Research Article

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Kinetics of the essential oil hydrodistillation from sweet flag (*Acorus calamus*) rhizomes: comparison of basic models

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Abstract

Background: This study investigated the hydrodistillation process for extracting essential oil from sweet flag (*Acorus calamus*) rhizomes. The primary objectives were to evaluate the influence of the water-to-rhizome ratio on both the yield and chemical composition of the essential oil and to model the extraction kinetics.

Results: The water-to-rhizome ratio was found to significantly influence the yield of essential oil, with the highest yield (1.36 ±0.01%) obtained at a ratio of 11:1. However, variations in this ratio did not result in significant changes in the chemical composition of the oil. A first-order kinetic model was identified as the most suitable for describing the hydrodistillation process, offering simplicity and strong agreement with experimental data.

Conclusion: The kinetic profile revealed a rapid initial increase in yield, corresponding to the washing phase — where readily accessible oil on the surface is removed—followed by a slower 'diffusion' phase, during which the essential oil gradually diffuses from the rhizome matrix into the distillate.

Keywords: essential oil; extraction; hydrodistillation; sweet flag; kinetics; modeling

INTRODUCTION

Essential oils are volatile substances with good smells obtained from diverse plants and their parts like flowers, leaves, stems, roots, or fruits, commonly by hydrodistillation methods. They are usually of complex composition, composed of secondary metabolites of plants. The demand for essential oils is surging across multiple industries, including food and beverage, aromatherapy, fragrance, cosmetics, and personal care. They are pivotal in flavoring, fragrance, and natural preservation of various products like fish, meat, sweets, pickles, and dairy due to their antibacterial properties. As consumer awareness shifts towards organic products, the market for plant-based essential oils is expected to grow further, driven by their health benefits in cosmetics, fragrance, and therapeutic applications. The global market value of essential oils is projected to increase from USD 23.74 billion in 2023 to USD 43.3 billion by 2030, growing at a compound annual growth rate (CAGR) of 9%.[1]

Extensive research into essential oil-bearing plants has significantly expanded their diversity and led to numerous commercially valuable herbal products with beneficial bioactive properties. Among them, sweet flag (*Acorus calamus*), a traditional herbal remedy, gained prominence in the late 1970s and 1980s due to studies highlighting its diverse biologically active compounds and increased commercial interest.[2] It is a perennial aromatic plant native to Central Asia, Europe, and North America.[3] Its scented leaves and rhizomes have been traditionally used medicinally for various ailments, including digestive problems, as a sedative, nerve tonic, antimicrobial agent, and expectorant.[4] The rhizome of the sweet flag (*Carami rhizoma*) is cylindrical, up to 2.5 cm thick, brown on the outside, and whitish on the inside, resembling a soft sponge.[3] Rhizomes of plants growing in water possess a well-developed aerenchyma, which constitutes the main portion of the primary bark and central cylinder.[5] The aerenchyma cells contain essential oil, starch, crystals, and occasionally tannins.[6]

Table 1 presents a comprehensive literature review on the essential oil extraction from sweet flag rhizomes using various

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Table 1. A review of the studies on essential oil extraction from sweet flag rhizomes

