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NOVEL METHODS FOR THE SEPARATION AND PURIFICATON OF BIOACTIVE SUBSTANCES

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DISSERTATION ABSTRACT

for the degree of PhD in the professional field
4.2. "Chemical Sciences,"

scientific discipline – Chemical and Biochemical
Engineering

Scientific advisor:

prof. Dragomir Yankov, PhD

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The dissertation is presented on 110 printed pages. It contains 19 tables and 37 figures. A total of 207 literary sources are cited.

The dissertation was prepared in the Biochemical Engineering Laboratory of the Institute of Chemical Engineering at the Bulgarian Academy of Sciences.

The defense of the dissertation will take place on ________________ at _______ h., at the Institute of Chemical Engineering, Bulgarian Academy of Sciences, Sofia, acad. G. Bonchev str., 103, entr. B, floor 2, Conference hall.

With immense gratitude, this work is dedicated to the family, friends, and scientists of the past and present. Without their support, I wouldn't have made it this far.

The author is grateful to his scientific supervisor, Prof. Dragomir Yankov, for the opportunity to work at the Institute of Chemical Engineering of the Bulgarian Academy of Sciences. For his openness, the opportunities he has provided, his calmness, and his constructive criticism of the author's work. The following helped the author develop his research capabilities.

The author is grateful to his colleagues at the Institute of Chemical Engineering of the Bulgarian Academy of Sciences, who welcomed him into their team, for their understanding, professional support, and assistance in preparing his dissertation.

With gratitude to all scientists around the world who have shared and continue to share their work, because of them, this dissertation exists.

A message to all future scientists – be bold and work together, only then will you carry the light of knowledge even through the darkest times.

Въведение

This dissertation investigates methods for extracting and separating bioactive substances to develop environmentally friendly technological approaches. The primary focus of the work is on the use of deep eutectic solvents (DES/NaDES) as extractants for liquid-liquid extraction, membrane methods (nanofiltration and reverse osmosis), and pertraction with a bulk liquid membrane.

The author has synthesised fifteen new hydrophobic DESs based on menthol. Part of these DES are composed of menthol-amine and were used to extract L-lactic acid from a model aqueous solution. The other DES, composed of menthol-salicylic acid, was used as an extractant to separate the cis- and trans-isomers of butenedioic acid. The physicochemical properties (density, viscosity, thermal stability) of the obtained DESs were described. The possibility of multiple uses of the solvents after regeneration is demonstrated.

The possibilities for dealcoholization of Mavrud red wine using membrane methods of nanofiltration and reverse osmosis under different operating conditions have been explored in the presented dissertation. The author examined the influence of the methods used and the operating mode on the content of organic acids and sugars in wine. The data obtained are new and show the application of membrane methods for effective control of the alcohol content in Mavrud wine.

In addition, the author studied the separation of the cis-trans isomers of butenedioic acid; for that purpose, a bulk liquid membrane pertraction was used. The study aimed to identify optimal process conditions. The experiments were conducted using a laboratory pertraction apparatus, developed at the Institute of Chemical Engineering. The findings indicate a correlation between the pH of the feed water and the characteristics of the organic phase used as the membrane.

The selected methods — liquid membrane pertraction, nanofiltration, and liquid-liquid extraction — constitute fundamental technological operations utilised in the chemical and biotechnology industries for the production of final products. The techniques proposed in the dissertation can be effectively implemented in waste-free, single-feed biorefineries, which play a significant role in the bio-circular economy. By carefully selecting parameters such as membrane type, solvent, pH, and temperature, these methods facilitate selective transfer and purification of target compounds while maintaining their structure, purity, and biological activity.

Aims and Objectives

Aims of the research.

1. To investigate environmentally friendly methods for the extraction and separation of biogenic carboxylic acids, aimed at enhancing the efficiency and selectivity of these processes.
2. To develop and optimize deep eutectic solvents (DES) for the extraction of:
    - L-lactic acid (application of DES menthol–amine);
    - Isomers of butenedioic acid (menthol–salicylic acid NaDES implemented).
3. To explore membrane separation techniques, including:
    - Pertraction in a laboratory apparatus with rotating discs for the separation of butenedioic acid isomers;
    - Nanofiltration of Mavrud red wine for partial dealcoholization, alongside an analysis of optimising in organic acids and sugars.

Research Objectives.

