



**NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY
POLITEHNICA BUCHAREST**
Faculty of Chemical Engineering and Biotechnology
Department of Chemical Engineering and Biochemistry
**DOCTORAL SCHOOL OF CHEMICAL ENGINEERING
AND BIOTECHNOLOGY**



DOCTORAL THESIS

-ABSTRACT-

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Scientific supervisor: **Prof. dr. ing. Oana Cristina PÂRVULESCU**

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**INTENSIFICATION OF MASS TRANSFER IN THE
EXTRACTION PROCESS OF BIOACTIVE
COMPOUNDS**

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PURPOSE, CONTEXT, AND STRUCTURE OF THE THESIS

In accordance with its title, the *general objective* of the doctoral thesis was to intensify mass transfer in the extraction processes (liquid-liquid and solid-liquid) of active compounds.

The *specific objectives* of the studies conducted were:

- to evaluate the effect of process factors on the performance and kinetics of the transfer process of indole-3-acetic acid (IAA) through a chloroform-based liquid membrane, using trioctylamine (TOA) as a carrier agent;
- optimization of ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) of chlorophyll pigments and carotenoids from mint leaves (*Mentha × piperita* L.);
- improvement of the extraction efficiency of thyme essential oil (*Thymus officinalis* L.) through the combined use of ultrasound treatment and microwave-assisted hydrodistillation (MWHd).

The thesis consists of two main parts, **1. LITERATURE REVIEW** and **2. ORIGINAL CONTRIBUTIONS**, followed by general conclusions and research perspectives, a list of works, and a bibliography.

The **LITERATURE REVIEW** contains important aspects of liquid-liquid and solid-liquid processes, *e.g.*, liquid membrane-facilitated percolation, separation of IAA from aqueous solutions, conventional and unconventional extraction methods, composition, uses, and therapeutic effects of mint and thyme, and extraction of bioactive compounds from these plants.

The **ORIGINAL CONTRIBUTIONS** section presents the results obtained in our own research on membrane-facilitated percolation (**chapter 2.1**) and the extraction of bioactive compounds from plants (**chapter 2.2**).

Chapter **2.1. MEMBRANE-FACILITATED PERTRACTION** comprises two main parts, *i.e.*, **2.1.1. Effects of process factors on the performance of the facilitated pertraction process** and **2.1.2. The kinetics of the facilitated pertraction process**, both referring to the transfer of IAA through a chloroform-based liquid membrane, using TOA as a carrier agent. The facilitated pertraction experiments were performed in a "tube-in-tube" transfer cell for 4 h at ambient temperature with mechanical stirring (200 rpm) of the inner tube. The inner tube contained the NaOH stripping solution, and the outer tube contained the membrane phase and the IAA feed solution.

Chapter **2.2. EXTRACTION OF BIOACTIVE COMPOUNDS FROM PLANTS** consists of two parts, *i.e.*, **2.2.1. Optimization of ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) of chlorophyll pigments and carotenoids from mint leaves (*Mentha × piperita* L.)** and **2.2.2. Improving the efficiency of essential oil extraction from thyme through the combined use of ultrasound treatment and microwave-assisted extraction.**

Subchapter **2.2.1.** presented the results obtained from the extraction of bioactive compounds from mint leaves in the presence of ultrasound (UAE) and microwaves (MAE), respectively. In accordance with a two-factor CCD experimental design with four experiments in the center of the design, twelve experiments were performed for each extraction method. The chlorophyll *a* (*CaC*) concentration, chlorophyll *b* (*CbC*) concentration, total carotenoid (*TCC*) concentration, and antibacterial activity of mint leaf extracts (evaluated by measuring the diameter of the inhibition zone (*d*)) were measured experimentally and predicted at different levels of the process factors, *i.e.*, the percentage mass concentration of ethanol in the extraction solvent ($c_{et} = 44\text{--}86\%$) and the liquid/solid ratio ($R_{LS} = 5.9\text{--}34.1$ mL/g).

Subsection **2.2.2.** presents the results obtained from the extraction of essential oil (EO) from thyme leaves and stems. The influence of ultrasonic pretreatment applied to the extraction mixture prior to microwave-assisted hydrodistillation (MWHD) on the EO extraction yield and the content of bioactive compounds, *i.e.*, thymol, γ -terpinene, *p*-cymene, α -terpinene, and β -pinene, was studied. Comparative extractions were also performed without pretreatment, both by MWHD and conventional hydrodistillation (CHD). The influence of process factors, *i.e.*, liquid/solid ratio, plant material particle size, type of ultrasonic equipment used for pretreatment, and ultrasonic processor amplitude, on the performance of the extraction process (MWHD with pretreatment, MWHD, and CHD) was evaluated.

Chapter **3** presents the general conclusions of the thesis and some research perspectives.

1.1. INTRODUCTION

Solid-liquid extraction and liquid-liquid extraction are processes for separating components from a solid or liquid phase based on differences in solubility in a solvent. The efficiency of the process depends on numerous factors, e.g. (Hernández et al., 2009):

- the nature and properties of the solvent;
- the initial composition of the solid/liquid phase;
- the contact surface between the solid/liquid phase and the extraction solvent;
- the liquid/solid ratio;
- the working temperature and pressure;
- the operating time;
- the type and characteristics of the extraction equipment.

Choosing the best extraction technique is essential for both quantitative and qualitative studies of bioactive compounds derived from plant sources (Sasidharan et al., 2011; Smith, 2003; Uwineza and Waśkiewicz, 2020). Over the past 50 years, new extraction techniques have been developed that are more efficient, faster, and environmentally friendly, e.g. (Gupta et al., 2012; Peng et al., 2021):

- ultrasound-assisted extraction (UAE);
- microwave-assisted extraction (MAE);
- supercritical fluid extraction (SFE);
- microwave-assisted hydrodistillation (MWHD);
- ultrasonic or enzymatic pretreatment prior to extraction.

2.1. FACILITATED PERTRACTION THROUGH LIQUID MEMBRANES

2.1.1. EFFECTS OF PROCESS FACTORS ON THE PERFORMANCE OF FACILITATED PERTRACTION

The paper has aimed at studying the transfer of indole 3-acetic acid (*IAA*) from a feed aqueous solution to a stripping aqueous solution of *NaOH* using a chloroform bulk liquid membrane and trioctylamine (*TOA*) as a ligand (*L*). The data presented in this chapter are published in the article:

Diaconu, I., Pârvulescu, O.C., **Topală, S.L.**, Dobre, T. (2021) *Effects of process factors on performances of liquid membrane-based transfer of indole-3-acetic acid*. Sci. Rep., 11(1), 23427, WOS:000727615800033 (Q1).

2.1.1.1. Materials and methods

IAA, chloroform, *TOA*, and *NaOH*, which were provided by Merck (Germany), were analytical grade reagents used without further purification.

2.1.1.2. Experimental setup and process parameters

A scheme of experimental setup used to study the transfer of *IAA* from the feed phase to stripping phase is shown in Fig. 8. The tube in tube setup consists of an outer glass tube, containing the feed solution (at the upper part) and membrane phase (at the bottom part), and an inner glass tube, containing the stripping solution. The internal diameter of outer tube was $D_{in}=0.042$ m, whereas the external and internal diameters of inner tube were $d=0.021$ m and $d_{in}=0.019$ m, respectively. The values of phase volumes were $V_F=20\times 10^{-6}$ m³, $V_M=50\times 10^{-6}$ m³, and $V_S=7\times 10^{-6}$ m³.

The mass transfer process in the experimental setup occurs as follows:

- *IAA* diffuses through *F* phase towards interface between *F* and *M* phases;
- *TOA* (*L*) diffuses through *M* phase towards *F-M* interface;
- *IAA* reacts with *L* at the interface forming the *IAA-L* complex;
- *IAA-L* complex diffuses through *M* phase and reaches the interface between *M* and *S* phases, where the decomplexation takes place;
- *IAA* is released into *S* phase and *L* diffuses back through *M* phase

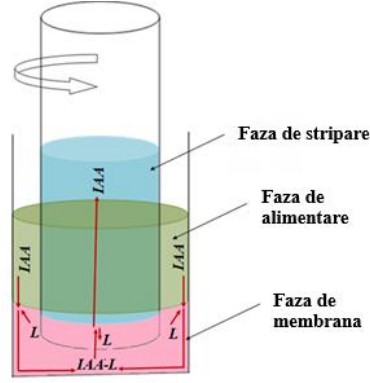


Figure 8. Scheme of experimental setup

Initial molar concentrations of *IAA*, *TOA*, and *NaOH* in *j* (*F*, *M*, *S*) phase, namely $c_{IAA,F0}$, $c_{L,M0}$, and $c_{NaOH,S0}$, were selected as process independent variables (factors). Twenty experimental runs were conducted at different levels of $c_{IAA,F0}$ (10^{-4} - 10^{-3} kmol/m³), $c_{L,M0}$ (10^{-2} and 10^{-1} kmol/m³), and $c_{NaOH,S0}$ (10^{-2} and 1 kmol/m³). Each experiment was performed for 4 h, at ambient temperature, under mechanical stirring (200 rpm) of inner tube.

Final (*f*) values of molar concentration of *IAA* in the feed ($c_{IAA,Ff,ex}$) and stripping solution ($c_{IAA,Sf,ex}$), corresponding to $\tau_f=14400$ s, were determined experimentally using a LAMBDA 750 spectrophotometer (PerkinElmer, USA). Extraction efficiency ($E_{F,ex}$), distribution coefficient ($K_{D,ex}$), and recovery efficiency ($E_{R,ex}$) are defined by Eqs. (1)-(3).

$$E_{F,ex} = 100 \frac{c_{IAA,F0} - c_{IAA,Ff,ex}}{c_{IAA,F0}} \quad (1)$$

$$K_{D,ex} = \frac{c_{IAA,F0} - c_{IAA,Ff,ex}}{c_{IAA,Ff,ex}} \quad (2)$$

$$E_{R,ex} = 100 \frac{c_{IAA,Sf,ex}}{c_{IAA,F0}} \quad (3)$$

Statistical analysis

Multiple regression analysis and correlation analysis were performed using XLSTAT 2019.1 (Excel).

2.1.1.3. Mathematical modelling

2.1.1.3.1. Statistical models

The effects of process factors ($c_{IAA,F0}$, $c_{NaOH,S0}$, and $c_{L,M0}$) on dependent variables (responses), *i.e.*, $c_{IAA,Ff}$, $c_{IAA,Sf}$, E_F , K_D , and E_R , were quantified using statistical models based on a 2^3 factorial plan^{35,36}. According to a 2^3 factorial plan, 8 experimental runs (1-8 in Table 2) were conducted at 2 levels (inferior and superior) of process factors. Dimensionless values of process factors are given by Eqs. (4)-(6), where $c_{IAA,F0,cp}=0.00055$ kmol/m³, $c_{NaOH,S0,cp}=0.505$ kmol/m³, and $c_{L,M0,cp}=0.055$ kmol/m³ are centre-points.

$$x_1 = \frac{c_{IAA,F0} - 0,00055}{0,00045} \quad (4)$$

$$x_2 = \frac{c_{NaOH,S0} - 0,505}{0,495} \quad (5)$$

$$x_3 = \frac{c_{L,M0} - 0,055}{0,045} \quad (6)$$

Moreover, 4 centre-point runs (9-12 in Table 2) were performed. Statistical models described by Eq. (7) link the process dimensionless factors, x_j ($j=1..3$), and their interactions (x_1x_2 , x_1x_3 , x_2x_3 , and $x_1x_2x_3$) to the process responses, y_i ($i=1..5$), *i.e.*, $y_1=c_{IAA,Ff} \times 10^4$, $y_2=c_{IAA,Sf} \times 10^3$, $y_3=E_F$, $y_4=K_D$, and $y_5=E_R$. Regression coefficients, β_{ki} ($k=1..8$, $i=1..5$), were determined based on experimental data summarized in Table 2.

$$y_i = \beta_{1i} + \beta_{2i}x_1 + \beta_{3i}x_2 + \beta_{4i}x_3 + \beta_{5i}x_1x_2 + \beta_{6i}x_1x_3 + \beta_{7i}x_2x_3 + \beta_{8i}x_1x_2x_3 \quad (7)$$

2.1.1.3.2. Mass transfer-based model

Some characteristic parameters of mass transfer process in the three-phase system, *i.e.*, association constants in membrane phase ($K_{as,M}$) and feed solution ($K_{as,F}$), repartition constants of species *IAA* and *L* (*TOA*) between membrane and feed solution ($R_{IAA,M/F}$ and $R_{L,M/F}$), repartition constant of species *L* between membrane and stripping solution ($R_{L,M/S}$), and extraction constant (K_{ext}), are defined by Eqs. (8)-(11), where $c_{s,p}$ (kmol/m³) represents the molar concentration of *s* (*IAA*, *L*, *IAA-L*) species in the *p* (*F*, *M*, *S*) phase.

$$K_{as,p} = \frac{c_{IAA-L,p}}{c_{IAA,p}c_{L,p}}, p = F, M \quad (8)$$

$$R_{IAA,M/F} = \frac{c_{IAA,M}}{c_{IAA,F}} \quad (9)$$

$$R_{L,M/p} = \frac{c_{L,M}}{c_{L,p}}, p = F, S \quad (10)$$

$$K_{ext} = \frac{c_{IAA-L,M}}{c_{IAA,F}c_{L,M}} \quad (11)$$

The partial mass balance of L species in the three-phase system, considering perfectly mixed phases, is given by Eq. (12), where V_p (m^3) is the volume of p (F, M, S) phase and $c_{L,M0}$ ($kmol/m^3$) the initial molar concentration of L species in the membrane phase. Dividing the terms of Eq. (12) to V_M , Eq. (13) was obtained, where $r_{M/p}$ ($p=F, S$) volume ratios are defined by Eq. (14).

$$c_{L,M0}V_M = (c_{L,M} + c_{IAA-L,M})V_M + (c_{L,F} + c_{IAA-L,F})V_F + c_{L,S}V_S \quad (12)$$

$$c_{L,M0} = (c_{L,M} + c_{IAA-L,M}) + \frac{(c_{L,F} + c_{IAA-L,F})}{r_{M/F}} + \frac{c_{L,S}}{r_{M/S}} \quad (13)$$

$$r_{M/p} = \frac{V_M}{V_p}, p = F, S \quad (14)$$

Substituting Eqs. (8)-(11) into Eq. (13), $c_{L,M0}$ is given by Eq. (15), where H is defined by Eq. (16). Assuming $H \approx 1$ ($R_{L,M/F}r_{M/F} \gg 1$ and $R_{L,M/S}r_{M/S} \gg 1$), $c_{IAA-L,M}$ can be expressed depending on $c_{L,M0}$, $c_{IAA,F}$, and K_{ext} using Eq. (17).