Method	Origin of the plant	Process conditions	Yield, %	Reference
HD-CA	India	Fresh rhizomes; 4 h	0.9%	Raina *et al.*[8]
	India	Dried rhizome (100 g)	2.8%	Marongiu *et al.*[13]
	India	Fresh rhizome (200 g), 8 h	3.5%	Joshi *et al.*[26]
	Iran	Dried, grinding, coarse powder rhizome (100 g), 3 h	-	Rahamoz-Haghighi *et al.*[23]
	India	Shade dried and powdered using mortar and pestle (20 g), water-to-rhizome ratio 10:1; 3 h	3.6–5.5%	Kasture *et al.*[27]
	India	Fresh rhizomes; 5 h	1.58 ± 0.19%	Verma *et al.*[28]
	India	Sun drying 240 h, shade drying 720 h, oven drying at 40°C for 60 h, and oven drying at 70 °C for 24 h; Dried rhizome 1000 g; distillation time: 6 h, 12 h (six hours for two successive days), 18 h (six hours for three successive days), 24 h (six hours for four successive days)	0.96–3.29%	Kumar *et al.*[9]
	India	Dried rhizome (250 g), 4 h	0.52%	Shukla *et al.*[29]
	India	Whole rhizome, 1–8 h	2.37%	Verma *et al.*[30]
	India	Fresh rhizomes (500 g); 3 h	1.2–4.8%	Parki *et al.*[31]
	India	Rhizomes were washed with distilled water, cut into small pieces, and shed dried for three days and in a hot air oven for 48 h (300 g), water-to-rhizome ratio: 10:1; 5 h	1.15%	Loying *et al.*[32]
	India	Dried rhizome	0.212–0.550%	Bhat *et al.*[33]
	Nepal	Dried rhizome (100 g), 4 h	0.8%	Joshi and Bashyal[34]
	Nepal	Washed with clean water, wet plant (200 g), water-to-rhizome ratio: 3:1, 6 h	0.9%	Khanal *et al.*[35]
	Nepal	Dried powdered rhizome (100 g), 4 h, 60 °C	1.67%	Aryal *et al.*[36]
	Thailand	Dried rhizome (1000 g), water-to-rhizome ratio:3:1, 8 h	n.a.	Phukhahad and Auamcharoen[20]
	Turkey	Dried rhizome, 3 h	1.26%	Süzgeç-Selçuk *et al.*[37]
	Serbia	Rhizomes dried at room temperature (50 g), water-to-rhizome ratio: 20:1	0.80–1.10%	Škobić *et al.*[7]
HD-DA	Iran	Dried rhizome (300 g), 3 h	0.05%	Wilczewska *et al.*[38]
HDE	China	Dried powder rhizome, solvent: *n*-hexane, 6 h	1.00%	Chen *et al.*[39]
SD	India	Dried rhizome (350 g)	0.4%	Marongiu *et al.*[13]
	Indonesia	Fresh rhizomes (10 kg)	0.1653%	Susanah *et al.*[11]
SDE	Nepal	Grinding rhizome (50 g), mix with distillate water, solvent: *n*-pentane: diethyl ether (1:1, v/v), 2 h	0.749%	Gyawali and Kim[14]
SEM	India	Oven drying at 40 °C, grinding, powdered rhizome (4 g), solvent: petroleum ether, chloroform, hexane, and ethyl acetate, 24 h	-	Devi and Ganjewala[15]
	Malaysia	Solvent: distilled water, methanol, and hexane, ratio 1:20, 3 h in a shaker at 60 ° C	-	Li and Wah[18]
	Indonesia	Dried rhizome (750 g), solvent: 96% ethanol, ratio 13.3:1, temperature 25 °C, 24 h	-	Susanah *et al.*[21]
	Indonesia	Oven drying at 50°C, grinding, powdered rhizome (150 g), solvent: 70% ethanol, time: 144 h (3 × 48 h)	11.81%	Hasanah *et al.*[17]
	Thailand	Dried rhizome (200 g), solvent: 70% ethanol, ratio: 10:1; time: 1 week	-	Dethoup *et al.*[16]
	Thailand	Dried rhizome (1000 g), solvent: ethanol, ratio: 3.5:1; time: 3 days	-	Phukhahad and Auamcharoen[20]
	Malaysia	Solvent: 95% ethanol, 0.5 and 1.0 g dried rhizome (particle size: 0.05–0.20 cm), solvent-to-solid ratio 100:1 and 50:1, temperature 40–45°C, 30 min	-	Omer *et al.*[19]
SES	India	Drying under shade, grinding, coarsely powdered rhizome (100 g), solvent: *n*-hexane, chloroform and methanol	-	Nalamwar *et al.*[22]
	Iran	Drying, grinding, coarse powder rhizome (100 g), solvent: ethanol and methanol, ratio:25:1; 24 h	-	Rahamoz-Haghighi *et al.*[23]
UAE	Malaysia	Solvent: 95% ethanol, 0.5 and 1.0 g dried rhizome (particle size: 0.05–0.20 cm), solvent-to-solid ratio 100:1 and 50:1, sonda: 20 kHz, power: 30–70%	-	Omer *et al.*[19]
SCE	India	Dried rhizome (250 g) pressure 9.0 MPa and temperature 45 °C	3.2%	Marongiu *et al.*[13]
SCE	India	Dried rhizome (200 g), optimal extraction parameters: pressure 35 MPa and temperature 55 °C, time: 40 min	4.12%	Yao *et al.*[25]

Table 1. Continued

Method	Origin of the plant	Process conditions	Yield, %	Reference
SCE	India	Dried powdered rhizome (diameter 0.5 mm), Pressure (100, 150, and 200 bar); temperature (45, 55, and 65 °C); 120 min/ best condition: 200 bar and 45 °C	0.73–3.15%	Shreelaxmi *et al.*[24]

Abbreviations: HD-CA, hydrodistillation – Clevenger apparatus; HD-DA, hydrodistillation – Deryng type apparatus; HDE, hydrodistillation + extraction; SCE, supercritical CO2 extraction; SD, steam distillation; SDE, steam distillation + extraction; SEM, solvent extraction – maceration; SES, solvent extraction – Soxhelet; UAE, ultrasound. assisted extraction.

methods. Sweet flag rhizomes typically contain essential oils ranging from 0.05% to 11.48%. This oil is rich in various compounds, particularly phenylpropanes, monoterpenes, and sesquiterpenes.[7] The composition of the essential oil varies with the plant's fertility; tetraploids often contain over 90% of the neurotoxic and carcinogenic β-asarone, whereas triploids have less than 5%, and diploids do not contain this component.[8] In essential oil production, achieving both high yield and quality is crucial, although there is often a trade-off between yield and purity. The predominant method for extracting essential oil from sweet flag rhizomes is hydrodistillation.[7-12] Alternative methods include steam distillation,[11,13] simultaneous hydrodistillation and extraction,[14] maceration,[15-21] Soxhlet extraction,[22,23] supercritical extraction,[13,24,25] and ultrasound-assisted extraction.[19] However, detailed investigations into the influence of hydrodistillation process parameters on sweet flag essential oil yield, physicochemical properties, and composition are still lacking.

