**b**) fraction 1 (0–2 min), (**c**) fraction 3 (10–30 min), and (**d**) fraction 6 (120–240 min).

A total of 89 components were identified in the control EO, accounting for 97.39% of the total EO. Among these, 51–85 components were present in the EO fractions, accounting for 97.04–99.75% of the total EO composition (Supplementary Materials, Table S1), confirming that not all EOCs were found in all the EO fractions obtained.

The main terpene class identified in the control EO was oxygen-containing sesquiterpenes (OCS; 51.12%), followed by MHs (19.68%), diterpene hydrocarbons (DHs; 18.65%), oxygen-containing diterpenes (OCD; 2.90%), oxygen-containing monoterpenes (OCM; 3.01%), and sesquiterpene hydrocarbons (SHs; 2.03%). On the other hand, the terpene classes present in the EO fractions decreased in the following order: MHs (91.79%) >> OCS (3.28%) ≈ OCM (2.96%) > SHs (0.93%) ≈ DHs (0.76%) > OCD (0.03%) in Fr1; MHs (45.23%) > OCS (27.44%) > DHs (10.38%) > OCM (8.98%) > SHs (5.51%) > OCD (0.68%) in Fr2; OCS (42.17%) > MHs (23.92%) > DHs (18.33%) > OCM (6.55%) > SHs (4.86%) > OCD (1.44%) in Fr3; OCS (49.73%) > DHs (22.78%) > MHs (15.47%) > OCM (4.00%) > SHs (2.83%) > OCD (2.23%) in Fr4; and OCS (50.85 and 62.39%), DHs (21.49 and 18.31%), MHs (17.48 and 10.09%), OCM (2.95 and 1.86%), OCD (2.95 and 3.77%), SHs (1.43 and 1.01%), in Fr5 and Fr6, respectively (Supplementary Materials, Table S1).

The major EOCs (≥4%) identified in the control EO were elemol (27.47%), phyllocladene (14.00%), α+β-eudesmol (13.10%), α-pinene (11.64%) and γ-eudesmol (6.55%) (Supplementary Materials, Table S1). On the other hand, fifteen major EOCs were identified in the EO fractions, as illustrated in Tables 2–7. The MHs α-pinene, camphene, sabinene, myrcene, and limonene were extracted at the beginning of the HD (0–2 min HDT) and accounted for 84% of the total EO fraction concentration. As expected, the MHs concentration decreased gradually as the process continued, reaching its lowest level in the EO fraction

obtained at 120–240 min HDT (Table 2). The OCM terpinen-4-ol and bornyl acetate were also distilled earlier in the HD process, but not as much as MHs. Terpinen-4-ol and bornyl acetate reached their maximum concentrations of 1.4 and 6.3% at 10–30 and 2–10 min HDT (Table 3), respectively.

Table 2. Concentration (%) of the main monoterpene hydrocarbons (MHs) in the fractions and control of Azorean *Cryptomeria japonica* foliage essential oil obtained by hydrodistillation.

HDTs (min)	α-Pinene	Camphene	Sabinene	Myrcene	Limonene	Other MHs	Total MHs
0–2	54.18 ^{a}	4.99 ^{a}	10.25 ^{a}	7.45 ^{a}	7.16 ^{a}	7.76 ^{a}	91.79 ^{a}
2–10	24.12 ^{b}	2.60 ^{b}	5.16 ^{b}	3.88 ^{b}	4.61 ^{b}	4.86 ^{b}	45.23 ^{b}
10–30	12.48 ^{c}	1.58 ^{c}	2.29 ^{c}	2.07 ^{c}	2.52 ^{c}	2.98 ^{c}	23.92 ^{c}
30–60	8.48 ^{e}	1.07 ^{d}	1.01 ^{d}	1.32 ^{cd}	1.52 ^{d}	2.07 ^{d}	15.47 ^{d}
60–120	10.15 ^{de}	1.17 ^{d}	0.79 ^{de}	1.45 ^{cd}	1.57 ^{d}	2.35 ^{d}	17.48 ^{d}
120–240	6.35 ^{f}	0.60 ^{e}	0.26 ^{e}	0.78 ^{d}	0.82 ^{e}	1.28 ^{e}	10.09 ^{e}
0–240 (control)	11.64 ^{cd}	1.07 ^{d}	1.77 ^{c}	1.61 ^{c}	1.74 ^{d}	1.85 ^{e}	19.68 ^{d}

Results are shown as means ± standard deviations (n = 3). Within each column, values followed by the same letter are not significantly different ($p < 0.05$). HDTs—hydrodistillation timeframes.