$$c_{L,M0} = c_{IAA-L,M} \left(1 + \frac{H}{K_{ext}c_{IAA,F}} \right) \quad (15)$$

$$H = 1 + \frac{K_{as,F}c_{IAA,F}}{R_{L,M/F}r_{M/F}} + \frac{1}{R_{L,M/F}r_{M/F}} + \frac{1}{R_{L,M/S}r_{M/S}} \quad (16)$$

$$c_{IAA-L,M} = \frac{c_{L,M0}}{1 + \frac{1}{K_{ext}c_{IAA,F}}} \quad (17)$$

Total molar flux of IAA ($J_{IAA,tot}$) is defined by Eq. (18) as sum of molar flux of free IAA (J_{IAA}) and molar flux of associated IAA (J_{IAA-L}). Assuming $J_{IAA} \ll J_{IAA-L}$, $J_{IAA,tot}$ [$kmol/(m^2 \cdot s)$] can be expressed by Eq. (19), where $k_{IAA-L,M}$ (m/s) represents the mass transfer coefficient of $IAA-L$ complex in the membrane phase. Substituting Eq. (17) into Eq. (19) and considering a stationary state characterized by a mean flux $J_{IAA,tot,m}$, Eq. (20) was obtained, where $c_{IAA,F,m}$ is a mean concentration of IAA in the feed phase. According to Eq. (20), $k_{IAA-L,M}$ and K_{ext} can be estimated

from the intercept and slope of the straight line given by a plot of $\frac{c_{L,M0}}{J_{IAA,tot,m}}$ vs. $\frac{1}{c_{IAA,F,m}}$. The parameters $k_{IAA-L,M}$ and K_{ext} were determined based on experimental data obtained in 16 experimental runs (1-8 and 13-20 in Table 2), which were performed at different levels of $c_{IAA,F0}$ (0.0001, 0.0003, 0.0006, and 0.001 kmol/m³), $c_{NaOH,S0}$ (0.01 and 1 kmol/m³), and $c_{L,M0}$ (0.01 and 0.1 kmol/m³).

$$J_{IAA,tot} = J_{IAA} + J_{IAA-L} \quad (18)$$

$$J_{IAA,tot} \approx J_{IAA-L} = k_{IAA-L,M} c_{IAA-L,M} \quad (19)$$

$$\frac{c_{L,M0}}{J_{IAA,tot,m}} = \frac{1}{k_{IAA-L,M}} + \frac{1}{k_{IAA-L,M} K_{ext}} \frac{1}{c_{IAA,F,m}} \quad (20)$$

2.1.1.4. Results and discussions

2.1.1.4.1. Statistical models

The values of dimensional and dimensionless factors and those of process responses determined experimentally, *i.e.*, $c_{IAA,Ff,ex}=0.02-1 \times 10^{-4}$ kmol/m³, $c_{IAA,Sf,ex}=0.22-2.58 \times 10^{-3}$ kmol/m³, $E_{F,ex}=90.0-97.9\%$, $K_{D,ex}=9.0-46.6$, and $E_{R,ex}=66.5-94.2\%$, are summarized in Table 2. Tabulated data highlight the following issues

- lower values of $c_{IAA,Ff,ex}$ at inferior levels of $c_{IAA,F0}$ and superior levels of $c_{NaOH,S0}$ and $c_{L,M0}$, the effect of $c_{IAA,F0}$ being significant (12.5-14.3 times);
- higher values of $c_{IAA,Sf,ex}$ at superior levels of $c_{IAA,F0}$ and $c_{NaOH,S0}$, the effect of $c_{IAA,F0}$ being significant (8.2-9.9 times);
- higher values of $E_{F,ex}$ and $K_{D,ex}$ at inferior level of $c_{IAA,F0}$ and superior levels of $c_{NaOH,S0}$ and $c_{L,M0}$; (higher values of $E_{R,ex}$ at inferior level of $c_{IAA,F0}$, superior level of $c_{NaOH,S0}$, and, except for runs 4 and 8, at superior level of $c_{L,M0}$).

An increase in extraction and recovery efficiencies and distribution coefficient with an increase in $c_{L,M0}$ and $c_{NaOH,S0}$ was reported in the related literature. A higher level of $c_{L,M0}$ leads to an increase in the concentration of *IAA-L* complex in the membrane phase, resulting in enhanced mass transfer of this complex. On the other hand, a higher value of $c_{NaOH,S0}$ determines enhanced mass transfer of *IAA-L* complex by increasing the concentration of *L* released in the membrane phase after decomplexation. Moreover, a decrease in extraction and recovery efficiencies with an increase in $c_{IAA,F0}$ was found by other researcher.

Table 2. Experimentation matrix for 2³ factorial experiment.

Exp.	$c_{IAA,F0}$ $\times 10^3$ (kmol/m ³)	$c_{NaOH,S0}$ $\times 10^3$ (kmol/m ³)	$c_{L,M0}$ $\times 10^3$ (kmol/m ³)	x_1	x_2	x_3	$c_{IAA,Ff,ex}$ $\times 10^4$ (kmol/m ³)	$c_{IAA,Sf,ex}$ $\times 10^3$ (kmol/m ³)	$E_{F,ex}$ (%)	$K_{D,ex}$	$E_{R,ex}$ (%)
1	0,1	10	10	-1	-1	-1	0,07	0,22	93,0	13,3	78,1
2	1	10	10	1	-1	-1	1,00	1,90	90,0	9,00	66,5
3	0,1	1000	10	-1	1	-1	0,03	0,26	97,0	32,3	91,0
4	1	1000	10	1	1	-1	0,42	2,58	95,8	22,8	90,2
5	0,1	10	100	-1	-1	1	0,04	0,24	96,0	24,0	85,1
6	1	10	100	1	-1	1	0,50	2,00	95,0	19,0	70,0
7	0,1	1000	100	-1	1	1	0,02	0,27	97,9	46,6	94,2
8	1	1000	100	1	1	1	0,28	2,37	97,2	34,7	83,0
9	0,55	505	55	0	0	0	0,25	1,11	95,5	21,3	70,6
10	0,55	505	55	0	0	0	0,26	1,08	95,3	20,3	68,7
11	0,55	505	55	0	0	0	0,23	1,15	95,8	22,8	73,2
12	0,55	505	55	0	0	0	0,24	1,13	95,7	22,3	71,9

Statistical models given by Eqs. (21)-(25) express the process responses depending on dimensionless factors and their interactions. Regression coefficients, β_{ki} ($k=1..8$, $i=1..5$), which were determined by processing the experimental data presented in Table 2, with their corresponding values of standard errors (SE_{ki}), t statistics (t_{ki}), and p -values (p_{ki}).

Tabulated results indicate that Eqs. (21)-(24) fit the data very well ($R^2 \geq 0.966$, $R^2_{adj} \geq 0.906$, $RSE \leq 3.050$, $F \geq 16.13$, $p \leq 0.009$), whereas Eq. (25) does not fit the data well ($R^2 = 0.674$, $R^2_{adj} = 0.104$, $RSE = 9.233$, $F = 1.182$, $p = 0.462$). Moreover, all factors and their binary and ternary interactions in Eq. (25) are statistically non-significant, *i.e.*, $p_{k5} > 0.05$ ($k=2..8$). Quadratic regression equation (26), where β_{k5} ($k=1..10$) are regression coefficients, was selected to express $y_5 = E_R$

$$y_1 = c_{IAA,Ff} \times 10^4 = 0,278 + 0,255x_1 - 0,107x_2 - 0,085x_3 - 0,093x_1x_2 - 0,075x_1x_3 + 0,048x_2x_3 + 0,042x_1x_2x_3 \quad (21)$$

$$y_2 = c_{IAA,Sf} \times 10^3 = 1,193 + 0,982x_1 + 0,139x_2 - 0,010x_3 + 0,123x_1x_2 - 0,017x_1x_3 - 0,040x_2x_3 - 0,037x_1x_2x_3 \quad (22)$$

$$y_3 = E_F = 95,35 - 0,737x_1 + 1,738x_2 + 1,288x_3 + 0,262x_1x_2 + 0,313x_1x_3 - 0,712x_2x_3 - 0,188x_1x_2x_3 \quad (23)$$

$$y_4 = K_D = 24,04 - 3,839x_1 + 8,899x_2 + 5,863x_3 - 1,518x_1x_2 - 0,387x_1x_3 + 0,685x_2x_3 - 0,208x_1x_2x_3 \quad (24)$$

$$y_5 = E_R = 78,53 - 4,821x_1 + 7,341x_2 + 0,805x_3 + 1,829x_1x_2 - 1,733x_1x_3 - 1,820x_2x_3 - 0,834x_1x_2x_3 \quad (25)$$

$$y_5 = E_R = \beta_{15} + \beta_{25}x_1 + \beta_{35}x_2 + \beta_{45}x_3 + \beta_{55}x_1x_2 + \beta_{65}x_1x_3 + \beta_{75}x_2x_3 + \beta_{85}x_1^2 + \beta_{95}x_2^2 + \beta_{105}x_3^2 \quad (26)$$

Statistical models expressed by Eq. (21) and Eqs. (27)-(30), obtained after removing statistically non-significant terms in Eqs. (22)-(24) and (26), along with their corresponding results of multiple regression analysis indicate the following aspects:

- $c_{IAA,Ff}$ decreases with a decrease in x_1 , x_2x_3 , and $x_1x_2x_3$ as well as with an increase in x_2 , x_3 , x_1x_2 , and x_1x_3 ;
- $c_{IAA,Sf}$ increases with an increase in x_1 , x_2 , and x_1x_2 ;
- E_F increases with a decrease in x_1 and x_2x_3 as well as with an increase in x_2 and x_3 ;
- higher levels of K_D correspond to lower values of x_1 and higher values of x_2 and x_3 ;
- higher levels of E_R correspond to lower values of x_1 and higher values of x_2 and x_2^2 ;
- x_1 has the most significant influence on $c_{IAA,Ff}$ and $c_{IAA,Sf}$, whereas x_2 has a major effect on E_F , K_D , and E_R ;
- Eq. (21) and Eqs. (27)-(30) fit the data very well ($R^2 \geq 0.905$, $R^2_{adj} \geq 0.870$, $RSE \leq 3.521$, $F \geq 25.47$, $p \leq 1.9E-04$).

The resulting equations after eliminating insignificant terms are:

$$y_2 = c_{IAA,Sf} \times 10^3 = 1,193 + 0,982x_1 + 0,139x_2 + 0,123x_1x_2 \quad (27)$$

$$y_3 = E_F = 95,35 - 0,737x_1 + 1,738x_2 + 1,288x_3 - 0,712x_2x_3 \quad (28)$$

$$y_4 = K_D = 24,04 - 3,839x_1 + 8,899x_2 + 5,863x_3 \quad (29)$$

$$y_5 = E_R = 71,11 - 4,821x_1 + 7,341x_2 + 11,13x_2^2 \quad (30)$$

2.1.1.4.2. Mass transfer-based model

The values of $c_{IAA,Sf,ex}$, $c_{IAA,F,m}$, and $J_{IAA,tot,m}$ at different levels of $c_{IAA,F0}$ (0.0001, 0.0003, 0.0006, and 0.001 kmol/m³), $c_{NaOH,S0}$ (0.01 and 1 kmol/m³), and $c_{L,M0}$ (0.01 and 0.1 kmol/m³) are presented in Table 14.

Table 14. Values of final molar concentration of IAA in the stripping solution, mean logarithmic concentration of IAA in the feed phase, and mean total flux of IAA at different levels of process factors

No.	Exp.	$c_{IAA,F0} \times 10^3$ (kmol/m ³)	$c_{NaOH,S0} \times 10^3$ (kmol/m ³)	$c_{L,M0} \times 10^3$ (kmol/m ³)	$c_{IAA,Sf,ex} \times 10^3$ (kmol/m ³)	$c_{IAA,F,m} \times 10^3$ (kmol/m ³)	$J_{IAA,tot,m} \times 10^9$ [kmol/(m ² ·s)]
1	1	0,1	10	10	0,22	0,035	0,382
2	13	0,3	10	10	0,63	0,109	1,087
3	14	0,6	10	10	1,20	0,223	2,057
4	2	1	10	10	1,90	0,391	3,258

5	3	0,1	1000	10	0,26	0,038	0,446
6	15	0,3	1000	10	0,75	0,128	1,279
7	16	0,6	1000	10	1,42	0,281	2,439
8	4	1	1000	10	2,58	0,388	4,418
9	5	0,1	10	100	0,24	0,045	0,416
10	17	0,3	10	100	0,69	0,146	1,190
11	18	0,6	10	100	1,30	0,320	2,234
12	6	1	10	100	2,00	0,581	3,429
13	7	0,1	1000	100	0,27	0,033	0,460
14	19	0,3	1000	100	0,77	0,117	1,323
15	20	0,6	1000	100	1,51	0,249	2,586
16	8	1	1000	100	2,37	0,468	4,066

$$c_{IAA,F,m} = \frac{c_{IAA,F0} - c_{IAA,Ff,ex}}{\ln\left(\frac{c_{IAA,F0}}{c_{IAA,Ff,ex}}\right)} \quad (31)$$

$$J_{IAA,tot,m} = 4 \frac{c_{IAA,Sf,ex} V_S}{\pi d_m^2 \tau_f} \quad (32)$$

According to Eq. (20), the values of mass transfer coefficient of *IAA-L* complex in the liquid membrane, $k_{IAA-L,M}=0.82-11.5 \times 10^{-7}$ m/s, and extraction constant, $K_{ext}=1033.91-1779.66$ m³/kmol, were obtained from the intercepts and the slopes of the straight lines given by the plots of $c_{L,M0}/J_{IAA,tot,m}$ vs. $1/c_{IAA,F,m}$ (Fig. 9). The levels of $k_{IAA-L,M}$ and K_{ext} , which are summarized in Table 15, indicate the following issues:

- (i) $k_{IAA-L,M}$ increases with an increase in $c_{NaOH,S0}$ (up to 5%) and decreases with an increase in $c_{L,M0}$ (about 14 times);
- (ii) K_{ext} increases with an increase in $c_{NaOH,S0}$ (up to 1.4 times) and $c_{L,M0}$ (up to 1.7 times).

The effect of x_2 and x_3 (dimensionless $c_{NaOH,S0}$ and $c_{L,M0}$ factors) on mass transfer coefficient and extraction constant can be predicted using Eqs. (33) and (34) ($R^2=1$, $RSE=0$). Multiple regression equation (34) indicates an increase in extraction constant with an increase in x_2 , x_3 , and x_2x_3 , the effect of x_3 being higher. Multiple regression equation (33) highlights that the effect of x_3 on $y=k_{IAA-L,M,calc}$ is over 30 times higher than the effects of x_2 and x_2x_3 . Eq. (35) was obtained by neglecting the contributions of x_2 and x_2x_3 in Eq. (33).