Continuous research into hydrodistillation methods, including their kinetics, is essential for designing, scaling up, and improving the recovery process, as well as establishing industry guidelines that minimize losses and enhance efficiency.[40] Understanding the kinetics of essential oil hydrodistillation methods can further optimize the process, improve energy efficiency, ensure product quality, and reduce production costs.[41] Despite its importance, the kinetics of essential oil hydrodistillation from sweet flag rhizomes remain unexplored. However, various mathematical models have been developed to describe the kinetics of essential oil hydrodistillation from other aromatic and medicinal plants. These models are either theoretically derived or empirically formulated. Common approaches include Fick's law of diffusion, chemical reaction kinetics, and parametric empirical equations to model essential oil extraction processes.[42-49]

In this study, the hydrodistillation process of essential oil extraction from sweet flag rhizomes was investigated. The primary objectives were to evaluate the influence of the water-to-rhizome ratio (hydromodule) on essential oil yield and to model the kinetics of essential oil hydrodistillation. The influence of the water-to-rhizome ratio was statistically assessed using the unbalanced one-way ANOVA, whereas the kinetics of hydrodistillation were examined using three established models, simplified adaptations of the model proposed by Marković *et al.*[43]

MATERIAL AND METHODS

Plant material

The sweet flag rhizomes were purchased from Malina Impex (Valjevo, Serbia). The identification of herbal materials used in this study was confirmed by dr Vanja Tadic, at the Institute for Medicinal Plant Research 'Dr Josif Pancic' (Belgrade, Serbia), where the voucher specimen was deposited. Cleaned and dried rhizomes were packed in paper bags and stored in a dry, dark place, away from direct sunlight. For the experiment, 100 g of rhizomes were ground three times for 20 s each using a 500 W BOSCH disintegrator, with 20-s intervals between each grinding session to prevent overheating. The mean particle size of the obtained powder was 1.88 mm.

Hydrodistillation

Hydrodistillation of essential oil from ground sweet flag rhizomes was performed using a standard Clevenger-type apparatus, which operates in a batchwise mode, equipped with a 2 L round-bottom flask, as described by Markovic *et al.*[43] The ground rhizomes were placed in the flask without pre-soaking, and distilled water was added to achieve the target water-to-rhizome ratios of 8:1, 9:1, 10:1, 11:1, or 12:1 g/mL. An initial water-to-rhizome ratio of 6:1 g/mL, as used in previous studies,[50,51] caused charring of the plant material during distillation. Thermal degradation was still observed at a 7:1 g/mL water-to-rhizome ratio, though less pronounced, and was absent at higher ratios. The optimal water-to-rhizome ratio was determined by maximizing essential oil yield while preventing thermal degradation. The process was conducted using a heating mantle and lasted for 240 min, starting from the appearance of the first drop of essential oil. The volume of the extracted essential oil was measured using the graduated section of the apparatus.

Analytical procedures

Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS)

The oil was analyzed by a Shimadzu GCMS-QP2010 mass spectrometer (Japan) equipped with a flame ionization detector and a GC-2010 gas chromatograph using a normalization procedure. The InertCap5 capillary column (60.0 m × 0.25 mm × 0.25 µm) was used for separation. Helium at a split ratio of 1:5 and a linear velocity of 35.2 cm/s was used as a carrier gas. The ion source temperature was 200 °C, the injector temperature was 250 °C, and the detector temperature was 300 °C, while the column temperature was linearly programmed from 60 to 260 °C (at the rate of 4 °C/min), held isothermally at 260°C for 10 min,[52] with a slight modification. The obtained samples were dissolved in ethanol (96% v/v) at a concentration of 1% and injected in an amount of 1 µL, split mode, 1:30.

The constituents were identified by comparison of their mass spectra to those from Wiley275 and NIST/NBS libraries, using different search engines, Probability Merge Search (PBM) included in the instrument's data station software, and the NIST 2.0 search program. The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1), compared to those from available literature,[53] and used as an

additional tool to approve MS findings. The relative proportion of EO components was expressed as percentages obtained by peak area normalization, with all relative response factors taken as one.

Kinetic modeling of sweet flag rhizome essential oil hydrodistillation

Given the dataset size of $n = 16$, models with a maximum of three parameters were selected to minimize the risk of overfitting. These models were simplified versions of the model proposed by Marković *et al.*,[43] which assumed that essential oil extraction occurred through three simultaneous processes: washing, unhindered diffusion, and hindered diffusion. Each process operated at its rate, reaching a maximum value at specific times from the beginning of the distillation.

The first considered model, referred to as Model I, was developed under the assumption that washing occurred instantaneously, followed by unhindered diffusion, whereas hindered diffusion was considered negligible. Furthermore, it was assumed that the maximum unhindered diffusion rate occurred at $t = 0$. Under these conditions, the model equation is as follows:

$$q(t) = \alpha + \beta\left(1 - e^{-t/\tau}\right) \tag{1}$$

where α and β are the amounts of essential oil extracted by washing and diffusion, respectively. The total amount of essential oil that can be extracted is $q_\infty = \alpha + \beta$, while 95% of q_∞ is extracted by time 3τ. This model was derived for conventional hydrodistillation by Milojević *et al.*[44]

The second model (Model II) was obtained by further simplifying the model derived by Marković *et al.*,[43] by assuming that the amounts of essential oil extracted by washing and hindered diffusion were negligible. Under these assumptions, the distillation rate can be expressed as:

$$\frac{dq(t)}{dt} = \frac{\beta}{\tau} e^{-|t-t_0|/\tau} \tag{2}$$

where τ is the distillation time constant and β/τ is the maximum distillation rate occurring at $t = t_0$. The model equation is as follows:

$$q(t) = \begin{cases} \beta e^{-t_0/\tau}\left(e^{t/\tau} - 1\right), t < t_0 \\ \beta\left(2 - e^{-t_0/\tau} - e^{-(t-t_0)/\tau}\right), t \geq t_0 \end{cases} \tag{3}$$

According to this model, the total amount of essential oil that can be extracted is $q_\infty = \beta\left(2 - e^{-t_0/\tau}\right)$, and approximately 95% of this total amount is extracted by time $t_0 + 3\tau$.