Table 3. Concentration (%) of the main oxygen-containing monoterpenes (OCM) in the fractions and control of Azorean *Cryptomeria japonica* foliage essential oil obtained by hydrodistillation.

HDTs (min)	Terpinen-4-ol	Bornyl Acetate	Other OCM	Total OCM
0–2	0.17 ^{d}	2.32 ^{c}	0.47 ^{cd}	2.96 ^{d}
2–10	0.97 ^{bc}	6.32 ^{a}	1.69 ^{a}	8.98 ^{a}
10–30	1.40 ^{a}	3.92 ^{b}	1.23 ^{b}	6.55 ^{b}
30–60	1.16 ^{ab}	2.11 ^{c}	0.73 ^{c}	4.00 ^{c}
60–120	0.98 ^{b}	1.48 ^{d}	0.49 ^{cd}	2.95 ^{d}
120–240	0.64 ^{c}	0.86 ^{e}	0.36 ^{d}	1.86 ^{e}
0–240 (control)	0.65 ^{c}	1.72 ^{d}	0.64 ^{c}	3.01 ^{d}

Results are shown as means ± standard deviations (n = 3). Within each column, values followed by the same letter are not significantly different ($p < 0.05$). HDTs—hydrodistillation timeframes.

Table 4. Concentration (%) of the main sesquiterpene hydrocarbons (SHs) in the fractions and control of Azorean *Cryptomeria japonica* foliage essential oil obtained by hydrodistillation.

HDTs (min)	δ-Cadinene	Other SHs	Total SHs
0–2	0.23 ^{e}	0.70 ^{e}	0.93 ^{e}
2–10	1.98 ^{b}	3.53 ^{b}	5.51 ^{a}
10–30	2.62 ^{a}	2.24 ^{a}	4.86 ^{a}
30–60	1.71 ^{b}	1.12 ^{b}	2.83 ^{b}
60–120	0.86 ^{c}	0.57 ^{c}	1.43 ^{d}
120–240	0.54 ^{d}	0.47 ^{d}	1.01 ^{e}
0–240 (control)	1.07 ^{c}	0.96 ^{c}	2.03 ^{c}

Results are shown as means ± standard deviations (n = 3). Within each column, values followed by the same letter are not significantly different ($p < 0.05$). HDTs—hydrodistillation timeframes.

The SHs class was found to a lesser extent, with δ-cadinene being the only relevant one to reach its maximum concentration (2.6%) at 10–30 min HDT (Table 4). On the contrary, OCS (Table 5) were the most widespread terpenes identified in the studied Az–CJF EO sample, as already highlighted. Elemol reached its peak concentration at 30–60 min HDT and remained stable until the end of HD. Germacrene-D-4-ol exhibited similar behaviour as OCM (Table 3), peaking at 2–10 min HDT and decreasing afterwards. γ-Eudesmol and α+β-eudesmol displayed the same trend, increasing their concentration gradually along the HD and reaching their maximum at 120–240 min HDT.

Table 5. Concentration (%) of the main oxygen-containing sesquiterpenes (OCS) in the fractions and control of Azorean *Cryptomeria japonica* foliage essential oil obtained by hydrodistillation.

HDTs (min)	Elemol	Germacrene-D-4-ol	γ-Eudesmol	α+β-Eudesmol	Other OCS	Total OCS
0–2	2.11 ^{e}	0.70 ^{c}	0.05 ^{e}	0.38 ^{f}	0.04 ^{e}	3.28 ^{e}
2–10	18.10 ^{d}	3.61 ^{a}	0.76 ^{e}	3.86 ^{e}	1.11 ^{d}	27.44 ^{d}
10–30	28.55 ^{ab}	1.43 ^{b}	2.14 ^{d}	8.05 ^{d}	2.00 ^{c}	42.17 ^{c}
30–60	30.07 ^{a}	0.18 ^{d}	4.38 ^{c}	11.99 ^{c}	3.11 ^{b}	49.73 ^{b}
60–120	25.62 ^{bc}	0.03 ^{d}	6.73 ^{b}	15.14 ^{b}	3.33 ^{b}	50.85 ^{b}
120–240	24.78 ^{c}	0.00 ^{d}	11.48 ^{a}	21.64 ^{a}	4.49 ^{a}	62.39 ^{a}
0–240 (control)	27.47 ^{abc}	0.97 ^{c}	6.55 ^{b}	13.1 ^{bc}	3.03 ^{b}	51.12 ^{b}