According to Eq. (35), the mass transfer coefficient is higher at lower levels of initial molar concentration of *TOA* in the membrane phase.

$$y = k_{IAA-L,M,calc} \times 10^7 = 6,0 + 0,158x_2 - 5,2x_3 - 0,142x_2x_3 \quad (33)$$

$$K_{ext,calc} = 1281,69 + 140,21x_2 + 232,67x_3 + 125,09x_2x_3 \quad (34)$$

$$y = 6,0 - 5,2x_3$$

(35)

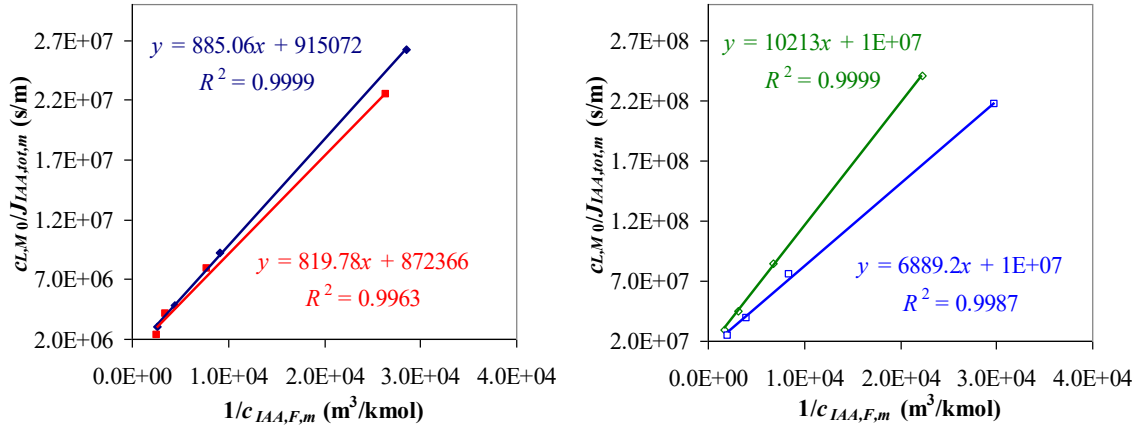


Figure 9. Variation of $c_{L,M0}/J_{IAA,tot,m}$ depending on $1/c_{IAA,F,m}$ under different operating conditions:

- ◆ $c_{L,M0}=0.01$ kmol/m³, $c_{NaOH,S0}=0.01$ kmol/m³; ■ $c_{L,M0}=0.01$ kmol/m³, $c_{NaOH,S0}=1$ kmol/m³;
 - ◇ $c_{L,M0}=0.1$ kmol/m³, $c_{NaOH,S0}=0.01$ kmol/m³; □ $c_{L,M0}=0.1$ kmol/m³, $c_{NaOH,S0}=1$ kmol/m³
- ($c_{IAA,F0}=10^{-4}$ - 10^{-3} kmol/m³, $\tau_f=14400$ s).

Table 15. Values of mass transfer coefficient of *IAA-L* complex in the liquid membrane and extraction constant at different levels of process factors

$c_{IAA,F0}$ (kmol/m ³)	$c_{NaOH,S0}$ (kmol/m ³)	$c_{L,M0}$ (kmol/m ³)	x_2	x_3	$k_{IAA-L,M} \times 10^7$ (m/s)	K_{ext} (m ³ /kmol)
10^{-4} - 10^{-3}	0,01	0,01	-1	-1	10,90	1033,91
10^{-4} - 10^{-3}	1	0,01	1	-1	11,50	1064,14
10^{-4} - 10^{-3}	0,01	0,1	-1	1	0,784	1249,06
10^{-4} - 10^{-3}	1	0,1	1	1	0,816	1779,66

2.1.1.5. Conclusion

Each experimental run was conducted for 4 h, at ambient temperature, under mechanical stirring (200 rpm) of inner tube.

The effects of dimensionless factors on process responses, *i.e.*, final values of molar concentration of *IAA* in the feed phase ($c_{IAA,Ff}$) and stripping solution ($c_{IAA,Sf}$), extraction efficiency (E_F), distribution coefficient (K_D), and recovery efficiency (E_R), were quantified using statistical models based on a 2³ factorial plan. Experimental values of process responses were as follows: $c_{IAA,Ff,ex}=0.02$ - 1×10^{-4} kmol/m³; $c_{IAA,Sf,ex}=0.22$ - 2.58×10^{-3} kmol/m³; $E_{F,ex}=90.0$ - 97.9% ; $K_{D,ex}=9.0$ - 46.6 ; $E_{R,ex}=66.5$ - 94.2%

Taking into account the statistically significant factors and their interactions, the results of regression analysis indicated the following aspects:

- all factors and their binary and ternary interactions influenced $c_{IAA,Ff}$;
- $c_{IAA,Sf}$ increased with an increase in $c_{IAA,F0}$, $c_{NaOH,S0}$, and their binary interaction;

- higher levels of E_F and K_D were obtained at low values of $c_{IAA,F0}$ and high values of $c_{L,M0}$ and $c_{NaOH,S0}$;
- higher levels of E_R were obtained at low values of $c_{IAA,F0}$ and high values of $c_{NaOH,S0}$;
- $c_{IAA,F0}$ had the most significant (positive) effect on $c_{IAA,Ff}$ and $c_{IAA,Sf}$, whereas $c_{NaOH,S0}$ had a major (positive) effect on E_F , K_D , and E_R .
- A very strong positive correlation ($r=0.95$) was found between E_F and K_D .

The results obtained in this study indicate that *IAA* was successfully transported through a chloroform BLM using *TOA* as a carrier. Mathematical models developed in the paper could be used to control and optimize the separation of *IAA* in systems containing liquid membranes.

2.1.2. KINETICS OF THE FACILITATED PERTRACTION PROCESS

This study aimed to evaluate the process factors on the kinetics of the transfer process of indole-3-acetic acid (*IAA*) through a chloroform-based liquid membrane, using trioctylamine (*TOA*) as a carrier agent. The data presented in this chapter will be published in the article:

Topală, S.L., Diaconu, I., Pârvulescu, O.C., Jidveian, R.S., Drăghici-Popa, A.M. (2025) Facilitated pertraction of indole 3-acetic acid in the presence of trioctylamine as a carrier. U.P.B. Sci. Bull. Series B 2024, 87(4) (accepted for publication).

2.1.2.1. Materials and methods

The materials and methods were those presented in chapter 2.1.1.1.

2.1.2.2. Experimental Setup and Process Parameters

The facilitated pertraction experiments were performed in the “tube-in-tube” transfer cell presented in chapter 2.1.1.2, using the same volumes of the 3 phases (F, M and S). The inner tube contained the stripping solution with NaOH, and the outer tube the membrane phase and the *IAA* feed solution. 6 experiments were performed, each of them taking place for 4 h, at ambient temperature and at different initial concentrations: $c_{IAA,F0} = 10^{-4}$ – 10^{-3} kmol/m³; $c_{L,M0} = 10^{-2}$ kmol/m³; $c_{NaOH,S0} = 10^{-2}$ – 1 kmol/m³.

The molar concentrations of *IAA* in the F and S phases at a time t , *i.e.*, $c_{IAA,F}(t)$ and $c_{IAA,S}(t)$, were measured using a spectrophotometer (UV1900i, Shimadzu, Kyoto, Japan), whereas the molar concentration of *IAA* in the M phase at a time t , *i.e.*, $c_{IAA,M}(t)$, was calculated using equation (36) based on the mass balance of *IAA* species in the membrane system. Specific amounts of *IAA* in

the F, M, and S phases at a time t , namely $R_F(t)$, $R_M(t)$, and $R_S(t)$, are defined by equations (37)–(39). Equation (40) was obtained by substituting them into equation (36).

$$c_{IAA,M}(t)V_M = c_{IAA,F0}V_F - c_{IAA,F}(t)V_F - c_{IAA,S}(t)V_S \quad (36)$$

$$R_F(t) = \frac{c_{IAA,F}(t)V_F}{c_{IAA,F0}V_F} = \frac{c_{IAA,F}(t)}{c_{IAA,F0}} \quad (37)$$

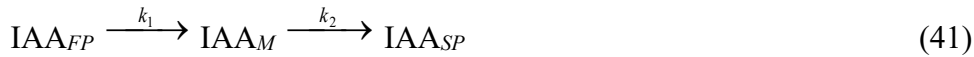
$$R_M(t) = \frac{c_{IAA,M}(t)V_M}{c_{IAA,F0}V_F} \quad (38)$$

$$R_S(t) = \frac{c_{IAA,S}(t)V_S}{c_{IAA,F0}V_F} \quad (39)$$

$$R_F(t) + R_M(t) + R_S(t) = 1 \quad (40)$$

2.1.2.3. Modelling

A kinetic model based on consecutive irreversible first-order reactions, as shown in scheme (41), and characterized by equations (42)–(44), where k_1 and k_2 are the rate constants, was selected to describe the mass transfer process of IAA species in the membrane system. Time variations of specific amounts of IAA in the F, M and S phases are given by equations (45)–(47). Equations (45) and (46) were obtained by integrating equations (42) and (43), whereas equation (47) resulted by substituting equations (45) and (46) into equation (40).



$$\frac{dR_F(t)}{dt} = -k_1 R_F(t) \quad (42)$$

$$\frac{dR_M(t)}{dt} = k_1 R_F(t) - k_2 R_M(t) \quad (43)$$

$$\frac{dR_S(t)}{dt} = k_2 R_M(t) \quad (44)$$

$$R_F(t) = e^{-k_1 t} \quad (45)$$

$$R_M(t) = \frac{k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \quad (46)$$

$$R_S(t) = 1 - R_F(t) - R_M(t) = 1 - \frac{1}{k_2 - k_1} (k_2 e^{-k_1 t} - k_1 e^{-k_2 t}) \quad (47)$$

$$R_{M,\max}(t_{\max}) = \left(\frac{k_1}{k_2}\right)^{-\frac{k_2}{k_1-k_2}} \quad (48)$$

$$t_{\max} = \frac{\ln\left(\frac{k_1}{k_2}\right)}{k_1 - k_2} \quad (49)$$

The kinetic constants k_1 and k_2 were determined from the experimental data using the Excel Solver. The extraction rate constant k_1 was calculated by minimizing the sum of squared residuals SSR_1 defined by equation (50), where $R_{F,ex}(t_i)$ and $R_{F,pred}(t_i)$ are the experimental and predicted values of specific amount of IAA in the F phase at $t_i = 1800i$ ($i = 0, 1 \dots 8$). The stripping rate constant k_2 was determined by minimizing the sum of squared residuals SSR_2 given by equation (51), where $R_{S,ex}(t_i)$ and $R_{S,pred}(t_i)$ are the experimental and predicted values of specific amount of IAA in the S phase at t_i .

$$SSR_1 = \sum_{i=0}^8 [R_{F,ex}(t_i) - R_{F,pred}(t_i)]^2 \quad (50)$$

$$SSR_2 = \sum_{i=0}^8 [R_{S,ex}(t_i) - R_{S,pred}(t_i)]^2 \quad (51)$$

Final values (at $t_f = 4 \text{ h} = 14400 \text{ s}$) of extraction, stripping, and recovery efficiencies of IAA, *i.e.*, E_{Ef} , E_{Sf} , and E_{Rf} , are given by equations (52)–(54), where $c_{IAA,Ff}$, $c_{IAA,Sf}$, R_{Ff} , and R_{Sf} are the final values of molar concentrations and specific amounts of IAA, respectively, in the F and S phases.

$$E_{Ef} = 100 \left(1 - \frac{c_{IAA,Ff}}{c_{IAA,F0}} \right) = 100(1 - R_{Ff}) \quad (52)$$

$$E_{Sf} = \frac{100c_{IAA,Sf}V_S}{(c_{IAA,F0} - R_{Ff}c_{IAA,F0})V_F} = \frac{100c_{IAA,Sf}V_S}{(1 - R_{Ff})c_{IAA,F0}V_F} = \frac{100R_{Sf}}{1 - R_{Ff}} = 10000 \frac{R_{Sf}}{E_{Ef}} \quad (53)$$

$$E_{Rf} = 100 \frac{c_{IAA,Sf}V_S}{c_{IAA,F0}V_F} = 100R_{Sf} = \frac{E_{Ef}E_{Sf}}{100} \quad (54)$$

2.1.2.4. Result and discussion

Characteristic parameters of equations (45)–(47), *i.e.*, k_1 , k_2 , $t_{\max}$ and $R_{M,\max}$, are specified in Tables 17 and 18, which also contain the values of sum of squared residuals (SSR_1 and SSR_2), total sum of squares [SST_1 and SST_2 given by equations (55) and (56)], and coefficient of determination (R_1^2 and R_2^2 defined by equations (57) and (58)].

$$SST_1 = \sum_{i=0}^8 \left[R_{F,ex}(t_i) - \frac{\sum_{i=0}^8 R_{F,ex}(t_i)}{9} \right]^2 \quad (55)$$

$$SST_2 = \sum_{i=0}^8 \left[R_{S,ex}(t_i) - \frac{\sum_{i=0}^8 R_{S,ex}(t_i)}{9} \right]^2 \quad (56)$$

$$R_1^2 = 1 - \frac{SSR_1}{SST_1} \quad (57)$$

$$R_2^2 = 1 - \frac{SSR_2}{SST_2} \quad (58)$$

Table 17. Characteristic parameters of the kinetic model ($C_{NaOH,S0} = 1 \text{ kmol/m}^3$)

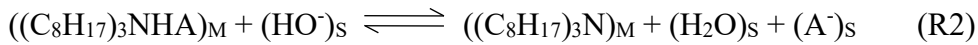
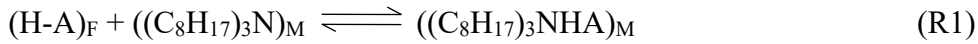
$C_{IAA,F0} \text{ (kmol/m}^3\text{)}$ Parametru	10^{-4}	3×10^{-4}	6×10^{-4}	10^{-3}
SSR_1	0,019	0,028	0,029	0,003
SST_1	0,757	0,935	0,896	0,825
R_1^2	0,975	0,970	0,967	0,996
$k_1 \times 10^4 \text{ (s}^{-1}\text{)}$	3,64	2,12	1,93	2,17
SSR_2	0,018	0,016	0,017	0,002
SST_2	0,863	0,752	0,724	0,658
R_2^2	0,980	0,979	0,977	0,998
$k_2 \times 10^4 \text{ (s}^{-1}\text{)}$	3,12	4,02	4,18	2,00
$R_{M,\max}$	0,397	0,258	0,238	0,383
$t_{\max} \text{ (s)}$	2968	3368	3434	4802

Tabel 18. Characteristic parameters of the kinetic model ($c_{IAA,F0} = 10^{-4}$ kmol/m³)

$c_{NaOH,S0}$ (kmol/m ³) \ Parametru	10^{-2}	10^{-1}	1
SSR_1	0,012	0,012	0,019
SST_1	0,858	0,807	0,757
R_1^2	0,986	0,985	0,975
$k_1 \times 10^4$ (s ⁻¹)	2,32	3,53	3,64
SSR_2	0,035	0,014	0,018
SST_2	0,669	0,798	0,863
R_2^2	0,948	0,983	0,980
$k_2 \times 10^4$ (s ⁻¹)	2,68	2,30	3,12
$R_{M,max}$	0,342	0,449	0,397
t_{max} (s)	4005	3485	2968

Tabulated results highlight a very good agreement between experimental and predicted data ($R_1^2 = 0.967$ – 0.996 and $R_2^2 = 0.948$ – 0.998). The extraction and stripping rate constants, $k_1 = (1.93$ – $3.64) \times 10^{-4}$ s⁻¹ and $k_2 = (2.00$ – $4.18) \times 10^{-4}$ s⁻¹, were similar, indicating that both reactions occurring at the interfaces between the F and M phases (R1) and M and S phases (R2) were rate-limiting steps in the mass transfer of IAA.