1. Synthesis and Characterization of the DES:
    - Formation of menthol-amine DES for the extraction of L-lactic acid;
    - Development of menthol-salicylic acid for the extraction of isomers of butenedioic acid
2. Investigation of Extraction Capacity of the DES:
    - Assessment of the extraction capacity of DES (menthol-amine) for L-lactic acid.
    - Evaluation of the selectivity of the deep eutectic solution (menthol-salicylic acid) towards the cis- and trans-isomers of butenedioic acid.
3. Analysis of Separation Using Membrane Processes:
    - Examination of the separation kinetics for butenedioic acid isomers through pertraction with a bulk liquid membrane in a rotary disc pertractor;
    - Examination of the kinetics involved in the separation of butenedioic acid isomers through pertraction in a rotary disc pertractor.

Contemporary state of the field

The literature review showed that the extraction of bioactive substances from natural raw materials is a rapidly developing field that combines principles of chemical and biochemical technologies.

Classic solid-liquid methods (maceration, percolation, Soxhlet extraction, etc.) remain the basis of phytochemical technologies. Nevertheless, they are characterised by high time and energy requirements and by limited selectivity in complex natural matrices. Innovative "green" approaches—ultrasonic, microwave, supercritical extraction, and extraction with deep eutectic solvents—offer higher efficiency, reduced processing time, and greater environmental sustainability. However, their industrial application is often limited by the high cost and complexity of the equipment.

Within this framework, liquid-liquid extraction and its various modifications have emerged as a versatile and selective methodology for the purification and concentration of target compounds. This approach operates effectively under mild physicochemical conditions, enhancing its applicability in diverse chemical processes. Membrane processes that combine the principles of liquid-liquid extraction with controlled transport through a selective barrier are particularly promising, as they reduce solvent consumption, facilitate automation, and allow integration into continuous process schemes.

Despite significant progress in membrane technologies, the influence of key physicochemical parameters (pH, concentration, time, composition, and membrane stability) on the mass transfer efficiency and selectivity of the process remains insufficiently clarified. Those parameters necessitate further experimental research to deepen the understanding of the behaviour of different systems in liquid-liquid and membrane extraction.

From an analytical perspective, high-performance liquid chromatography (HPLC) has established itself as a key tool for evaluating extraction processes, enabling precise identification and quantification of bioactive compounds. Combining HPLC analysis with varying extraction parameters offers an opportunity to optimise and model mass transfer processes.

This dissertation focuses on the experimental investigation and optimisation of liquid-liquid and membrane processes for the extraction of bioactive substances, utilising HPLC analysis as a dependable criterion for assessing efficiency and selectivity. The overarching aim is to develop environmentally sustainable and energy-efficient technologies for the extraction and purification of biologically active natural products.

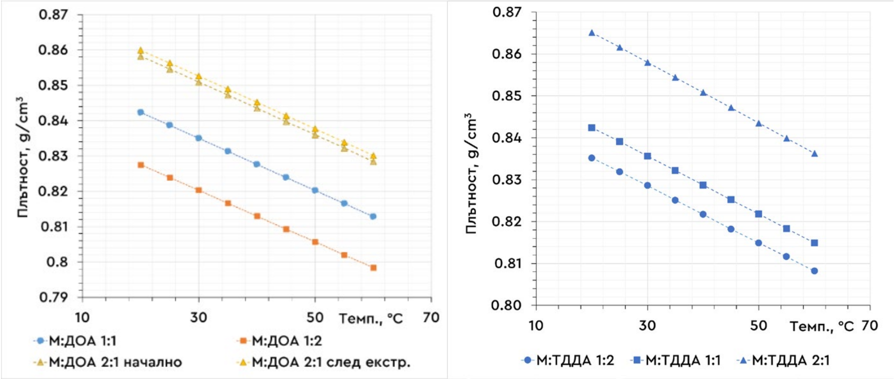
Extraction and separation of carboxylic acids

1. DES as extractants for carboxylic acids

1.1. Characterisation of the synthesised DES menthol-amine

Density

The change in density with temperature in DES from the menthol-secondary/tertiary amine system does not differ from that of other liquids. The density of all studied DES decreases with increasing temperature over the range 20-60 °C.



(a) ***(б)***

***Fig. 1**. Influence of the temperature on the density of the synthesised DES:*
***(a)** DES M-DOA и **(б)** DES M-TDDA.*

The other distinct variation in density is the ratio of components in the eutectic system. It is observed that a higher menthol content in the DES composition increases density, whereas an increase in amines decreases density. Examples of this include M/DOA and M/TDDA. Figure 1(a) illustrates the changes in density as a function of temperature and DES composition. Figure 1(b) shows changes in density as a function of temperature and the DES component ratio.

Dynamic viscosity

The results presented in Figure 2 show the dependence of dynamic viscosity on temperature relative to the ratio of components in DEPs (e.g., the mentholamine-dioctylamine solution). The curves obtained confirm that the dynamic viscosity of the solutions studied decreases with increasing temperature.