Results are shown as means ± standard deviations (n = 3). Within each column, values followed by the same letter are not significantly different ($p < 0.05$). HDTs—hydrodistillation timeframes.

Table 6. Concentration (%) of the main diterpene hydrocarbons (DHs) in the fractions and control of Azorean *Cryptomeria japonica* foliage essential oil obtained by hydrodistillation.

HDTs (min)	Phyllocladene	Rosa-5,15-diene	Other DHs	Total DHs
0–2	0.58 ^{d}	0.10 ^{d}	0.08 ^{c}	0.76 ^{e}
2–10	7.21 ^{c}	1.43 ^{c}	1.74 ^{b}	10.38 ^{d}
10–30	12.94 ^{b}	2.39 ^{ab}	3.00 ^{ab}	18.33 ^{c}
30–60	16.42 ^{a}	2.73 ^{a}	3.63 ^{a}	22.78 ^{a}
60–120	16.12 ^{a}	2.26 ^{b}	3.11 ^{ab}	21.49 ^{ab}
120–240	14.20 ^{ab}	1.66 ^{c}	2.45 ^{ab}	18.31 ^{bc}
0–240 (control)	14.00 ^{ab}	2.11 ^{b}	2.54 ^{ab}	18.65 ^{bc}

Results are shown as means ± standard deviations (n = 3). Within each column, values followed by the same letter are not significantly different ($p < 0.05$). HDTs—hydrodistillation timeframes.

Table 7. Concentration (%) of the main oxygen-containing diterpenes (OCD) in the fractions and control of Azorean *Cryptomeria japonica* foliage essential oil obtained by hydrodistillation.

HDTs (min)	Nezukol	Other OCD	Total OCD
0–2	0.03 ^{f}	0.00 ^{c}	0.03 ^{f}
2–10	0.66 ^{e}	0.02 ^{c}	0.68 ^{e}
10–30	1.39 ^{d}	0.05 ^{c}	1.44 ^{d}
30–60	2.12 ^{c}	0.11 ^{b}	2.23 ^{c}
60–120	2.75 ^{b}	0.20 ^{b}	2.95 ^{b}
120–240	3.47 ^{a}	0.30 ^{a}	3.77 ^{a}
0–240 (control)	2.59 ^{b}	0.31 ^{a}	2.9 ^{b}

Results are shown as means ± standard deviations (n = 3). Within each column, values followed by the same letter are not significantly different ($p < 0.05$). HDTs—hydrodistillation timeframes.

The DHs, a significant class of the studied Az–CJF EO sample, were also affected by HDTs (Table 6). Phyllocladene and rosa-5,15-diene concentrations peaked at 30–60 min HDT, slightly decreasing in later HDTs (Table 6). On the other hand, the amount of nezukol, a major component of the OCD class, gradually increased throughout the HD process, reaching its maximum at 120–240 min HDT (Table 7).

Overall, different HDTs significantly influence the composition of Az–CJF EO. The MHs class dominated in Fr1 and Fr2, demonstrating a fivefold and 2.3-fold increase in content compared to the control EO, respectively. α-Pinene was the major MH in these two fractions, representing almost 50% of the total MHs. On the contrary, OCS dominated in Frs. 3–6, as in the control EO. In these four samples, elemol was the major OCS, followed by α+β-eudesmol, and γ-eudesmol. Furthermore, among all the studied EO samples, we observed that: (i) Fr2 and Fr3 (distilled at 2–30 min HDT) were the richest in OCM (mainly bornyl acetate) and SHs (mainly δ-cadinene); (ii) Fr4 and Fr5 (distilled at 30–120 min HDT)