The values of k_1 were lower than those obtained in a previous study performed at the same levels of $c_{IAA,F0}$ and $c_{NaOH,S0}$, but using tributylphosphate (TBF) and trioctylphosphine oxide (TOPO) as carriers, *i.e.*, $k_1 = (5.12$ – $11.35) \times 10^{-4}$ s⁻¹, whereas the values of k_2 were generally higher than those reported for TBF and TOPO, *i.e.*, $k_2 = (1.16$ – $2.41) \times 10^{-4}$ s⁻¹. Moreover, t_{max} increased with an increase in $c_{IAA,F0}$ and a decrease in $c_{NaOH,S0}$.



The effects of $c_{IAA,F0}$ (10^{-4} – 10^{-3} kmol/m³) and $c_{NaOH,S0}$ (10^{-2} – 1 kmol/m³) on $R_{Ff,ex}$, $R_{Mf,ex}$, $R_{Sf,ex}$, $E_{Ef,ex}$, $E_{Sf,ex}$, and $E_{Rf,ex}$ ($c_{L,M0} = 10^{-2}$ kmol/m³, $v = 200$ rpm, and $t_f = 4$ h) are shown in Figs. 11 and 12. The maximum value of $R_{Sf,ex}$ ($R_{Sf,ex,max} = 0.911$), corresponding to maximum values of $E_{Ef,ex}$ (94.81%), $E_{Sf,ex}$ (96.08%), and $E_{Rf,ex}$ (91.09%), was obtained at the minimum level of $c_{IAA,F0}$ (10^{-4} kmol/m³) and maximum level of $c_{NaOH,S0}$ (1 kmol/m³).

The values of $R_{Sf,ex}$ for the other levels of $c_{IAA,F0}$ (3×10^{-4} – 10^{-3} kmol/m³), *i.e.*, 0.790–0.882 kmol/m³, were 3–15% lower than $R_{Sf,ex,max} = 0.911$ (Fig. 11a). An increase in $c_{IAA,F0}$ (from 10^{-4}

kmol/m^3 to 10^{-3} kmol/m^3) had a negative effect on $E_{Sf,ex}$ and $E_{Rf,ex}$ (which decreased from 96.08% to 84.54% and from 91.09% to 79.04%, respectively), but a negligible effect on $E_{Ef,ex}$ (93.17–94.81%) (Fig. 11b).

The values of $R_{Sf,ex}$ for the other levels of $c_{NaOH,S0}$ (10^{-2} kmol/m^3 and 10^{-1} kmol/m^3), *i.e.*, 0.781 kmol/m^3 and 0.851 kmol/m^3 , were significantly lower (7.1% and 16.6%, respectively) than $R_{Sf,ex,max} = 0.911$ (Fig. 12a). An increase in $c_{NaOH,S0}$ (from 10^{-2} kmol/m^3 to 1 kmol/m^3) had a significant positive effect on $E_{Sf,ex}$ and $E_{Rf,ex}$ (which increased from 83.87% to 96.08% and from 78.14% to 91.09%, respectively), but a negligible effect on $E_{Ef,ex}$ (93.17–94.81%) (Fig. 12b)

Furthermore, $E_{Sf,ex}$ and $E_{Rf,ex}$ were very strongly positively correlated ($r = 0.998$).

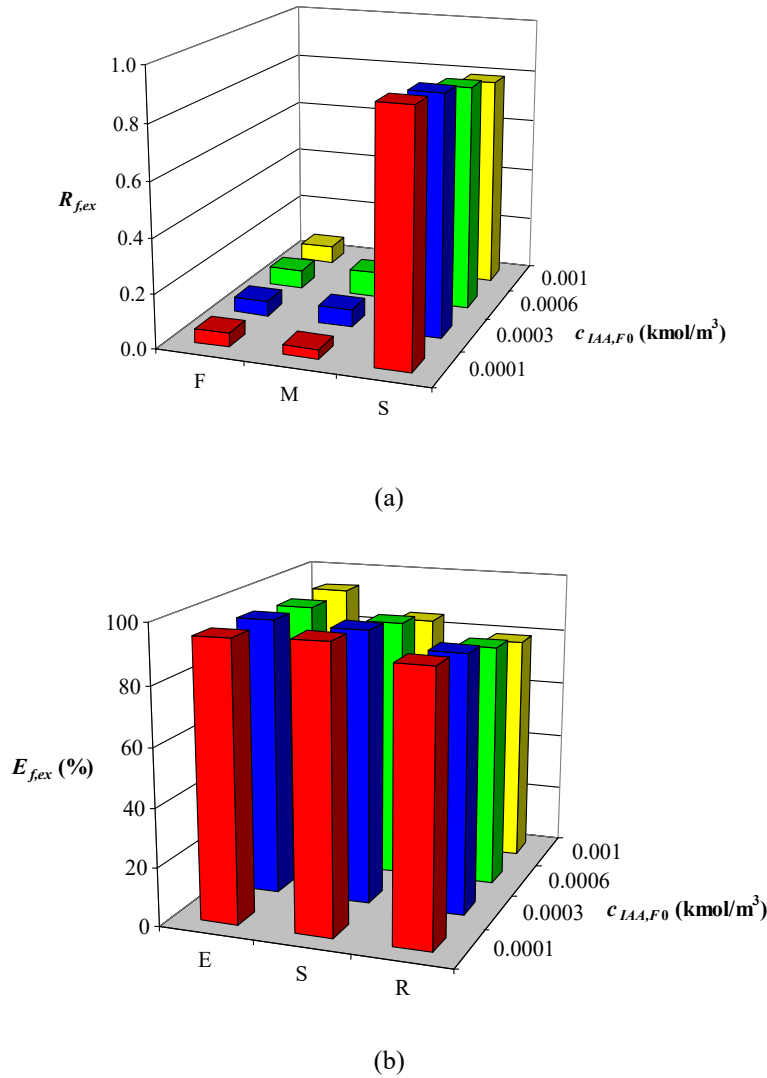


Figure 11. Effects of initial concentration of IAA in the feed phase ($c_{IAA,F0}$) on the final values of: (a) specific amounts of IAA in the feed (F), liquid membrane (M), and stripping (S) phases; (b) extraction (E), stripping (S), and recovery (R) efficiencies of IAA ($c_{L,M0} = 10^{-2} \text{ kmol/m}^3$, $c_{NaOH,S0} = 1 \text{ kmol/m}^3$, $v = 200 \text{ rpm}$, and $t_f = 4 \text{ h}$)

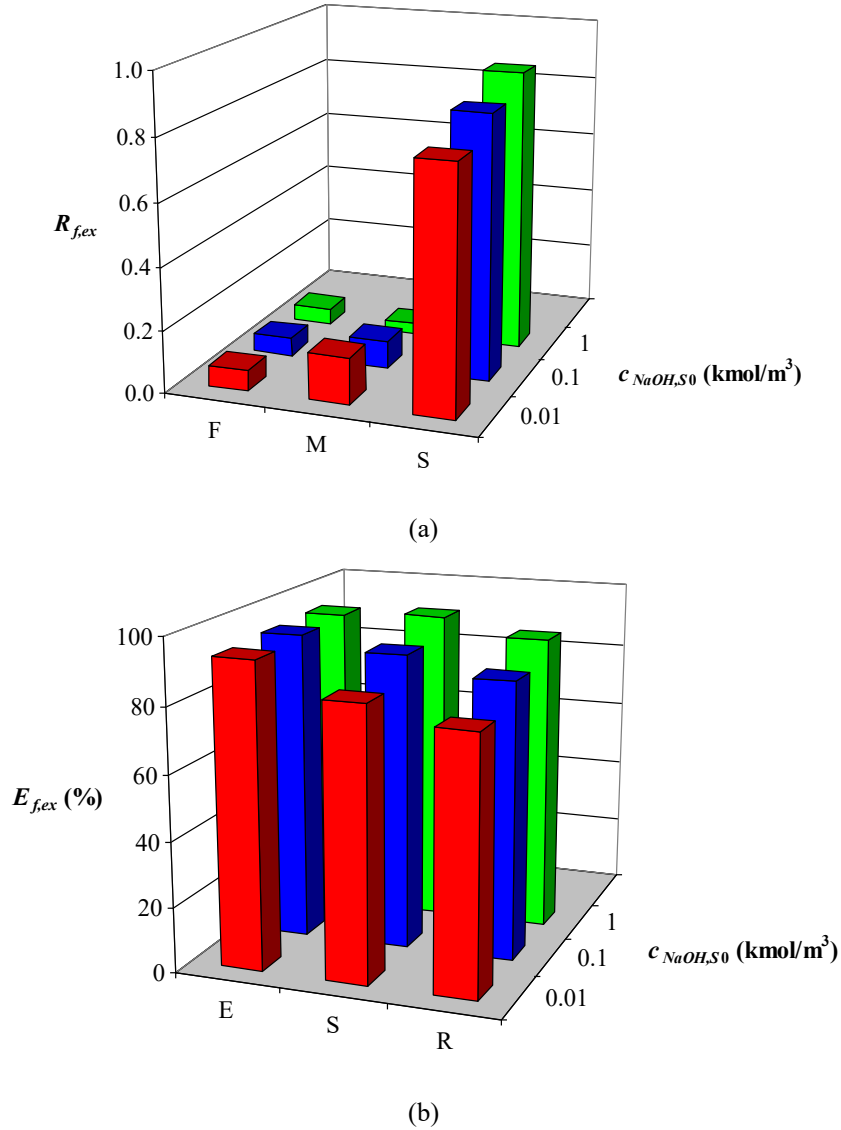


Figure 12. Effects of initial concentration of NaOH in the stripping phase ($c_{NaOH,S0}$) on the final values of: (a) specific amounts of IAA in the feed (F), liquid membrane (M), and stripping (S) phases; (b) extraction (E), stripping (S), and recovery (R) efficiencies of IAA ($c_{IAA,F0} = 10^{-4}$ kmol/m³, $c_{L,M0} = 10^{-2}$ kmol/m³, $v = 200$ rpm, and $t_f = 4$ h).

2.1.1.5. Conclusions

A kinetic model assuming consecutive irreversible first-order reactions was used to predict the specific yields of IAA in the F, M, and S phases. The adjustable parameters of the model in terms of extraction and stripping rate constants, *i.e.*, $k_1 = (1.93\text{--}3.64) \times 10^{-4} \text{ s}^{-1}$ and $k_2 = (2.00\text{--}4.18) \times 10^{-4} \text{ s}^{-1}$, were regressed from experimental data. The values of k_1 and k_2 were similar, suggesting that both chemical reactions occurring at the interfaces between the F and M phases (complexation) and M and S phases (decomplexation), respectively, were rate-limiting steps. The results obtained indicated a good agreement between experimental and predicted data ($R^2 = 0.948\text{--}0.998$).

The maximum final value (at $t_f = 4$ h) of specific amount of IAA in the S phase ($R_{Sf,ex} = 0.911$), corresponding to maximum final values of extraction, stripping, and recovery efficiencies ($E_{Ef,ex} = 94.81\%$, $E_{Sf,ex} = 96.08\%$, and $E_{Rf,ex} = 91.09\%$), was obtained at $c_{IAA,F0} = 10^{-4}$ kmol/m³ and $c_{NaOH,S0} = 1$ kmol/m³.

2.2. EXTRACTION OF BIOACTIVE COMPOUNDS FROM PLANTS

2.2.1. OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION AND MICROWAVE-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM ROMANIAN PEPPERMINT (*MENTHA × PIPERITA* L.)

Acest studiu a avut ca scop optimizarea extracției asistate de ultrasunete (UAE) și a extracției asistate de microunde (MAE) a pigmentilor de clorofilă și carotenoizi din frunzele de mentă românească (*Mentha × piperita* L.), utilizând soluții apoase de etanol ca solvenți de extracție. The data presented in this chapter will be published in the article:

Roșu, A.M., Aruș, V.A., Răducanu, D., Platon, N., Georgescu, A.M., Muntianu, G., Nistor, I.D., Topală, S.L., Pârvulescu, O.C. (2025) *Optimization of ultrasound-assisted extraction and microwave-assisted extraction of bioactive compounds from Romanian peppermint (Mentha × piperita L.)*. (manuscript in progress)..

2.2.1.1. Materials and methods

2.2.1.1.1. Plant material

Peppermint leaves in dried form were provided by Dacia Plant (Romania). The plant material was ground, passed through a 20-mesh (0.841 mm) sieve, and crushed with a pestle in a mortar

2.2.1.1.2. Extraction procedure

According to a CCD with 2 independent variables (factors) and 4 center-point runs, 12 experimental runs were performed for each extraction method at 5 levels of process factors, i.e., mass percentage of ethanol ($c_{et} = 40\text{--}90\%$) and liquid (extraction solvent)/solid (dried plant material) ratio ($R_{LS} = 6\text{--}34$ mL/g) (Egri, 2024; Mot, 2024). The levels of dimensional factors and those of dimensionless factors (X_1 and X_2), which were calculated using Equations (57) and (58), are summarized in Table 19.

Table 19. Levels of dimensional and dimensionless extraction factors for UAE and MAE.

Exp.	c_{et} (%)	R_{LS} (mL/g)	X_1	X_2
1	50	10	-1	-1
2	50	30	-1	1
3	80	10	1	-1
4	80	30	1	1
5	65	20	0	0
6	65	20	0	0
7	44	20	-1,414	0
8	86	20	1,414	0
9	65	5,9	0	-1,414
10	65	34,1	0	1,414
11	65	20	0	0
12	65	20	0	0

$$X_1 = \frac{c_{et} - 65}{15} \quad (57)$$

$$X_2 = \frac{R_{LS} - 20}{10} \quad (58)$$

Chlorophyll *a*, chlorophyll *b*, and total carotenoid concentrations (*CaC*, *CbC*, and *TCC*) were determined by an UV-3100PC Spectrophotometer (VWR International, Avantor, Inc., Radnor, Pennsylvania, U.S.) at wavelengths of 470, 645, and 662 nm, respectively, using Equations (59)–(61), where A_{470} , A_{645} , and A_{662} are the extract absorbances at 470, 649 și 664 nm. The analyses were performed in triplicate, and the results were expressed as g/L.

$$CaC = 13,36A_{664} - 5,19A_{649} \quad (59)$$

$$CbC = 27,43A_{649} - 8,12A_{664} \quad (60)$$

$$TCC = \frac{1000A_{470} - 2,13CaC - 97,63CbC}{209} \quad (61)$$

2.2.1.2. Antibacterial activity of peppermint hydroalcoholic extracts

The Kirby-Bauer disk diffusion test was used to evaluate the antibacterial activity of peppermint extracts against *Escherichia coli* (Hudzicki, 2009).