The third considered model (Model III) was obtained by simplifying Eqs. (2) and (3), assuming that the maximum distillation rate occurred at $t_0 = 0$. Therefore, the model equation is as follows:

$$q(t) = \beta\left(1 - e^{-t/\tau}\right) \tag{4}$$

The total amount of essential oil that can be extracted is $q_\infty = \beta$, with 95% of it extracted by time 3τ. This model has already been verified for some hydrodistillation processes.[43,45,47]

The parameters of the kinetic models are determined by minimizing the sum of squared differences between the experimentally obtained values and the predicted values (*i.e.*, the Sum of Squared Errors – SSE) using the Levenberg–Marquardt algorithm

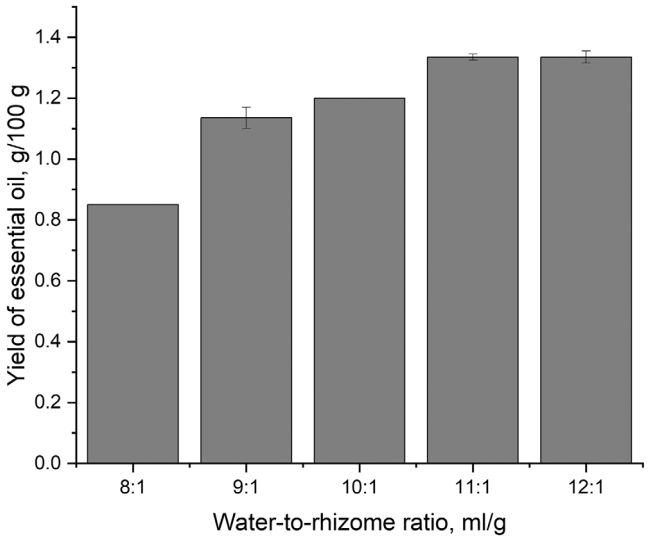

Figure 1. Effect of water-to-rhizome ratio on essential oil yield.

implemented in the function nlsLM from the package minpack.lm in R software. The root mean square error (RMSE), coefficient of determination (R^2), and mean relative percentage deviation (MRPD) are used to assess the goodness of model fit:

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\mathrm{SSE}} = \sqrt{\frac{1}{n}\sum_{k=1}^{n}\left(q_k - \overline{q}_k\right)^2} \tag{5}$$

$$R^2 = 1 - \frac{\mathrm{SSE}}{\sum_{k=1}^{n}\left(q_k - \overline{q}\right)^2} \tag{6}$$

$$\mathrm{MRPD} = \frac{100}{n}\sum_{k=1}^{n}\frac{\left|q_k - \overline{q}_k\right|}{\overline{q}_k} \tag{7}$$

where n is the size of the dataset, $\overline{q}_k$ and q_k are the experimental and model values of essential oil yield, respectively, while q is the mean of the experimental values. The RMSE was chosen over

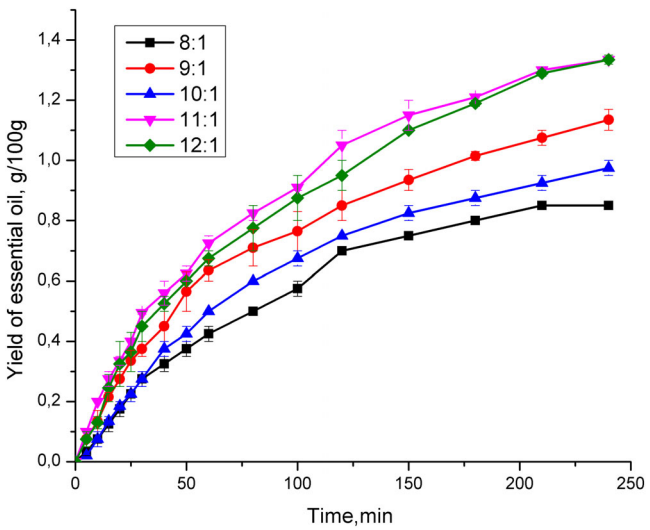

Figure 2. Effect of a water-to-rhizome ratio on the hydrodistillation of essential oil from sweet flag rhizomes.

Table 2. Model I: values of the kinetic model parameters, R^2, RMSE, MRPD, and q_∞

Hydromodule	α, [g]	β, [g/100 g]	τ, [min]	q_∞, [g/100 g]	RMSE	MRPD, %	R^2
8:1	0	0.94***	97.3***	0.94	0.017	±6.6	0.996
9:1	0.04.	1.13***	88.1***	1.17	0.024	±5.7	0.995
10:1	0.05**	1.29***	113.7***	1.34	0.019	±6.1	0.997
11:1	0.07**	1.36***	94.7***	1.43	0.024	±5.1	0.996
12:1	0.06*	1.41***	107.8***	1.46	0.032	±9.8	0.994

Note: Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '.