in DHs (mainly phyllocladene); and (iii) Fr6 (distilled at 120–240 min HDT) in OCD (mainly nezukol). Previous reports on Az–CJF EO differ from the present results by highlighting MHs are the predominant class, with α-pinene being the major compound [1,30,31]. Nonetheless, it is not an unprecedented event, since in Figueiredo et al.'s [30] study, an Azorean *C. japonica* EO sample obtained by HD from branches and foliage also contained high amounts of the OCS elemol (subcluster Id). In addition, of the 76 components present in all 105 EO samples obtained by SD from *C. japonica* (branches and foliage) collected in different Azorean Islands, the authors [30] selected 20 as representative and characteristic Azorean *C. japonica* EOCs. α-Pinene, sabinene, myrcene, limonene, terpinen-4-ol, bornyl acetate, elemol, α-eudesmol, β-eudesmol, phyllocladene, and nezukol, which are among the major EOCs identified in our EO fractions, are within the selected 20 components [30], confirming the homogeneity of the EO composition from Azorean *C. japonica* aerial parts.

The effect of HDTs on the CJF EO fraction composition can be attributed to various factors influencing the distillation order of EOCs during HD, such as (i) volatility: EOCs with lower boiling points and higher vapour pressures tend to be distilled earlier in the HD process; (ii) molecular weight: EOCs with lower molecular weight generally have higher vapour pressure; (iii) chemical structure: EOCs with polar functional groups or hydrogen bonding capabilities may interact with HD medium (water), causing them to be distilled later.

3.4. Kinetics Regression Models

Figures 3–7 illustrate the relationship between HDTs (excluding the 0–240 min) and the following parameters: EO yield (%), HDR (EO mg/min), and individual concentration (%) of major EOCs, while also showing the fitted model that best describes the mentioned relationships. The fitted model can be used for prediction purposes, as described below.

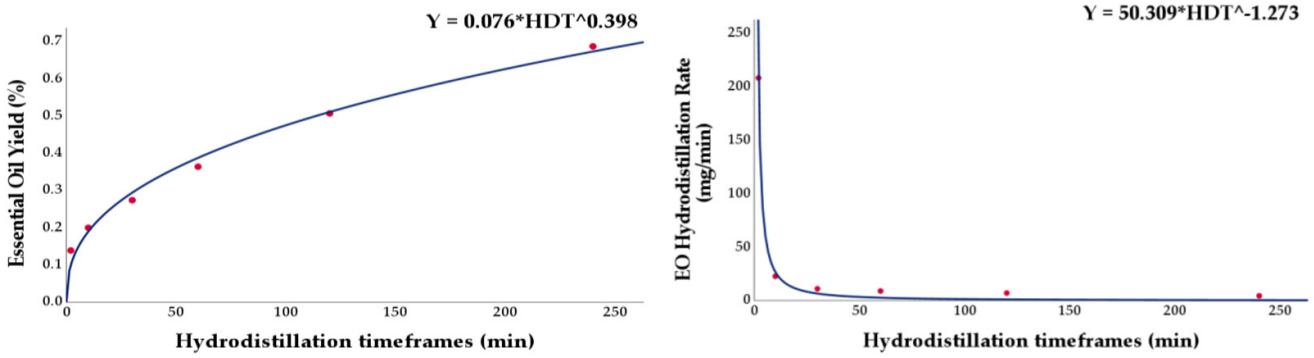

Figure 3. Plot of hydrodistillation timeframes (HDTs) vs. (**a**) essential oil (EO) cumulative yield and (**b**) EO hydrodistillation rate along with the fitted Power regression models.

The relationships between HDTs and EO yield (Figure 3a), and HDTs and EO HDR (Figure 3b), were adequately modelled by the Power model (Equation (1)).

The relationship between HDTs and major MHs (Figure 4) was also adequately modelled by the Power model (Equation (1)).

Similarly, Semerdjieva et al. [27] used the Power model to describe the relationship between HDTs and the α-pinene, sabinene, β-pinene, and limonene contents in *Juniperus* sp. EO. In the present study, MHs camphene and myrcene were also adequately modelled by the Power model, suggesting that the MHs class always follows this regression model during HD.