The discs used for testing the hydroalcoholic extracts were prepared from Whatman type regenerated cellulose filter membranes (0.45 μm pore size and 5 mm diameter), were sterilized by autoclaving, dried, and kept in a desiccator until use. Mueller-Hinton agar (MHA) was used as the

culture medium, and Mueller-Hinton broth (MHB) was used to prepare the inoculum. MHA and MHB were supplied by Merck KGaA (Darmstadt, Germany).

Antibacterial activity was determined by measuring the diameter (d) of the zone of inhibition around the disc using a digital caliper (Verke V86000, Poland). All tests were performed in triplicate. Antibacterial effects of peppermint extracts obtained by UAE and MAE against *E. coli* for several experimental runs (Table 19) can be visualized in Fig. 13.

Activitatea antibacteriană a fost determinată prin măsurarea diametrului zonei de inhibiție (d) cu ajutorul unui șubler digital (Verke V86000, Polonia). Toate testele au fost realizate în triplu exemplar. Efectele antibacteriene ale extractelor de mentă obținute prin UAE și MAE împotriva *E. coli* pentru câteva experimente (tabelul 19) pot fi vizualizate în figura 13.

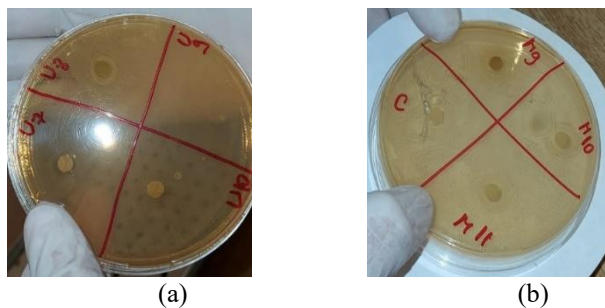


Figure 13. The antibacterial effect of mint leaf extracts obtained by UAE (a) and MAE (b) against *E. coli*

2.2.1.3. Experimental Design, Statistical Analysis and Process Optimization

CCD was selected as experimental design. All measurements were conducted in triplicate, and the results were presented as either mean values or mean values $\pm$ standard deviations (SD). Statistical analysis and process factor optimization were performed using STATISTICA 10 software (Stat Soft Inc., Tulsa, USA)

2.2.1.4. Results and Discussion

2.2.1.4.1. UAE

Effects of dimensionless factors of UAE (X_1 și X_2) and their interactions on the predicted response variables ($Y_{j,pr,UAE}$, $j = 1 \dots 4$), i.e.: $Y_{1,pr,UAE} = CaC_{pr,UAE}$; $Y_{2,pr,UAE} = CbC_{pr,UAE}$; $Y_{3,pr,UAE} = TCC_{pr,UAE}$; $Y_{4,pr,UAE} = d_{pr,UAE}$. were quantified using the statistical models described by the equation (62).

$$Y_{j,pr,UAE} = a_{0j} + a_{1j}X_1 + a_{11j}X_1^2 + a_{2j}X_2 + a_{22j}X_2^2 + a_{12j}X_1X_2, \quad j = 1 \dots 4 \quad (62)$$

The values of regression coefficients in Equation (62) (a_{0j} , a_{1j} , a_{11j} , a_{2j} , a_{22j} , and a_{12j}) were determined from the mean experimental values (corresponding to triplicate measurements) of UAE response variables ($Y_{j,m,UAE}$, $j = 1 \dots 4$) summarized in Table 20.

The values of regression coefficients in Equation (62), coefficients of determination (R_j^2), adjusted coefficients of determination ($R_{j,adj}^2$), F statistic (F_j), and p_j -value for F_j at different levels of dimensional and dimensionless process factors, which are specified in Table 20, emphasize the following aspects:

- a significant positive effect of X_1 on $Y_{1,pr,UAE}$ and $Y_{2,pr,UAE}$;
- a significant positive effect of X_2 and significant negative effects of X_1 and X_2^2 on $Y_{3,pr,UAE}$; surface and contour plots highlight an increase in $Y_{3,pr,UAE}$ with an increase in X_2 and a decrease in X_1 ;
- a significant positive effect of X_1 and a significant negative effect of X_2 on $Y_{4,pr,UAE}$;
- a good agreement between experimental and predicted values of process response variables ($0.788 \leq R_j^2 \leq 0.875$, $0.610 \leq R_{j,adj}^2 \leq 0.770$, $4.447 \leq F_j \leq 8.361$, and $0.011 \leq p_j \leq 0.049$ for $j = 1 \dots 4$).

Table 20. Mean experimental values of process response variables ($Y_{j,m,UAE}$, $j = 1 \dots 4$) and related values of regression coefficients (b_{0j} , b_{1j} , b_{11j} , b_{2j} , b_{22j} , and b_{12j}), coefficient of determination (R_j^2), adjusted coefficient of determination ($R_{j,adj}^2$), F statistic (F_j), and p_j -value for F_j at different levels of dimensional and dimensionless process factors of UAE.

Exp.	C_{et} (%)	R_{LS} (ml/g)	X_1	X_2	$Y_{1,m,UAE} =$ $CaC_{m,UAE}$ (mg/L)	$Y_{2,m,UAE} =$ $CbC_{m,UAE}$ (mg/L)	$Y_{3,m,UAE} =$ $TCC_{m,UAE}$ (mg/L)	$Y_{4,m,UAE} =$ $d_{m,UAE}$ (mm)
1	50	10	-1	-1	13,02	8,799	6,484	9,54
2	50	30	-1	1	1,214	0,809	4,948	9,12
3	80	10	1	-1	38,72	27,05	9,495	13,7
4	80	30	1	1	22,40	16,18	7,304	11,8
5	65	20	0	0	21,86	15,81	7,231	10,5
6	65	20	0	0	22,05	15,94	7,256	10,8
7	44	20	-1,414	0	5,421	3,737	5,464	8,30
8	86	20	1,414	0	32,71	23,05	8,688	10,5
9	65	5,86	0	-1,414	10,65	7,218	6,165	12,6
10	65	34,1	0	1,414	13,38	9,038	6,532	8,78
11	65	20	0	0	23,43	16,86	7,442	11,1
12	65	20	0	0	22,14	16,00	7,269	11,3
j					1	2	3	4
a_{0j}					22,37	16,154	7,300	10,91
a_{1j}					10,69	7,615	1,241	1,232
a_{11j}					-0,827	-0,769	-0,026	-0,515

a_{2j}	-3,033	-2,036	-0,401	-0,964
a_{22j}	-4,354	-3,401	-0,389	0,145
a_{12j}	-1,129	-0,721	-0,164	-0,360
R_j^2	0,875	0,884	0,850	0,805
$R_{j,adj}^2$	0,770	0,788	0,724	0,642
F_j	8,361	9,161	6,784	4,945
p_j	0,011	0,009	0,019	0,039

Statistically significant regression coefficients are highlighted in bold

The optimal levels of dimensionless factors, which were obtained based on desirability function (f) approach [59], were $X_{1,opt,UAE} = 1.414$ ($c_{et,opt,UAE} = 86\%$) and $X_{2,opt,UAE} = -0.707$ ($R_{LS,opt,UAE} = 12.9$ mL/g), for which $f_{UAE} = 0.918$. The values of the response variables predicted by Equation (62) at $X_{1,opt,UAE}$ and $X_{2,opt,UAE}$, i.e., $Y_{j,pr,opt,UAE}$ ($j = 1 \dots 4$), are summarized in Table 21. To validate the statistical models described by Equation (62), three UAE experimental runs were performed at optimal levels of process factors. The mean values of experimental response variables under optimal conditions ($Y_{j,m,opt,UAE}$), related standard deviations (SD_j), and values of percentage prediction error (ε_j) defined by Equation (63) are given in Table 21. The values of percentage prediction error ($5.78\% \leq \varepsilon_j \leq 9.90\%$) and results of t -test ($p_j \geq 0.06$) indicate that $Y_{j,m,opt,UAE}$ and $Y_{j,pr,opt,UAE}$ were not significantly different, which proves the validity of statistical models.

$$\varepsilon_j = 100 \frac{Y_{j,m,opt,UAE} - Y_{j,pr,opt,UAE}}{Y_{j,m,opt,UAE}}, \quad j = 1 \dots 4 \quad (63)$$

Table 21. Predicted and experimental values of response variables at optimal levels of UAE process factors ($c_{et,opt,UAE} = 86\%$ and $R_{LS,opt,UAE} = 12.9$ mL/g).

j	Response variable		Optimal Value		Percentage Prediction error
	Symbol	Units	Predicted	Experimental	
			$Y_{j,pr,opt,MAE}$	$Y_{j,m,opt,MAE} \pm SD_j$	ε_j (%)
1	CaC_{UAE}	mg/L	36,93	$39,20 \pm 3,757$	5,78
2	CbC_{UAE}	mg/L	25,85	$28,69 \pm 2,888$	9,9
3	TCC_{UAE}	mg/L	9,256	$10,19 \pm 0,954$	9,18
4	d_{UAE}	mm	12,73	$14,10 \pm 1,176$	9,74

2.2.1.1.3. MAE

Effects of dimensionless factors of MAE (X_1 și X_2) and their interactions on the predicted response variables ($Y_{j,pr,MAE}$, $j = 1 \dots 4$), i.e.: $Y_{1,pr,MAE} = CaC_{pr,MAE}$; $Y_{2,pr,MAE} = CbC_{pr,MAE}$; $Y_{3,pr,MAE} = TCC_{pr,MAE}$; $Y_{4,pr,MAE} = d_{pr,MAE}$ were quantified using the statistical models described by the equation (64).

$$Y_{j,pr,MAE} = b_{0j} + b_{1j}X_1 + b_{11j}X_1^2 + b_{2j}X_2 + b_{22j}X_2^2 + b_{12j}X_1X_2, \quad j=1\ldots 4 \quad (64)$$

The values of regression coefficients in Equation (64) (b_{0j} , b_{1j} , b_{11j} , b_{2j} , b_{22j} , and b_{12j}) were determined from the mean experimental values of MAE response variables ($Y_{j,m,MAE}$, $j = 1\ldots 4$) summarized in Table 22. Pearson correlation coefficients (r) indicated:

- very strong positive correlations between $Y_{1,m,MAE} = CaC_{m,MAE}$, $Y_{2,m,MAE} = CbC_{m,MAE}$, and $Y_{3,m,MAE} = TCC_{m,MAE}$ ($r = 0.9565\text{--}0.9996$);
- strong positive correlations between $Y_{4,m,MAE} = d_{m,MAE}$ and $Y_{1,m,MAE}$ ($r = 0.6381$) and $Y_{4,m,MAE}$ and $Y_{2,m,MAE}$ ($r = 0.6315$);
- a moderate positive correlation between $Y_{4,m,MAE}$ and $Y_{3,m,MAE}$ ($r = 0.5486$).

The values of regression coefficients in Equation (64), R_j^2 , $R_{j,adj}^2$, F statistic (F_j), and p_j -value for F_j at different levels of dimensional and dimensionless process factors, are specified in Table 22.

Table 22. Mean experimental values of process response variables ($Y_{j,m,MAE}$, $j = 1\ldots 4$) and related values of regression coefficients (b_{0j} , b_{1j} , b_{11j} , b_{2j} , b_{22j} , and b_{12j}), coefficient of determination (R_j^2), adjusted coefficient of determination ($R_{j,adj}^2$), F statistic (F_j), and p_j -value for F_j at different levels of dimensional and dimensionless process factors of MAE.

Exp.	c_{et} (%)	R_{LS} (mL/g)	X_1	X_2	$Y_{1,m,MAE} =$ $CaC_{m,MAE}$ (mg/L)	$Y_{2,m,MAE} =$ $CbC_{m,MAE}$ (mg/L)	$Y_{3,m,MAE} =$ $TCC_{m,MAE}$ (mg/L)	$Y_{4,m,MAE} =$ $d_{m,MAE}$ (mm)
1	50	10	−1	−1	4,732	3,960	12,18	11,60
2	50	30	−1	1	6,216	2,140	9,199	11,24
3	80	10	1	−1	34,97	50,94	0,328	14,08
4	80	30	1	1	23,77	17,91	3,634	11,88
5	65	20	0	0	28,88	20,54	7,106	11,52
6	65	20	0	0	29,94	25,32	5,248	11,62
7	44	20	−1,414	0	6,859	9,860	12,08	10,74
8	86	20	1,414	0	32,28	39,81	0,107	12,14
9	65	5,86	0	−1,414	35,21	63,88	4,317	13,60
10	65	34,1	0	1,414	16,81	14,33	4,973	11,62
11	65	20	0	0	32,68	34,97	2,467	11,70
12	65	20	0	0	31,19	30,18	3,945	11,78
j					1	2	3	4
b_{0j}					30,67	21,69	9,132	11,655
b_{1j}					10,47	7,482	1,291	0,637
b_{11j}					-6,894	-4,958	-0,992	-0,064
b_{2j}					-4,467	-2,976	-0,600	-0,670
b_{22j}					-3,674	-2,532	-0,789	0,521
b_{12j}					-3,172	-2,113	-0,665	-0,460

R_j^2	0,924	0,924	0,934	0,973
$R_{j,adj}^2$	0,861	0,860	0,879	0,950
F_j	14,60	14,54	16,94	43,05
p_j	0,003	0,003	0,002	0,0001

Coeficienții de regresie semnificativi statistic sunt evidențiați cu caractere albine

The results presented in Table 22 highlight the following aspects:

- a significant positive effect of X_1 and significant negative effects of X_2 and X_1^2 on $Y_{1,pr,MAE}$, $Y_{2,pr,MAE}$, and $Y_{3,pr,MAE}$, as well as a significant negative effect of X_2^2 on $Y_{3,pr,MAE}$, the effect of X_1 being more important;
- significant positive effects of X_1 and X_2^2 , as well as significant negative effect of X_2 and X_1X_2 on $Y_{4,pr,MAE}$; an increase in $Y_{4,pr,MAE}$ with an increase in X_1 as well as a decrease in $Y_{4,pr,MAE}$ with an increase in X_2 from -1.414 to 0 and almost constant values for X_2 in the range 0–1.414;
- a good agreement between experimental and predicted values of process response variables ($0.924 \leq R_j^2 \leq 0.973$, $0.860 \leq R_{j,adj}^2 \leq 0.950$, $14.54 \leq F_j \leq 43.05$, and $0.0001 \leq p_j \leq 0.003$ for $j = 1 \dots 4$).

The optimal levels of dimensionless factors, obtained based on desirability function (f) approach, were $X_{1,opt,MAE} = 1.414$ ($c_{et,opt,MAE} = 86\%$) and $X_{2,opt,MAE} = -1.414$ ($R_{LS,opt,MAE} = 5.9$ mL/g), for which $f_{MAE} = 0.990$.