Table 3. Model II: values of the kinetic model parameters, R^2, RMSE, MRPD, and q_∞

Hydromodule	t_0, [min]	β, [g/100 g]	τ, [min]	q_∞, [g/100 g]	RMSE	MRPD, %	R^2
8:1	6.6*	0.89***	97.8***	0.95	0.017	±5.7	0.996
9:1	0	1.14	79.3***	1.14	0.027	±4.4	0.993
10:1	0	1.28	96.1***	1.28	0.027	±6.7	0.994
11:1	0	1.36	79.0***	1.36	0.035	±6.6	0.992
12:1	0	1.40	91.8***	1.40	0.037	±6.3	0.991

Note: Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '.

Table 4. Model III: values of the kinetic model parameters, R^2, RMSE, MRPD, and q_∞

Hydromodule	$\beta = q_\infty$, [g/100 g]	τ, [min]	RMSE	MRPD, %	R^2
8:1	0.95***	99.4***	0.017	±6.4	0.996
9:1	1.14***	79.3***	0.027	±4.4	0.993
10:1	1.28***	96.1***	0.027	±6.7	0.994
11:1	1.36***	79.0***	0.035	±6.6	0.992
12:1	1.40***	91.8***	0.037	±6.3	0.991

Note: Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '.

the SSE because the latter was highly sensitive to the size of the dataset, making it less suitable for comparative analysis in this case. On the other hand, the MRPD metric should not be used as the sole criterion for model comparison, as the experimental values at the beginning of the distillation process were very small, potentially skewing results. Akaike's information criterion (*AIC*) was used to identify the best model among the five developed, each featuring a different number of parameters but based on the same data set. This criterion does not provide information about the quality of the model in an absolute sense. *AIC* is defined as follows:

$$\mathrm{AIC} = -2\log(L) + 2K \tag{8}$$

where L is the maximum value of the credibility function, and K is the number of model parameters. Since these were small data sets, *i.e.*, $n/K < 40$, the corrected *AIC* number, *AICc*, was used:

$$\mathrm{AIC_c} = \mathrm{AIC} + \frac{2K(K+1)}{n-K-1} \tag{9}$$

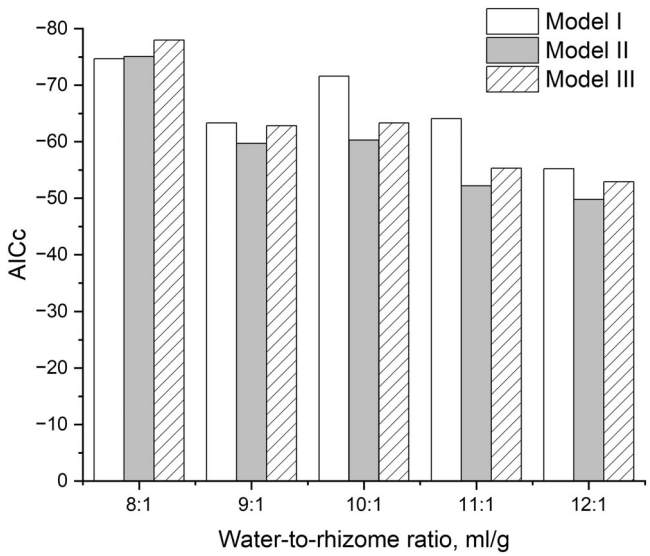

Figure 3. Variation of $\mathrm{AIC_c}$ with the water-to-rhizome ratio.