The best regression models were exponential decay (Equation (2)) and logarithmic (Equation (3)) for other major EOCs such as elemol, phyllocladene and rosa-5,15-diene (Figure 5), α+β-eudesmol, and nezukol (Figure 6).

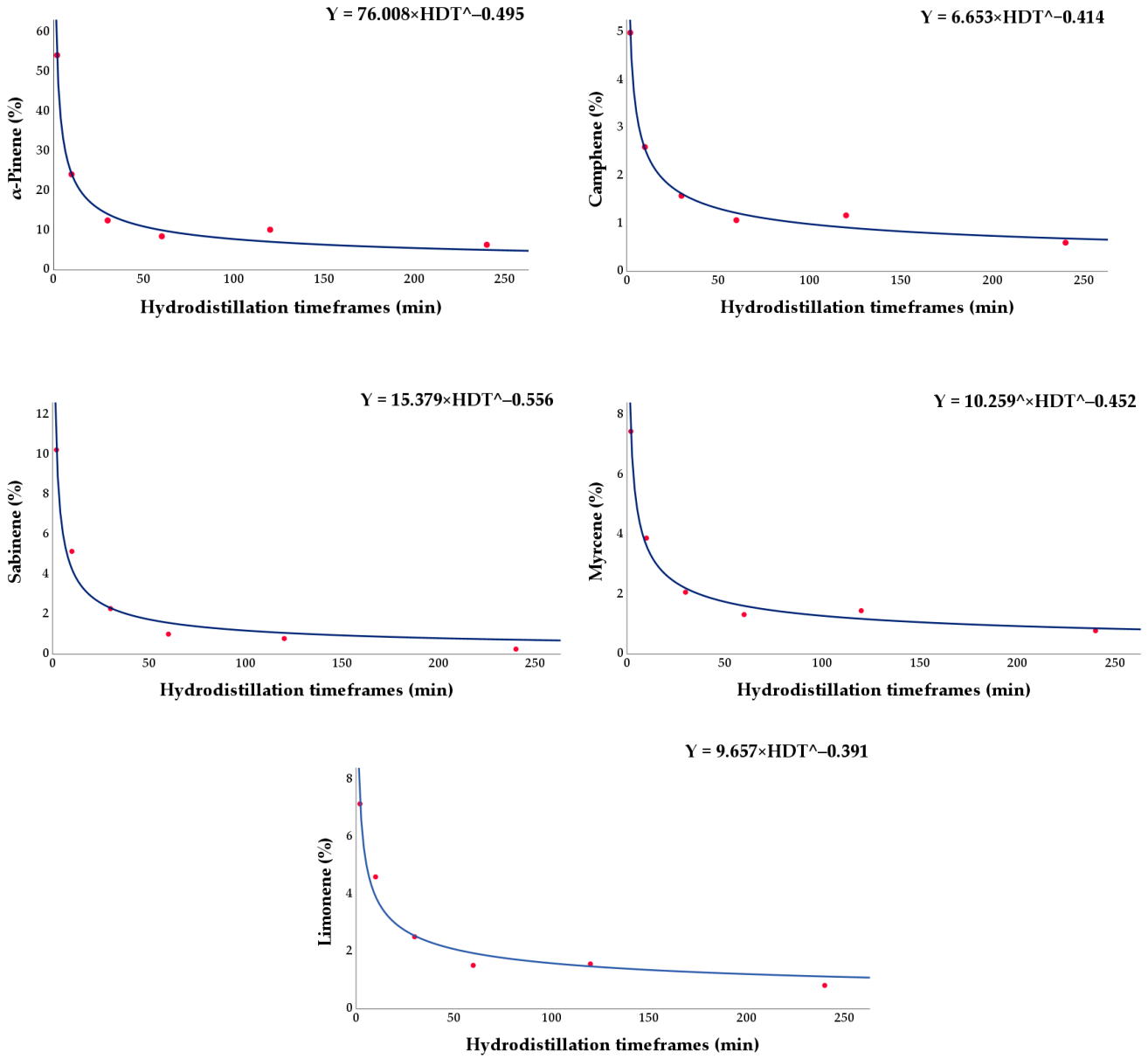

Figure 4. Plot of hydrodistillation timeframes (HDTs) vs. the concentration of α-pinene, camphene, sabinene, myrcene, and limonene (monoterpene hydrocarbons) along with the fitted Power regression model.