The values of the response variables predicted by Equation (64) at $X_{1,opt,MAE}$ and $X_{2,opt,MAE}$, i.e., $Y_{j,pr,opt,MAE}$ ($j = 1 \dots 4$), are summarized in Table 23. To validate the statistical models described by Equation (64), three MAE experimental runs were performed at optimal levels of process factors. The mean values of experimental response variables under optimal conditions ($Y_{j,m,opt,MAE}$), related SD_j and ε_j defined by Equation (65) are given in Table 23. The values of percentage prediction error ($-5.41\% \leq \varepsilon_j \leq 8.37\%$) and results of t -test ($p_j \geq 0.09$) indicate that $Y_{j,m,opt,MAE}$ and $Y_{j,pr,opt,MAE}$ were not significantly different, which proves the validity of statistical models.

$$\varepsilon_j = 100 \frac{Y_{j,m,opt,MAE} - Y_{j,pr,opt,MAE}}{Y_{j,m,opt,MAE}}, \quad j = 1 \dots 4 \quad (65)$$

Tabelul 23. Predicted and experimental values of response variables at optimal levels of MAE process factors ($C_{et,opt,MAE} = 86\%$ and $R_{LS,opt,MAE} = 5.9$ mL/g).

j	Response variable		Optimal Value		Percentage Prediction error
	Symbol	Units	Predicted	Experimental	
			$Y_{j,pr,opt,MAE}$	$Y_{j,m,opt,MAE} \pm SD_j$	ε_j (%)
1	CaC_{MAE}	mg/L	37,00	$38,78 \pm 3,165$	4,58
2	CbC_{MAE}	mg/L	25,72	$28,07 \pm 2,579$	8,37
3	TCC_{MAE}	mg/L	9,574	$10,31 \pm 0,841$	7,16
4	d_{MAE}	mm	15,34	$14,55 \pm 1,301$	-5,41

The results of one-way ANOVA (at $\alpha = 0.05$) indicated that $Y_{j,m,opt,MAE}$ and $Y_{j,m,opt,UAE}$ were not significantly different ($p \geq 0.340$) for $j = 1 \dots 4$.

2.2.1.5. Comparison with data reported in the literature

The mean values of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and total carotenoid concentrations (CaC , CbC , CtC , and TCC) in dried leaves of Romanian peppermint, which were obtained in the experiments of UAE and MAE ($c_{et} = 44\text{--}86\%$ and $R_{LS} = 5.9\text{--}34.1$ mL/g) performed in this study, are summarized in Table 24. In addition to the extraction method and conditions, these values can be significantly affected by other factors, *e.g.*, drying type and conditions, cultivar (cv.) type and origin of peppermint.

Table 24. Mean values of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and total carotenoid concentrations reported in this study..

Technique	UAE	MAE
CaC_m	1,214–39,20 mg/L = 36,42–672,1 mg/kg DM	4,732–38,78 mg/L = 47,32–713,1 mg/kg DM
CbC_m	0,809–28,69 mg/L = 24,26–485,3 mg/kg DM	3,165–28,07 mg/L = 31,65–512,6 mg/kg DM
$CtC_m = CaC_m + CbC_m$	2,023–67,89 mg/L = 60,69–1157 mg/kg DM	7,897–66,85 mg/L = 78,97–1226 mg/kg DM
TCC_m	4,948–10,19 mg/L = 36,13–222,7 mg/kg DM	5,415–10,31 mg/L = 52,87–224,6 mg/kg DM

Xylia et al. (2024) obtained the following mean values of CtC in dried leaves of peppermint from Limassol (Cyprus): 910 mg/kg DM for sun drying, 890 mg/kg DM for oven drying (at 42 °C), and 810 mg/kg DM for shade drying. The values reported by Uribe et al. (2015) and Xylia et al. (2024) are within the range found in our study, *i.e.*, $CtC_m = 60.69\text{--}1226$ mg/kg DM.

2.2.1.6. Conclusions

Quantifying the effects of extraction process factors on its performance and optimizing process factors are essential to achieve high process efficiency. This study aimed at optimizing the UAE and MAE of chlorophyll pigments and carotenoids from Romanian peppermint leaves using aqueous solutions of ethanol as extraction solvents. Chlorophyll *a*, chlorophyll *b*, and total carotenoid concentrations of peppermint leaf extracts (*CaC*, *CbC*, and *TCC*), as well as antibacterial activity of peppermint leaf extracts against *E. coli*, evaluated based on the Kirby-Bauer disk diffusion test by measuring the diameter of the zone of inhibition (*d*), were experimentally determined and predicted at various levels of process factors, i.e., mass percentage of ethanol ($c_{et} = 44\text{--}86\%$) and liquid/solid ratio ($R_{LS} = 5.9\text{--}34.1\text{ mL/g}$), according to a CCD. Each UAE and MAE experimental run was performed for 30 min.

Second-order polynomial models obtained based on experimental data for UAE ($CaC_{m,UAE} = 1.214\text{--}38.72\text{ mg/L}$, $CbC_{m,UAE} = 0.809\text{--}27.05\text{ mg/L}$, $TCC_{m,UAE} = 4.948\text{--}9.495\text{ mg/L}$, and $d_{m,UAE} = 8.300\text{--}13.66\text{ mm}$) highlighted a significant positive effect of dimensionless c_{et} (X_1) on all response variables and a significant negative effect of dimensionless R_{LS} (X_2) on $d_{pr,UAE}$. Under optimal conditions of UAE ($c_{et,opt,UAE} = 86\%$ and $R_{LS,opt,UAE} = 12.9\text{ mL/g}$), the mean values of experimental response variables were as follows: $CaC_{m,opt,UAE} = 39.20\text{ mg/L}$, $CbC_{m,opt,UAE} = 28.69\text{ mg/L}$, $TCC_{m,opt,UAE} = 10.19\text{ mg/L}$, and $d_{m,opt,UAE} = 14.10\text{ mm}$.

2.2.2. ENHANCING THE EXTRACTION PROCESS EFFICIENCY OF THYME ESSENTIAL OIL BY COMBINED ULTRASOUND AND MICROWAVE TECHNIQUES

The aim of this study was to improve the extraction efficiency of essential oil (EO) from thyme (*Thymus officinalis* L.) by increasing the extraction yield and the quantity of valuable components (thymol) by applying ultrasonic pretreatment to the plant material followed by microwave-assisted hydrodistillation. The data presented in this chapter were published in the article:

Gavrila, A.I., Chisega-Negrila, C.G., Maholea, L., Gavrila, M.L., Pârvulescu, O.C., Popa, I. (2023) Enhancing the extraction process efficiency of thyme essential oil by combined ultrasound and microwave techniques. *Agronomy*, 13 (9), art. no. 2331, 1–14, WOS:

001093126200001 (Q1).

2.2.2.1. Materials and Methods

2.2.2.1.1. Materials

Fresh thyme (stems and leaves) was purchased from Hofigal (Bucharest, Romania). The fresh leaves and stems were chopped into pieces of 1–2 cm, then dried in an air flow heating oven (Memmert UNE 500 Universal Oven, Memmert GmbH +Co. KG, Schwabach, Germany) at 60 °C to a constant weight. Part of the dried vegetal material was used as such, and another part was ground using an electric grinder and screened to a particle size under 0.1 cm. The ground thyme was dosed in samples of 100 g (in sealed plastic container) and stored at 4–5 °C until extraction of EO.

2.2.2.1.2. Essential Oil Extraction Procedure

The extraction of EO from thyme was performed by MWHD using a multimode microwave oven (Plazmatronika, Plazmatronika NT SP Z.O.O., Wrocław, Poland), which has a frequency of 2450 MHz.

Steam produced in the reactor carrying the thyme EO was directed to a modified Neo Clevenger trap with a 10 mL graduated tube. The extractions were carried out at different ratios of solvent to plant: 8/1, 10/1, and 12/1 (v/w). The particle size of vegetal material was varied, as follows: pieces of 1–2 cm or particles under 0.1 cm. The solvent used was distilled water and the mixture was subjected to extraction until no EO was obtained (80 min). The protocols for the MWHD method are described in our previous work. Comparative extraction of thyme EO by conventional hydro-distillation (CHD) method was also performed. The conventional extraction was carried out for 180 min (after this time no EO was obtained) at a solvent to plant ratio of 12/1 (v/w). The separated EO was kept at 4 °C until GC-MS analysis. The extraction yield was expressed as grams of EO per 100 grams of dry matter (g EO/100 g DM).

2.2.2.1.3. Ultrasound Pre-Treatment of Plant Material

Before MWHD, the vegetal material mixed with the solvent (distilled water) was subjected to ultrasound pre-treatment for 30 min. The pre-treatment was performed using either an

ultrasound bath (ES375H Bench Top Ultrasonic Tank, Hilsonic Ultrasonic Cleaners, Birkenhead, UK) with a volume of 3 L, a power of 120 W, and a frequency of 40 kHz, either an ultrasonic processor (Vibracell VCX-750, Sonics & Materials, Inc., Newtown, CT, USA) with a power of 750 W, a frequency of 20 kHz, and a titanium horn with a diameter of 13 mm. The sonication of the extraction mixture, using the ultrasonic processor, was applied with a duty cycle of 5 s on and 5 s off for an amplitude of 50% or 70%. The sonication for the ultrasonic bath was applied continuously. Control samples without any pre-treatment were directly subjected to MWHD or CHD.

2.2.2.1.4. GC-MS Analysis of the Thyme Essential Oil

The separated EO was analyzed by GC-MS method. The GC-MS apparatus, a Thermo Electron Corporation Focus GC system (Thermo Fisher Scientific S.p.A., Milan, Italy), is described in our previous work Calinescu et al. [17]. The main constituents of thyme EO (thymol, -terpinene, p-cymene, -terpinene, and -pinene) were identified according to retention times of known standards. Identification of the other constituents of thyme EO was performed by comparing the samples' spectral peaks with spectra from Wiley database. The main constituents of thyme EO were chosen based on preliminary tests that were part of a research project (PN-III-P2-PED-2019-2118, project: "IMUNOSTIM", no. 381PED/2020). Absolute amounts of the individual components were calculated based on GC peak areas, and were expressed as milligrams per 100 grams of dry matter (mg/100 g DM).

2.2.2.2. Statistical Analysis

All measurements were performed in triplicate, and the data were expressed with standard deviation (SD) as mean value SD for triplicate of samples ($n = 3$). All the results achieved at different levels of process factors were subjected to univariate one-way ANOVA and multivariate principal component analysis (PCA). Statistical analysis of the data was performed using the multiple comparison Duncan's post hoc tests in order to determine the significant statistical differences between the averages of the main components of two or more independent groups. To evaluate the strength of linear correlations between dependent variables, the Pearson correlation coefficient (r) was used. The differences were considered statistically significant at $p < 0.05$.

Statistical analysis was conducted using XLSTAT Version 2019.1 (Lumivero, Addinsoft, New York, NY, USA).

2.2.2.2. Results

2.2.2.3.1. Microwave-Assisted Hydro-Distillation (MWHD) vs. Conventional Hydro-Distillation (CHD)

The first step to optimize the extraction process of thyme EO consists of kinetics study of the control samples obtained by MWHD and CHD (Figure 1). As shown in Figure 1, the time required to achieve the reflux temperature (the time after the EO starts to be collected into the Clevenger apparatus) for MWHD (10 min) is lower compared with CHD (60 min). This can be explained by the volumetric heating of microwaves compared with the conventional one, which occurs by convection. The MWHD leads to a rapid increase in EO content in the first 40 min (which corresponds to 83% of the total yield), achieving a maximum amount after 70 min (1.73 0.03 g EO/100 g DM). For CHD, the EO content increases slowly, obtaining 1.65 0.06 g EO/100 g DM after 170 min. Further, the experiments were performed by MWHD.

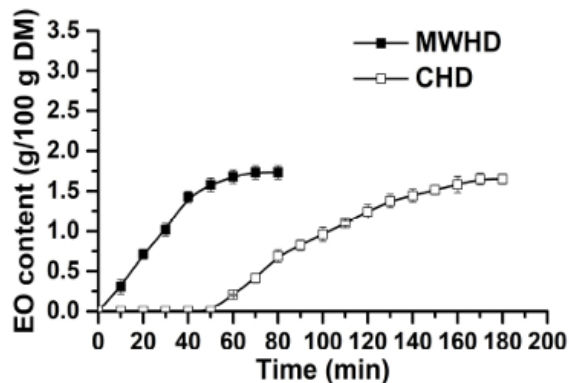


Figure 17. Kinetics study of microwave-assisted hydro-distillation (MWHD) vs. conventional hydro distillation (CHD) for a solvent to plant ratio of 12/1 (v/w) and a particle size of 1–2 cm.

The thyme EO was analyzed and identified by GC-MS. The results, for both methods (MWHD and CHD), are shown in Table 25.

Table 25. Chemical composition of thyme essential oil (EO) obtained by microwave-assisted hydrodistillation (MWHD) and conventional hydrodistillation (CHD), for a solvent-to-plant ratio of 12/1 (v/w) and a particle size of 1–2 cm

RT (min)	Compound	CAS	BP (°C)	Component content (mg/100 g DM)	
				MWHD	CHD
9,043	α -Pinen	80-56-8	155	13 $\pm$ 0,2 ^b	15 $\pm$ 0,7 ^a
9,205	Camfen	79-92-5	159	16 $\pm$ 0,2 ^b	26 $\pm$ 1,2 ^a
9,941	Sabinen	3387-41-5	163	7 $\pm$ 0,1 ^b	11 $\pm$ 0,5 ^a
10,039	β-Pinen	127-91-3	165	33 $\pm$ 0,3 ^b	41 $\pm$ 1,8 ^a
10,568	α-Terpinen	99-86-5	173	57 $\pm$ 1,1 ^b	68 $\pm$ 2,9 ^a
10,613	p-Cimen	99-87-6	177	88 $\pm$ 3,0 ^b	110 $\pm$ 4,8 ^a
10,783	Eucaliptol	470-82-6	176	6 $\pm$ 0,1 ^b	11 $\pm$ 0,5 ^a
11,245	γ-Terpinen	99-85-4	183	523 $\pm$ 37,4 ^a	573 $\pm$ 24,9 ^a
13,071	Terpinen-4-ol	562-74-3	219	-	14 $\pm$ 0,6 ^a
14,657	Timol	89-83-8	232	969 $\pm$ 28,9 ^a	689 $\pm$ 29,9 ^b
16,724	β -(E)-Cariofilen	87-44-5	254	4 $\pm$ 0,1 ^b	11 $\pm$ 0,5 ^a
17,616	γ -Cadinen	39029-41-9	271	3 $\pm$ 0,1 ^b	4 $\pm$ 0,2 ^a

RT—retentiontime;CAS—CAS registrynumber; BP—boilingpoint.

For both methods, five main compounds were identified, i.e., thymol, γ -terpinene, p-cymene, α -terpinene, and β -pinene, which represent between 93% and 97% of the total amount of UE obtained. The results specified in Table 25 indicate a fairly similar composition of the EU obtained by the two extraction methods.