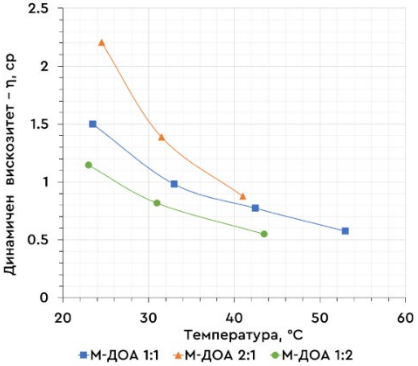
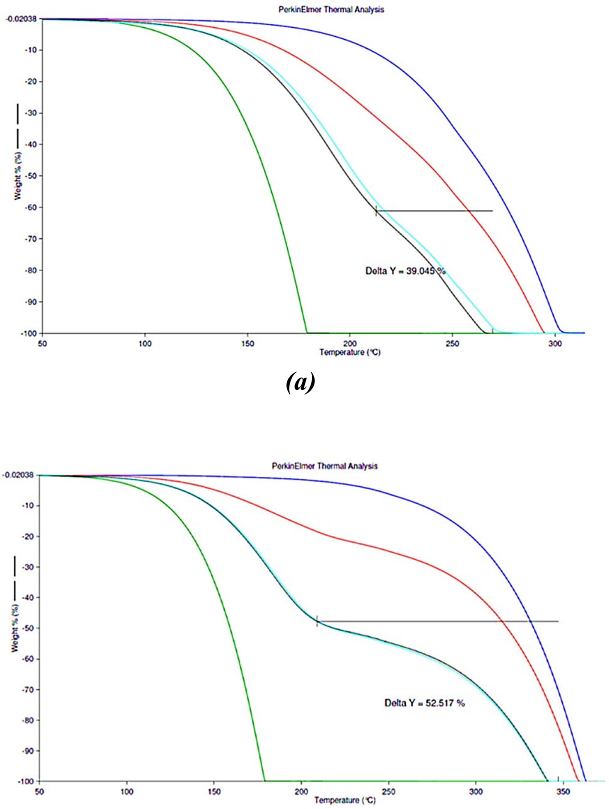


Fig.2. *Dynamic viscosity (η) of DES M-DOA relative to temperature change.*

The analysis shows that viscosity depends on the molar ratio of the components in the DESs under consideration. The increased menthol content in the system increases viscosity. The dynamic viscosity decreases with increasing DOA content in the solution system.

Thermogravimetric analysis

Figure 3 presents the thermal decomposition profiles obtained from TGA analysis of two of the DESs used for extraction – menthol–dioctylamine (DOA), which are in a molar ratio (1:2); menthol–trioctylamine (TOA) with a molar ratio (1:2). The results of the analysis allow an assessment of the stability of eutectic systems at increasing temperatures. The change in the sample's mass with increasing temperature is an indicator of the stability of the bonds in the eutectic system. The study also includes a eutectic system that has undergone re-extraction.



(a)

(b)

***Fig. 3**. Curves of the thermal decomposition of substances from TGA analysis of DES - **(a)** M-DOA 1:2 ; **(b)** M-TOA.*
*Colour legend – **green** – menthol; **blue** – tertiary amine; **red** – eutectic composition; **ultramarine** – after extraction; **black** – after re-extraction*

Comparison between the menthol curve and the DES curves clearly shows that the decomposition of the eutectic mixture proceeds in a completely different manner from that of the simple substance. Which means that more energy is required to destroy the eutectic solution. The curves show that the decomposition of menthol begins at around 100°C and ends at around 170°C, while the decomposition of amines begins at above 200°C and ends at least at 300°C (DOA) or at much higher temperatures (TOA-300°C; THA and TDDA - 460°C). The decomposition temperature (T_{deg}) and degree of decomposition of eutectic mixtures are shown in Table 1.

Table 1. Thermal properties of menthol and the amines used and their eutectic mixtures.

Compound	T, °C	DES	T_{deg}, °C	First stage of decomposition, %
L-ментол	175	M/DOA 1:1	280	
ДОА	305	M/DOA 1:2	290	
ТОА	365	M/DOA 1:2	360	26,5
ТХА	465	M/THA 1:1	275	
ТДДА	470	M/THA 1:2	290	
		M/TDDA 1:1	460	30
		M/TDDA 1:2	460	18

Analysis of the TGA results, in the form of thermal curves, for the eutectic mixtures menthol/amine shows that their decomposition occurs at much higher temperatures than menthol, but at lower temperatures than the corresponding amine.