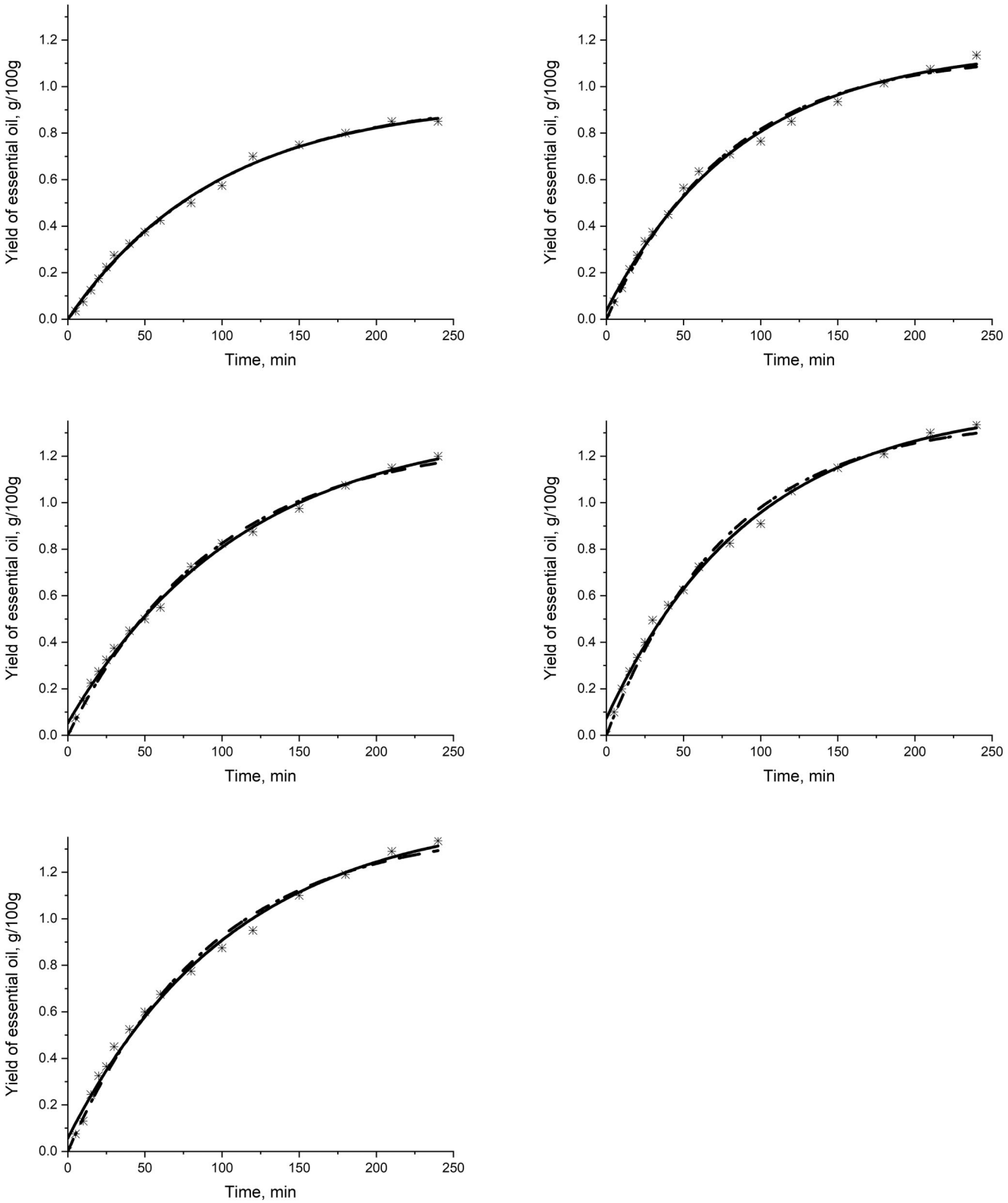

Figure 4. Fitting the model to the experimental data: (a) 8:1, (b) 9:1, (c) 10:1, (d) 11:1, and (e) 12:1 (Experimental points – ×, Model I – solid line, Model II – dashed line, and Model III – dotted line).

RESULTS AND DISCUSSION

Effect of the water-to-rhizome ratio on essential oil yield

Figure 1 shows a progressive increase in essential oil yield, from 0.85 ± 0.00 g/100 g to 1.34 ± 0.01 g/100, as the water-to-rhizome ratio increased from 8:1 to 11:1 mL/g. Beyond this point, increasing the water-to-rhizome ratio to 12:1 mL/g did not result in further changes to the yield. The observed improvement is likely due to enhanced water-plant material

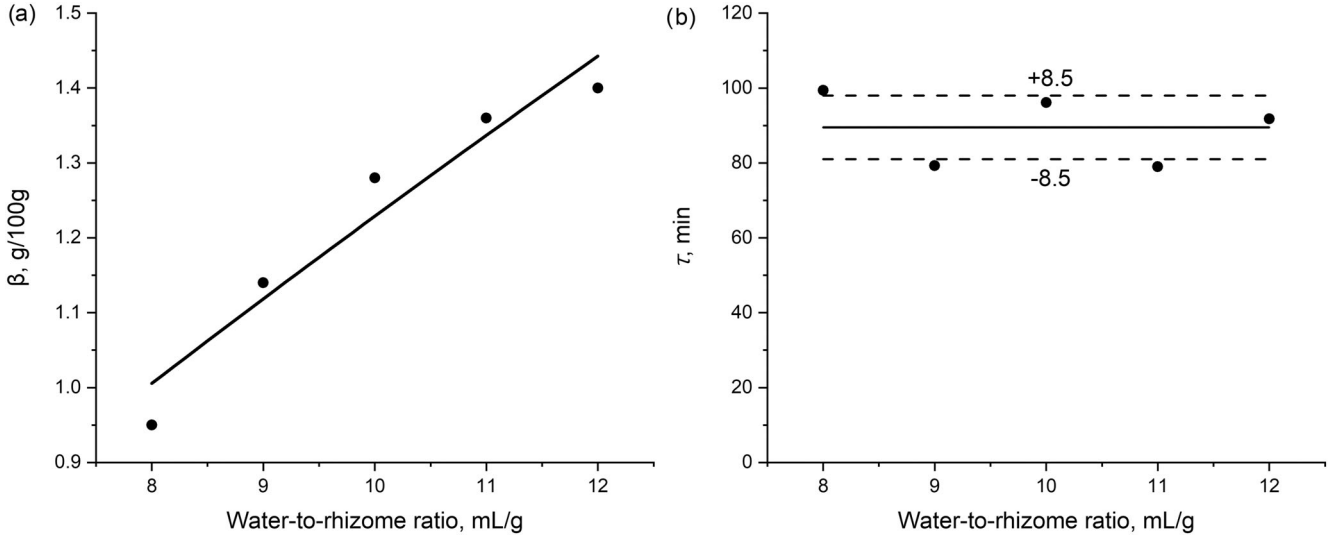

Figure 5. Dependence of Model III's parameters (a) *b* and (b) *t* on the water-to-rhizome ratio.