Thymol is extracted more efficiently by MWHD, while γ -terpinene is extracted more efficiently by CHD. This difference can be explained by the bioconversion of γ -terpinene to thymol, a process influenced by several factors, including the extraction method (Zarshenas et al., 2014). The same behavior is observed for p-cymene, α -terpinene, and β -pinene, which are extracted more efficiently by CHD.

Krause et al. (2021) proposed a biosynthetic pathway for thymol, starting with the cyclization of geranyl diphosphate, forming γ -terpinene. This is oxidized by the enzyme P450 monooxygenase into cyclohexadienol intermediates. These products are unstable and are dehydrogenated by a dehydrogenase/reductase enzyme into allylic ketone intermediates. Subsequently, through keto-enolic tautomerism, thymol is formed (Krause et al., 2021).

2.2.2.3.2. Influence of Solvent to Plant Ratio on the Extraction of Thyme Essential Oil

The next step to optimize the EO extraction from thyme was to study the influence of solvent to plant ratio. To establish the solvent volume required to achieve as much EO as possible, different ratios of solvent to plant material were used. Moreover, to avoid degradation of thyme leaves due to direct microwave irradiation, an adequate amount of water is required. As shown in

Figure 18a, the amount of EO increases with the increase in the water volume. The most efficient solvent to plant ratio for the extraction of thyme EO by MWHD is 12/1 (v/w).

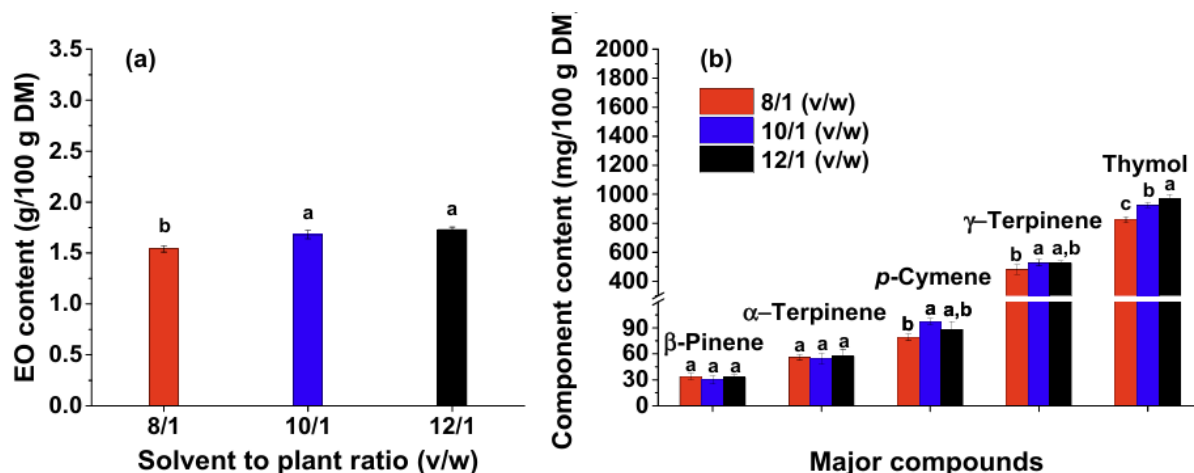


Figure 18. Influence of solvent to plant ratio on the extraction of thyme EO by microwave-assisted hydro-distillation (MWHD) for a particle size of 1–2 cm: (a) essential oil (EO) content; (b) major compounds identified by GC-MS. Different letters (a–c) within graph show the significant difference between groups analyzed by ANOVA ($p < 0.05$) and Duncan's post hoc t-tests.

The resulted EO, for all solvent to plant ratios, was analyzed by GC-MS. The main compounds of thyme EO identified by GC-MS are shown in Figure 18b. It can be noticed that the EO component content is similar for all three ratios and the amount of thymol is directly proportional with the solvent to plant ratio. Thus, a high amount of water is required to entrain the constituents with high boiling points.

The ANOVA analysis showed that by increasing the solvent to plant ratio from 8/1 to 10/1, the EO content increases significantly ($p < 0.05$). By the further increase in the solvent to plant ratio, the EO content increases non-significantly (Figure 18a). Regarding the thymol extraction, the ANOVA analysis showed that by increasing the solvent to plant ratio, the thymol concentration increased significantly ($p < 0.05$, Figure 18b) that by increasing the solvent to plant ratio from 8/1 to 10/1, the EO content increases significantly ($p < 0.05$).

Although the amount of EO is similar for the 10/1 and 12/1 ratios, further experiments were carried out for the 12/1 (v/w) ratio because the amount of the target compound (thymol) is higher compared to the other ratios used (969 ± 28.90 mg thymol/100 g DM for the 12/1 ratio compared to 926 ± 16.93 and 824 ± 20.67 mg thymol/100 g DM for the 10/1 and 8/1 ratios, respectively).

2.2.2.3.3. Influence of Ultrasound Equipment on the Extraction of Thyme Essential Oil

Prior to the extraction of thyme EO by MWHD, the extraction mixture was subjected to ultrasound pre-treatment for 30 min using two types of equipment: an ultrasound bath (ES375H Bench Top Ultrasonic Tank, Hilsonic Ultrasonic Cleaners, Birkenhead, UK) and an ultrasonic processor (Vibracell VCX-750, Sonics & Materials, Inc., Newtown, CT, USA).

It can be noticed (Figure 19a) that the ultrasound pre-treatment of the extraction mixture leads to higher amounts of EO compared with the experiments without pre-treatment, for both the ultrasound bath (approximately 6% higher— 1.83 ± 0.09 g EO/100 g DM) and the horn (approximately 21% higher— 2.10 ± 0.03 g EO/100 g DM). This could be due to the cavitation phenomenon, which can stimulate the disruption of cell walls and increase the mass transfer rate. In the ultrasonic bath, the cavitation occurs uncontrollably. The ultrasound energy is unequally dispersed in the bath and has low intensity. On the other hand, for the horn, the cavitation has a high localized intensity, and implicitly, the sonication process is more efficient. Therefore, the higher amount of EO achieved when the ultrasound pre-treatment is carried out using the Vibracell equipment (an increase of approximately 15%) could be due to these differences between ultrasonic baths and horns.

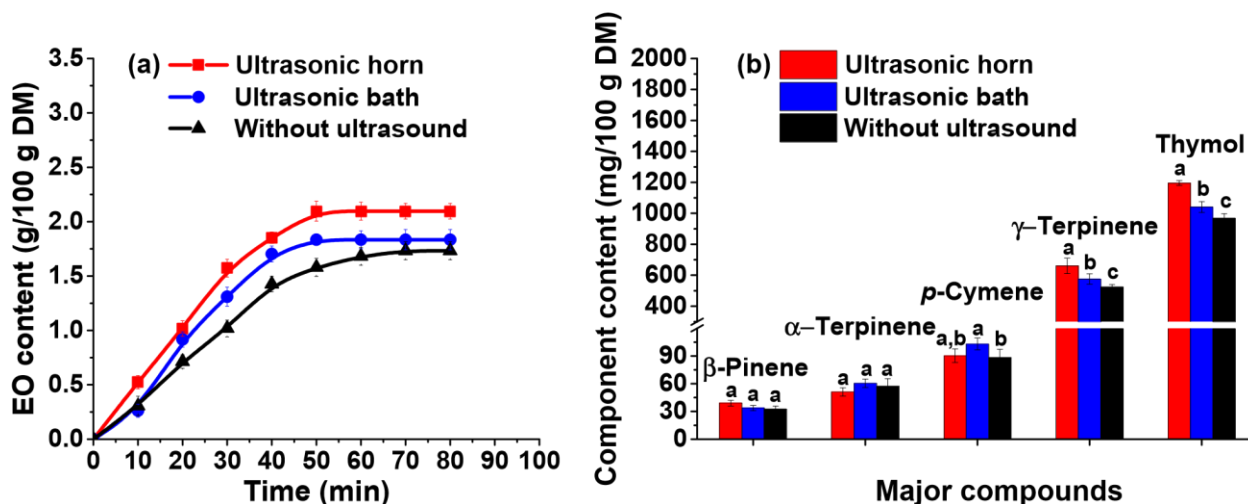


Figure 19 Influence of ultrasound pre-treatment on the extraction of thyme EO by microwave-assisted hydro-distillation (MWHD) for a solvent to plant ratio of 12/1 (v/w), a particle size of 1–2 cm, and an amplitude of 50%: (a) essential oil (EO) content; (b) major compounds identified by GC-MS. Different letters (a–c) within graph show the significant difference between groups resulted from ANOVA ($p < 0.05$) and Duncan's post hoc t-tests.

In addition, the ultrasound pre-treatment of the extraction mixture has a beneficial effect on the extraction time, achieving a maximum amount of EO in only 50 min compared with the extraction without pre-treatment when a maximum amount is achieved after 70 min. Further, the experiments were performed using the Vibracell ultrasonic processor to pre-treat the extraction mixture.

The GC-MS analyses of the EO obtained by consecutive use of ultrasound and microwave irradiation are shown in Figure 3b. It can be noticed that the major constituents of the EO are similar for all three methods (the extraction with ultrasound pre-treatment using both ultrasonic horn and bath and the extraction without pre-treatment). As shown in Figure 3b, the thymol is extracted more efficiently when the ultrasound pre-treatment is carried out using the ultrasonic probe (1196 ± 15.35 and 1040 ± 35.62 mg/100 g DM for ultrasonic probe and bath, respectively). Instead, *p*-cymene is obtained in lower amounts (90 ± 7.28 and 103 ± 6.49 mg/100 g DM for ultrasonic probe and bath, respectively). This could be due to the transformation of some compounds during ultrasound irradiation, such as isomerization, oxidation, and degradation of dimmers and polymers [35]. Also, in water, the cavitation phenomenon can lead to the formation of free radicals ($H\bullet$, $OH\bullet$) which can cause the transformation of the EO constituents (Ince et al., 2001).

2.2.2.3.4. Influence of Ultrasound Amplitude on the Extraction of Thyme Essential Oil

The influence of ultrasound amplitude on the extraction process efficiency was also studied. Amplitudes of 50 and 70% were chosen. As shown in Figure 20a, the amount of thyme EO is directly proportional to the ultrasound amplitude. The EO content increases by 6% compared with the pre-treatment performed at an amplitude of 50% (2.10 ± 0.03 and 2.22 ± 0.06 g EO/100 g DM for an amplitude of 50 and 70%, respectively).

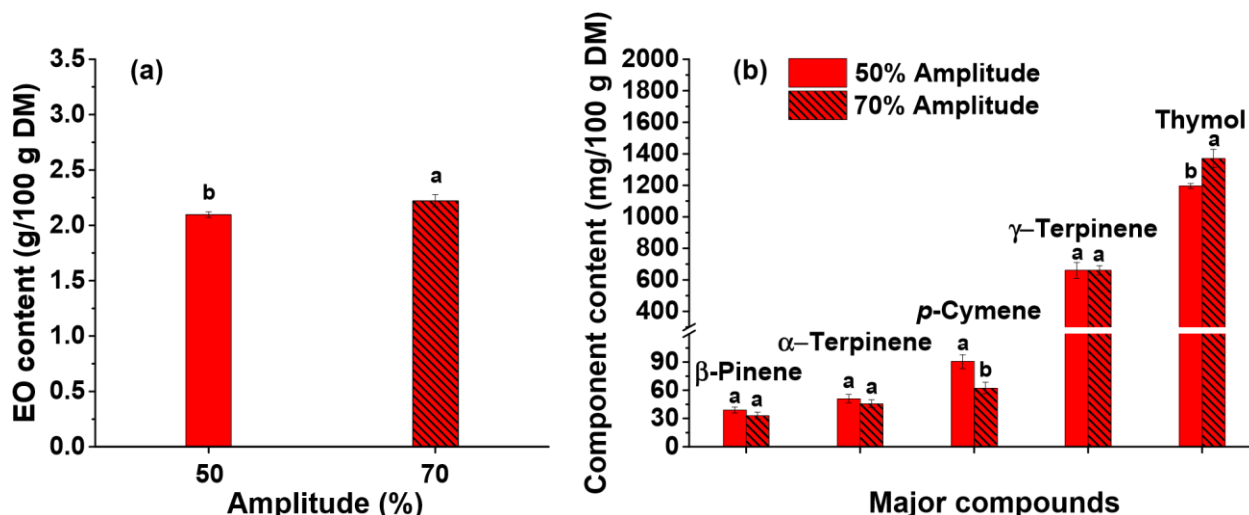


Figura 20. Influence of ultrasound amplitude on the extraction of thyme EO by microwave-assisted hydro-distillation (MWHd) for a solvent to plant ratio of 12/1 (v/w) and a particle size of 1–2 cm using the Vibracell ultrasonic processor: (a) essential oil (EO) content; (b) major compounds identified by GC-MS. Different letters (a,b) within graph show the significant difference between groups from one-way ANOVA ($p < 0.05$) and Duncan's post hoc *t*-tests.

As shown in Figure 4b, the targeted compound, thymol, is extracted more efficiently when an amplitude of 70% is used (1196 ± 15.35 and 1370 ± 58.78 mg/100 g DM for an amplitude of 50 and 70%, respectively). Statistical analysis confirmed that by increasing the ultrasonic amplitude from 50 to 70%, the EO and thymol contents increase significantly ($p < 0.05$). However, the content of *p*-cymene is inversely proportional with the ultrasound power, achieving higher amounts for an amplitude of 50%. This can be due to an accentuated transformation of some constituents at high ultrasound powers.

2.2.2.3.5. Influence of Plant Particle Size on UE Extraction

As shown in Figure 5a, the particle sizes of the thyme leaves influence to a large extent the EO content. For a particle size under 0.1 cm, higher amounts of EO are achieved for both ultrasound pre-treatment (for both amplitudes applied, *i.e.*, 50 and 70%) and extraction without pre-treatment. Using smaller particles, the EO content is 23% higher when an ultrasound pre-treatment (with an amplitude of 70%— 2.67 ± 0.06 g EO/100 g DM) of the extraction mixture is applied, compared with the extraction without pre-treatment (2.18 ± 0.07 g EO/100 g DM). The ANOVA analysis showed that EO content increased significantly ($p < 0.05$) by decreasing the plant material particles size.

These strategies (the ultrasound pre-treatment using an ultrasonic probe and milling the plant material to a particle size under 0.1 cm) lead to a higher extraction yield compared with other

studies. For example, Kowalski et al., after applying ultrasonic pre-treatment using an ultrasound bath, followed by conventional steam distillation method, achieved an extraction yield of 2.15 g EO/100 g DM.