On the other hand, the relationship between HDTs and γ-eudesmol content (Figure 7) was delineated by a linear regression model (Equation (4)).

Relative to the other four major EOCs (terpinen-4-ol, bornyl acetate, δ-cadinene, and germacrene-D-4-ol), there was no linear or NLIN regression model that could describe the relationship between its concentration and the HDTs.

Overall, specific EO fractions can be targeted in Az–CJF EO by varying the HDT. For example, Fr1, Fr4, and Fr6 were rich in α-pinene, phyllocladene, and elemol + eudesmol isomers (α, β and γ), respectively, which were the major EOCs identified in the studied EO sample, accounting for 54%, 17%, and 64%, respectively, of the total EO composition. Thus, the present study results may be a significant finding for the *C. japonica* EO industry.

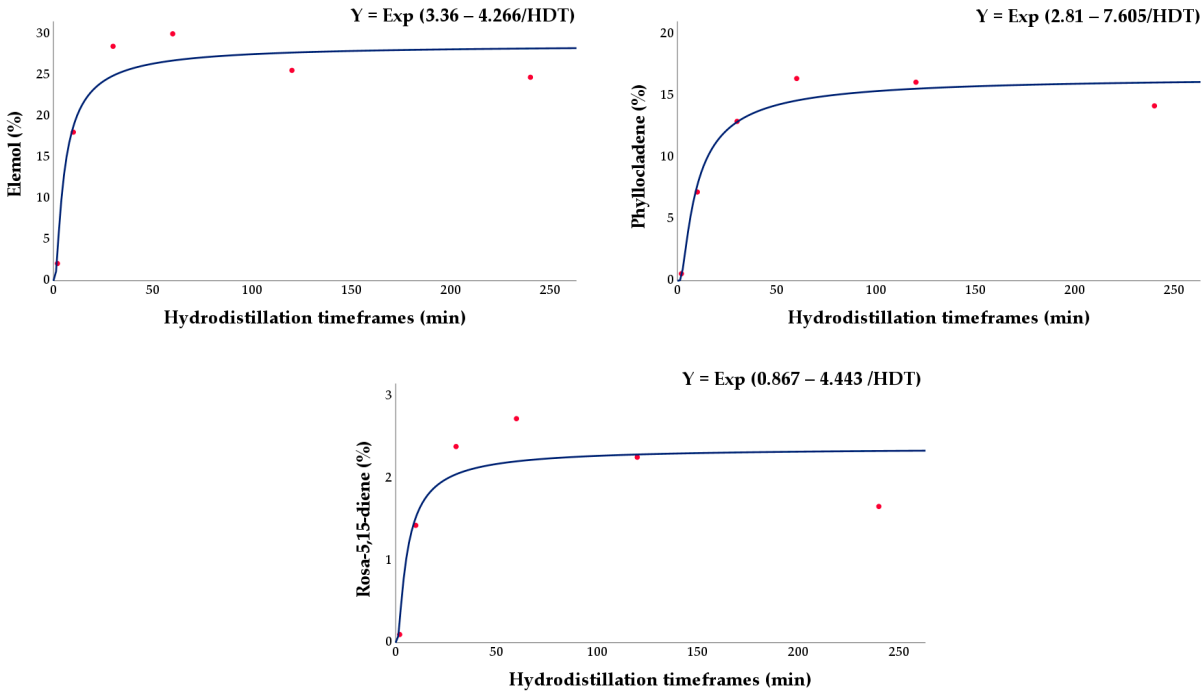

Figure 5. Plot of hydrodistillation timeframes (HDTs) vs. the concentration of elemol (oxygen-containing sesquiterpene), phyllocladene, and rosa-5,15-diene (diterpene hydrocarbons) along with the fitted exponential regression model.