interaction and reduced mass transfer resistance, which facilitate the release of volatile compounds during hydrodistillation.[54] At the water-to-rhizome ratio of 12:1, the plateau in yield may be attributed to the saturation of solubilization and/or the chemical modification of some components involving water molecules.[44] Statistical analysis *via* one-way ANOVA confirms the significant influence of the water-to-rhizome ratio on the extraction efficiency of essential oil from *A. calamus* rhizomes ($F = 118.6 > p = 0.0001$).

Mechanism of sweet flag essential oil distillation

Figure 2 shows the typical variations in essential oil yield throughout the hydrodistillation process, highlighting distinct fast and slow distillation stages, commonly referred to as the washing and diffusion phases. The rapid initial increase in essential oil yield corresponds to the 'washing' stage, where essential oil present on the external surfaces of rhizome particles is quickly extracted. In contrast, the subsequent slower distillation stage involves the diffusion of essential oil from the interior of the rhizome matrix into the bulk liquid phase. This process is influenced by the anatomy of sweet flag rhizomes, characterized by developed aerenchyma, primary bark, and central cylinder.[5] Grinding disrupts some oil-containing cells within the aerenchyma, releasing a part of the oil onto the external surfaces, which is rapidly distilled off during the initial phase of hydrodistillation. Meanwhile, essential oil from intact cells gradually diffuses through cell membranes and structural barriers to reach the outer surfaces and the bulk liquid phase during the slower diffusion stage.

Kinetic essential oil extraction

The parameter values for the proposed models, along with statistical criteria such as R^2, RMSE, and MRPD, are presented in Tables 2, 2, 3, and 4. The graphical representation of AIC_c is shown in Fig. 3. All three models provided an excellent fit to the experimental data, as illustrated in Fig. 4. This is further confirmed by high R^2 values (>0.990) and low RMSE values (ranging from 0.017 to 0.037). Additionally, MRPD values ranged from 4% to 10%, with most staying below ±7%. As a result, all three models effectively describe the time variation of essential oil yield, with Model I showing the best overall agreement with the experimental data. Model I also achieved the lowest AIC_c values, ranging from −74.7 to −55.2, except for the case of a 1:8 water-to-rhizome ratio. In contrast, Model II, with AIC_c values of −75.1 and −49.8, demonstrated the poorest fit to the experimental data. Model III exhibited AIC_c values between −78.0 and −52.9.

Statistical criteria indicate that Model I, with all parameters statistically significant, fits the data very well. However, Models II and III do not perform any worse. They have somewhat lower R^2 values. Overall, Model III appears to be the most suitable for modeling the extraction kinetics of sweet flag essential oil due to its simplicity and excellent agreement with the experimental data. Fig. 5 indicates that the parameter β (= q_∞) increases with increasing the water-to-rhizome ratio, while the parameter *t* seems to vary around a mean value (89.1 ± 8.5 min), indicating that the diffusion rate does not depend on the water-to-rhizome ratio.

It is evident from Fig. 5 that the parameter β increases with the water-to-rhizome ratio, gradually leveling off as it approaches a maximum value at higher ratios. To model this behavior, a hyperbolic function is used:

$$\beta = \frac{a \cdot x}{b + x} \tag{10}$$

where *x* is the water-to-rhizome ratio, *a* is the highest value β can reach, and *b* shows when β stops increasing. The parameters *a* and *b* were estimated through non-linear regression, with values of $a = 11.04$ [g/100 g] and $b = 79.84$ [mL/g] ($R^2 = 0.916$, MRPD = ±3.3%).

CHEMICAL COMPOSITION OF THE ESSENTIAL OILS

The chemical analysis revealed the presence of 53 components. In Table 5, only components found in the essential oil of *A. calamus* rhizome that have a content higher than 1% by weight were presented. The variation of the water-to-rhizome ratio did not significantly affect the essential oil composition of sweet flag rhizomes. The main components present in the oil were shyobunone

Table 5. Composition (% mas) of components in essential oil (total = 53 compounds)