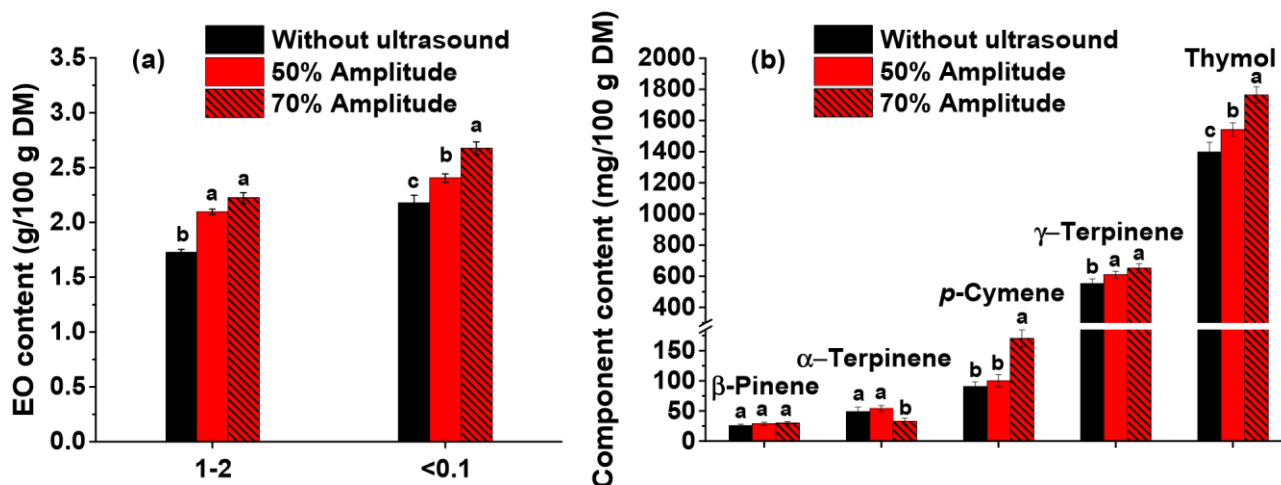


Figure 21. Influence of leaf particle size on the extraction of thyme EO by microwave-assisted hydrodistillation (MWHd) for a solvent to plant ratio of 12/1 (v/w): (a) essential oil (EO) content; (b) major compounds identified by GC-MS for a particle size under 0.1 cm. Letters (a–c) within graph show the significant difference between groups' ANOVA ($p < 0.05$) and Duncan's post hoc t -tests.

The major component content of thyme EO for a granulation of the leaves under 0.1 cm is similar with the one for a particle size of 1–2 cm. However, the amount of the targeted compound (thymol) is higher for the herb milled to a granulation under 0.1 cm (see Figures 19b, 20b and 21b). This behavior can be noticed for all three methods: the extraction with ultrasound pre-treatment applying both 50 and 70% amplitudes and the extraction without pre-treatment (1542 ± 42.54 and 1764 ± 52.68 mg/100 g DM for an amplitude of 50 and 70%, respectively, and 1396 ± 62.7 mg/100 g DM for the extraction without pre-treatment).

As shown in Figure 21b, the thymol content is higher when an ultrasound pre-treatment with an amplitude of 70% is applied, while the α -terpinene content is lower (54 ± 4.91 and 33 ± 5.27 mg/100 g DM for an amplitude of 50 and 70%, respectively).

This behavior of α -terpinene can be also observed for the extraction with thyme leaves of a 1–2 cm particle size (51 ± 4.5 and 46 ± 4.02 mg/100 g DM for an amplitude of 50 and 70%, respectively). Moreover, the p -cymene content is higher for an amplitude of 70% compared with 50% when the plant is milled (Figure 20b— 100 ± 9.9 and 170 ± 14.06 mg/100 g DM for an amplitude of 50 and 70%, respectively), while for the extraction performed with leaves of 1–2 cm

particle size, an opposite behavior is observed (Figure 20b— 90 ± 7.28 and 63 ± 5.99 mg/100 g DM for an amplitude of 50 and 70%, respectively). This could be due to a possible oxidation of some components (such as α -terpinene and γ -terpinene) yielding *p*-cymene. However, further research is required for possible oxidation under combined ultrasound and microwave treatments.

2.2.2.3.6. *Principal Component Analysis (PCA)*

To evaluate the relationship between the extraction method and the EU composition, principal component analysis (PCA) was performed. PCA revealed two eigenvalues greater than 1, corresponding to principal components PC1 (3.55) and PC2 (1.04), which explain 90.68% of the variability ($70.21\% + 20.47\%$).

The concentrations of the major compounds (β -pinene, α -terpinene, *p*-cymene, γ -terpinene, and thymol) were considered as variables, and these concentrations were noted in italics, i.e., β -pinene, α -terpinene, *p*-cymene, γ -terpinene, and thymol.

2.2.1.7 Concluzii

The purpose of this study was to investigate the influence of the ultrasound pretreatment of thyme leaves, before MWHD, on the thymol content and on the EO extraction yield. Comparative extractions, without pre-treatment, by both MWHD and CHD were also performed. The influence of several parameters (solvent to plant ratio, particle size of the vegetal material, ultrasound equipment to pre-treat the extraction mixture, and amplitude of the ultrasonic processor) on the extraction of thyme EO by MWHD was investigated.

The composition of the EO resulted from all methods was analyzed by GSMS, the major constituents being thymol, γ -terpinene, *p*-cymene, α -terpinene, and β -pinene. The results showed that the ultrasound pre-treatment of the extraction mixture enhances the EO content, which can be extracted from thyme leaves. Moreover, by combining the strategies of ultrasound pre-treatment using an ultrasonic probe and milling the plant material to a particle size under 0.1 cm, a maximum yield of 2.67 ± 0.06 g EO/100 g DM was achieved. These strategies were also favorable for the targeted constituent, thymol, leading to a maximum content of 1764 ± 52.68 mg/100 g DM.

GENERAL CONCLUSIONS AND RESEARCH PERSPECTIVES

The doctoral thesis entitled ***INTENSIFICATION OF MASS TRANSFER IN THE EXTRACTION PROCESS OF BIOACTIVE COMPOUNDS*** comprises two main parts, *i.e.*, **1. LITERATURE REVIEW**, which contains relevant aspects about liquid-liquid and solid-liquid extraction processes, indole-3-acetic acid (IAA), the composition, uses, and therapeutic effects of mint and thyme, and the extraction of bioactive compounds from these plants, and **2. ORIGINAL CONTRIBUTIONS**, which presents the results obtained in our own research on the liquid membrane-facilitated extraction of IAA (Chapter 2.1) and the extraction of bioactive compounds from mint and thyme leaves (Chapter 2.2).

In subchapter **2.1.1** the results of multiple regression analysis and correlation analysis indicated the following aspects:

- all dimensionless factors and their binary and tertiary interactions significantly influenced the $c_{IAA,Ff}$ concentration;
- $c_{IAA,Ff}$ increased with the increase of the dimensionless factors $c_{IAA,F0}$ (x_1) and $c_{NaOH,S0}$ (x_2) and their interaction (x_1x_2);
- higher values of the E_F and K_D parameters were obtained at lower values of the dimensionless factor $c_{IAA,F0}$ (x_1) and higher values of the dimensionless factors $c_{L,M0}$ (x_3) and $c_{NaOH,S0}$;
- higher values of ER efficiency were obtained at lower values of factor x_1 and higher values of factor x_2 ;
- factor x_1 had a strong positive effect on $c_{IAA,Ff}$ and $c_{IAA,Sf}$ concentrations, and factor x_2 had a strong positive effect on E_F , K_D , and E_R parameters;
- E_F and K_D parameters were very strongly positively correlated ($r = 0.95$).

In subsection **2.1.2. Kinetics of the facilitated pertraction process**, the specific quantities of IAA in the feeding (F), liquid membrane (M), and stripping (S) phases were predicted based on a kinetic model that assumes consecutive irreversible first-order reactions. The adjustable parameters of the model, *i.e.*, the rate constants of the extraction and stripping processes, $k_1 = (1,93\text{--}3,64) \times 10^{-4} \text{ s}^{-1}$ and $k_2 = (2,00\text{--}4,18) \times 10^{-4} \text{ s}^{-1}$, were identified based on experimental data. The similar values of the kinetic parameters k_1 and k_2 suggest that both the complexation reaction at

the interface between phases F and M and the decomplexation reaction at the interface between phases M and S were rate-determining steps. The results indicated good agreement between the experimental and predicted data ($R^2 = 0.948\text{--}0.998$).

The maximum final value (at $t_f = 4$ h) of the specific amount of IAA in phase S ($R_{Sf,ex} = 0,911$), corresponding to the maximum values of extraction efficiency ($E_{Ef,ex} = 94,81\%$), stripping ($E_{Sf,ex} = 96,08\%$), and recovery ($E_{Rf,ex} = 91,09\%$) was obtained at $c_{IAA,F0} = 10^{-4}$ kmol/m³ and $c_{NaOH,S0} = 1$ kmol/m³.

In subsection 2.2.1., the regression models obtained based on experimental data for UAE ($CaC_{m,UAE} = 1,214\text{--}38,72$ mg/L, $CbC_{m,UAE} = 0,809\text{--}27,05$ mg/L, $TCC_{m,UAE} = 4,948\text{--}9,495$ mg/L și $d_{m,UAE} = 8,300\text{--}13,66$ mm) showed a significant positive effect of the dimensionless factor c_{et} (X_1) on all process responses and a significant negative effect of the dimensionless factor R_{LS} (X_2) on $d_{pr,UAE}$. At the optimal levels of the UAE process factors ($c_{et,opt,UAE} = 86\%$ și $R_{LS,opt,UAE} = 12,9$ mL/g), the average values of the responses were: $CaC_{m,opt,UAE} = 39,20$ mg/L, $CbC_{m,opt,UAE} = 28,69$ mg/L, $TCC_{m,opt,UAE} = 10,19$ mg/L și $d_{m,opt,UAE} = 14,10$ mm.

Regression models obtained based on experimental data for MAE ($CaC_{m,MAE} = 4,732\text{--}35,21$ mg/L, $CbC_{m,MAE} = 3,165\text{--}24,71$ mg/L, $TCC_{m,MAE} = 5,415\text{--}9,751$ mg/L și $d_{m,MAE} = 10,74\text{--}14,08$ mm) indicated the following:

- the dimensionless factor c_{et} (X_1) had a significant positive effect on all process responses, and X_1^2 had a significant negative effect on the variables $CaC_{pr,MAE}$, $CbC_{pr,MAE}$ și $TCC_{pr,MAE}$;
- the dimensionless factor R_{LS} (X_2) had a significant negative effect on all responses, X_2^2 had a significant negative effect on the variable $TCC_{pr,MAE}$ and a significant positive effect on $d_{pr,MAE}$, and the interaction X_1X_2 had a significant negative effect on $d_{pr,MAE}$.

At the optimal levels of the MAE process factors ($c_{et,opt,MAE} = 86\%$ and $R_{LS,opt,MAE} = 5,9$ mL/g), the average response values were: $CaC_{m,opt,MAE} = 38,78$ mg/L, $CbC_{m,opt,MAE} = 28,07$ mg/L, $TCC_{m,opt,MAE} = 10,31$ mg/L and $d_{m,opt,MAE} = 14,55$ mm.

The average values of CaC_{opt} , CbC_{opt} , TCC_{opt} și d_{opt} , and d_{opt} for UAE and MAE were not significantly different. Mint extracts prepared under optimal operating conditions could be used as ingredients in food, personal care products, cosmetics, or pharmaceuticals.

In subsection 2.2.2. **Improving the efficiency of essential oil extraction from thyme through the combined use of ultrasound treatment and microwave-assisted extraction**, the influence of ultrasound pretreatment applied to thyme leaves and stems prior to microwave-

assisted hydrodistillation (MWHD) was studied in relation to the essential oil (EO) extraction yield and the content of bioactive compounds.. The influence of process factors, i.e., liquid/solid ratio, plant material particle size, type of ultrasonic equipment used for pretreatment, and ultrasonic processor amplitude, on the performance of the extraction process was evaluated.

The main research objectives/directions I am pursuing further relate to:

- facilitated extraction of bioactive compounds using different liquid membranes and transport agents;
- extraction of bioactive compounds from other medicinal and aromatic plants using different conventional and unconventional techniques;
- integration of extracts into food matrices and/or cosmetic formulations.

LIST OF WORKS

Works published in ISI-indexed/rated journals:

1. Diaconu, I., Pârvulescu, O.C., Topală, S.L., Dobre, T. (2021) Effects of process factors on performances of liquid membrane-based transfer of indole-3-acetic acid. *Sci. Rep.*, 11(1), 23427, WOS:000727615800033 (Q1), FI (2021) =4.38.
2. Gavrilă, A.I., Chisega-Negrila, C.G., Maholea, L., Gavrilă, M.L., Pârvulescu, O.C., Popa, I. (2023) Enhancing the extraction process efficiency of thyme essential oil by combined ultrasound and microwave techniques. *Agronomy*, 13 (9), art. no. 2331, 1–14, WOS: 001093126200001 (Q1), FI (2023) = 3.3.
3. Topală, S.L., Diaconu, I., Pârvulescu, O.C., Jidveian, R.S., Drăghici-Popa, A.M. (2025) Facilitated extraction of indole 3-acetic acid in the presence of trioctylamine as a carrier. *U.P.B. Sci. Bull. Series B* 2025, 87(4) (accepted for publication) FI (2024) = 0.3.
4. Roșu, A.M., Aruș, V.A., Răducanu, D., Platon, N., Georgescu, A.M., Muntianu, G., Nistor, I.D., Topală, S.L., Pârvulescu, O.C. (2025) Optimization of ultrasound-assisted extraction and microwave-assisted extraction of bioactive compounds from Romanian peppermint (*Mentha × piperita* L.). *Agronomy* (Q1) (submitted for publication).

IMPACT FACTOR: 7.98

Oral presentations (O)/posters (P) at international conferences/symposiums:

1. Topală, S.L., Pârvulescu, O.C., Diaconu, I., Badea, G.I., Radu, D.A. (2022) Removal of emerging pollutants from wastewater using membrane techniques. 22nd Romanian International Conference on Chemistry and Chemical Engineering (RICCCE), Section 5: ENVIRONMENTAL MONITORING AND PROTECTION, September 7-9, 2022, Sinaia, Romania (P).
2. Topală, S.L., Diaconu, I., Tudor, C.A., Cernica, G., Orbeci, C., Pârvulescu, O.C. (2024) Efficiency and selectivity of ionic liquids in the removal of emerging pollutants through bulk liquid membranes. 23rd Romanian International Conference on Chemistry and Chemical Engineering (RICCCE), Section 6: SUSTAINABILITY AND ENVIRONMENTAL ENGINEERING, September 4-7, 2024, Mamaia, Romania (P).
3. Gavrilă, A.I., Trifan, A., Popa, I., Maholea, L., Feizula, E. (2024) Ultrasound-assisted extraction of neuroprotective phytochemicals from *Melissa officinalis* L. 23rd Romanian International Conference on Chemistry and Chemical Engineering (RICCCE), Section 3: GREEN ENERGY AND CHEMICAL CONCEPTS, September 4-7, 2024, Mamaia, Romania (P).

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