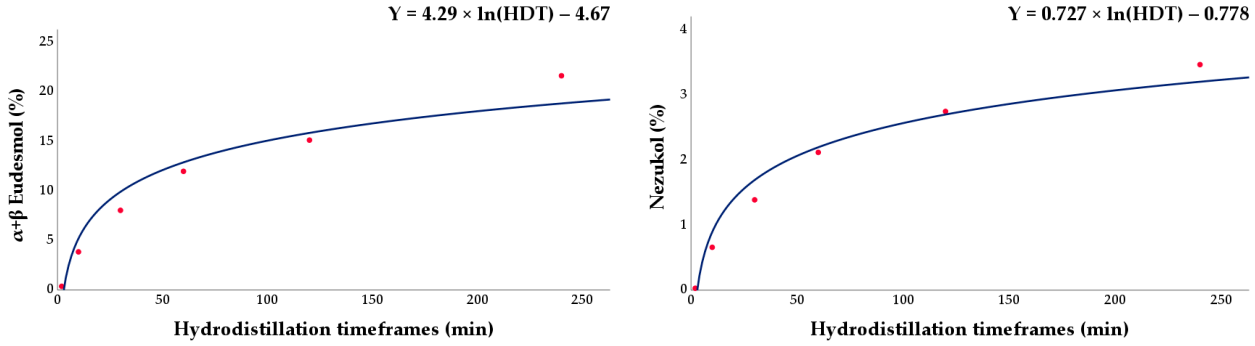

Figure 6. Plot of hydrodistillation timeframes (HDTs) vs. the concentration of α+β-eudesmol (oxygen-containing sesquiterpenes) and nezukol (oxygen-containing diterpene) along with the fitted logarithmic regression model.

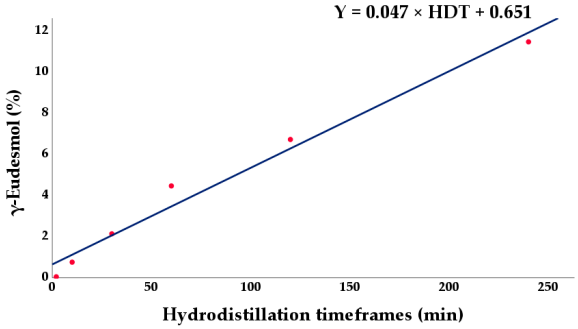

Figure 7. Plot of hydrodistillation timeframes (HDTs) vs. the concentration of γ-eudesmol (oxygen-containing sesquiterpene) along with the fitted linear regression model.

4. Conclusions

To the best of our knowledge, no prior studies on HD use in fractionating EOs from *C. japonica* have been reported. In the present study, a sequential separation of EOCs during HD of Azorean *C. japonica* fresh foliage was performed using six distinct HDTs. The results clearly demonstrated that HDTs can be a tool for obtaining EO fractions with specific compositions from CJF. Thus, the valuable findings presented here offer EO producers and processors an opportunity to exert greater control over *C. japonica* EO compositions by manipulating HDT, which saves time and energy by identifying specific time points during the HD process where higher yields of desired EOCs are extracted. The regression models presented here can also serve as a valuable benchmark for comparing literature reports. Furthermore, studies on a laboratory scale could be an incentive for the *C. japonica* EO industry to optimize SD timeframes, obtain EO fractions with differential compositions and, thus, produce products with higher added value.

Ongoing bioactivity screening studies into these novel Az–CJF EO fractions can help to meet different market demands compared to the typical crude CJF EO. Furthermore, they will contribute to the biovalorisation of wastes from abundant Azorean resources (such as the CJBR) and the local circular economy.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations10090483/s1, Table S1: GC/MS analysis of the control and fractions of Azorean *Cryptomeria japonica* foliage essential oil obtained by HD.

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Abbreviations

Az–CJF, Azorean *Cryptomeria japonica* foliage; CJBR, *Cryptomeria japonica* biomass residues; CJF, *Cryptomeria japonica* foliage; DHs, diterpene hydrocarbons; EO, essential oil; EOC, essential oil component; Fr, fraction; f.w., fresh weight; GC–MS, gas chromatography–mass spectroscopy; HD, hydrodistillation; HDR, hydrodistillation rate; HDT, hydrodistillation timeframe; MHs, monoterpene hydrocarbons; NLIN, nonlinear; OCD, oxygen-containing diterpenes; OCM, oxygen-containing monoterpenes; OCS, oxygen-containing sesquiterpenes; RI, retention indices; SHs, sesquiterpene hydrocarbons; SD, steam distillation; TIC, total ion current.

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