Components	RI	Water-to-rhizome ratio 8:1 %m/m	9:1 %m/m	10:1 %m/m	11:1 %m/m	12:1 %m/m
Camphene, $C_{10}H_{16}$	946	2.35	2.53	1.93	1.61	1.23
Camphor, $C_{10}H_{16}O$	1141	4.40	4.63	4.27	4.08	3.29
β – Gurjunene, $C_{15}H_{24}$	1431	0.94	0.96	0.80	0.89	1.00
β – Selinene, $C_{15}H_{24}$	1489	1.31	1.37	1.16	1.28	1.31
α-Selinene, $C_{15}H_{24}$	1498	2.37	2,32	1.82	2.21	2.32
Shyobunone, $C_{15}H_{24}O$	1502	5.77	5.99	5.20	5.54	5.36
δ – Cadinene, $C_{15}H_{24}$	1522	2.46	3.10	2.31	2.64	2.73
Spatlulenol, $C_{15}H_{24}O$	1577	1.62	1.77	1.85	1.75	1.70
β-Asarone ((*Z*)-asarone), $C_{12}H_{16}O_3$	1616	11.71	10.86	11.81	11.84	11.45
β-Cedrene epoxide, $C_{15}H_{24}O$	1621	10.22	9.18	9.30	9.75	9.83
Muurola-4,10(14)-dien-1-beta-ol, $C_{15}H_{24}O$ (α-acorenol, MW222, RI 1630)	1630	2.71	2.84	2.75	2.89	2.83
epi- α-Cadinol, $C_{15}H_{26}O$	1640	1.64	1.79	1.68	1.67	1.73
epi-a-Muurolol, $C_{15}H_{26}O$	1642	1.90	1.89	1.82	1.84	1.87
α-Selin-11-en-4-ol, $C_{15}H_{26}O$	1658	/	0.97	1.16	1.08	1.11
(*E*)-Asarone, MW208, $C_{12}H_{16}O_3$	1675	1.02	1.12	1.08	1.10	1.20
Spiro[4.5]decan-7-one,1,8-dimethyl-4-(1-methyl ethyl)-, $C_{15}H_{26}O$	1682	1.03	1.25	1.10	1.15	1.24
Spiro[4.5]dec-8-en-7-ol,4,8-dimethyl-1-(1-methylethyl)-(Trichoacorenol)	1687	3.33	3.40	3.54	3.46	3.54
Acorenone, $C_{15}H_{24}O$	1693	1.53	1.58	1.78	1.61	1.62
Acorenone B,$C_{15}H_{24}O$	1703	18.14	19.13	18.54	18.48	19.79
Isocalamendiol, $C_{15}H_{26}O_2$	1760	4.02	3.76	4.61	4.47	4.76
Iso-acorenone, $C_{15}H_{24}O$	1813	2.73	2.97	3.50	3.29	2.73
Monoterpene hydrocarbons		2.35	2.53	1.93	1.61	1.23
Oxygenated monoterpenes		4.4	4.63	4.27	4.08	3.29
Sesquiterpene hydrocarbons		7.08	7.75	6.09	7.02	7.36
Oxygenated sesquiterpenes		54.64	56.52	56.83	56.98	58.11
Phenylpropanoids		12.73	11.98	12.89	12.94	12.65

(5.20–5.99%), β-asarone (10.86–11.84%), β-cedrene epoxide (9.18–10.22%), and 1,7-*cis*-acarenone (18.14–19.79%).

The composition of essential oil obtained by hydrodistillation of the rhizome from triploid *A. calamus* was investigated in detail only in a few studies.[7,38,55-57] Mazza[57] analyzed the European triploid *A. calamus*, identifying acorenone (8.1%), isoshyobunone (6.3%), β-gurjunene (6.7%), calamendiol (5.2%), and β-asarone (5.2%) as the main components. Dušek *et al.*[54] reported a β-asarone content ranging from 9 to 21%, while Škobić *et al.*[7] reported β-asarone levels between 4.82% and 17.98% in cultivated and wild *A. calamus*. Essential oils obtained from sweet flag originating from Italy and Poland exhibited a similar composition, with the highest concentrations of β-azarone (11% and 10.4% in Italian and Polish samples, respectively).[38,55]

The essential oil of *A. calamus* from Estonia was characterized by high concentrations of acorenone (22.4–27.5%), shyobunone (8.2–13.7%), pre-isocalamendiol (8.1%), iso-acorone (2.8–5.0%), dehydroisocalamendiol (3.5–4.5%), epishyobunone (3.3–3.9%), and β-asarone (9.2–10.2%). Notably, none of the examined essential oils contained β-cedrene epoxides, though β-cedrene has been identified in some studies as a component of *A. calamus* rhizome essential oil.[7,10] It might be hypothesized that β-cedrene undergoes epoxidation during extended hydrodistillation, leading to the formation of β-cedrene epoxides.

Sweet flag essential oils are predominantly composed of oxygenated sesquiterpenes. Škobić *et al.*[7] reported a similar finding, with these compounds comprising approximately 45% of the oil composition. Furthermore, essential oils extracted under lower water-to-rhizome ratios exhibited higher proportions of monoterpenes and their oxygenated derivatives, while those obtained under higher water-to-rhizome ratios showed reduced levels of oxygenated sesquiterpenes.

CONCLUSION

The effect of the water-to-rhizome ratio on the yield, chemical composition, and extraction kinetics of sweet flag essential oil by HD was thoroughly investigated. The water-to-rhizome ratio significantly affected the yield of sweet flag essential oil, with the best yield obtained at an 11:1 water-to-rhizome ratio. While all three models tested showed good agreement with the experimental data, the simplest Model III, *i.e.*, the first-order kinetics model, was recommended as the most suitable for describing the kinetics of the extraction process. The water-to-rhizome ratio did not significantly affect the chemical composition of the essential oil. The main components present in the oil were identified as shyobunone (5.20–5.99%), β-asarone (10.86–11.84%), β-cedrene epoxide (9.18–10.22%), and 1,7-cis-acarenone (18.14–19.79%).

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DATA AVAILABILITY STATEMENT

Data available upon request from the corresponding author.

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