The thermal decomposition curve of DES menthol-TOA shows an additional phenomenon – thermal decomposition occurs in two stages. The following is a consequence of DES's physicochemical properties and suggests the partial decomposition of the complex formed by its two components. The complex is formed as a result of hydrogen bonds between the donor (amine) and the acceptor (menthol) or transformation of the type of complexes.

Investigation of DES mentholamine using Fourier transform infrared spectroscopy

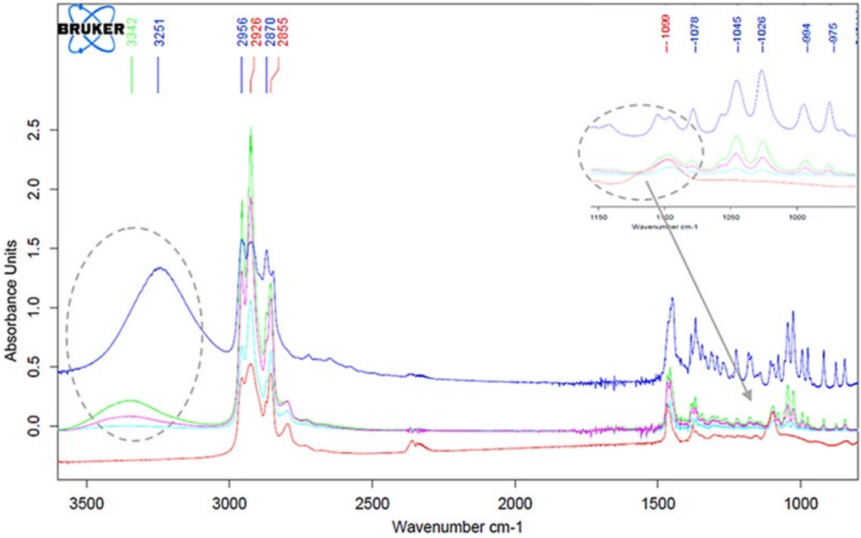


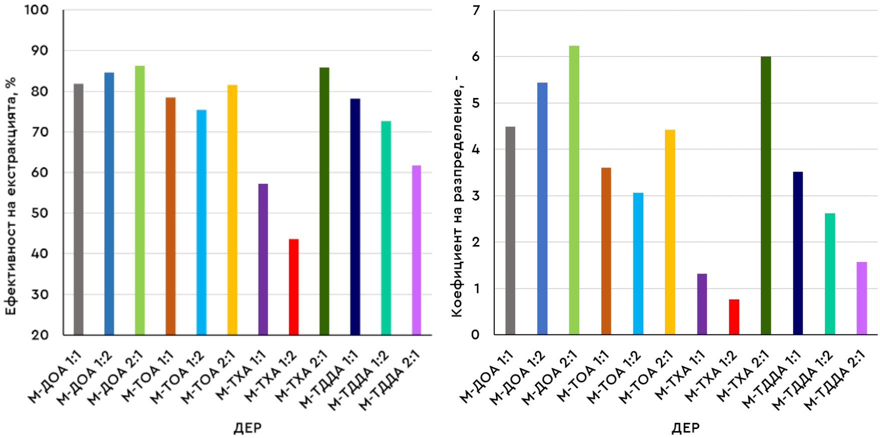
Fig. 4. *A plot of the transmittance vs. wave numbers of the menthol (blue), TOA (red), and M/TOA eutectic mixtures at 2:1 (green), 1:1 (magenta), and 1:2 (cyan) molar ratio. The inset shows a scale-up in the region below 1150 cm^{-1} .*

Figure 4 shows that the infrared spectra of the eutectic solution of menthol and trioctylamine in different molar ratios do not differ significantly.

In the IR spectrum of menthol (blue line), the characteristic wave range around 3250 cm^{-1}, corresponding to the hydroxyl group, is observed. The ranges corresponding to the C-H groups are located in the region from 2800 cm^{-1} to about 2990 cm^{-1}, and the ranges of valence vibrations for the C-H groups are located below 1500 cm^{-1}. In the TOA spectrum (red), the valence vibrations for CH2 are located at 1470 cm^{-1} and for CH_3 at 1380 cm^{-1}. At 1099 cm^{-1}, the valence vibration range for the C-N group can be observed. The spectra of the eutectic mixtures of menthol/amine in the valence vibration range for the O-H group are significantly shifted to 3340 cm^{-1}, which can be attributed to the formation of a hydrogen bond between the hydroxyl group of menthol and the electron pair of the amino group of TOA, acting as a hydrogen bond donor. A similar effect is observed here, judging by the small but noticeable shift in the range – from 1450 cm^{-1} to 1465 cm^{-1}. In addition, the valence vibration range for C-N at 1099 cm^{-1} is slightly shifted to 1078 cm^{-1} and its intensity becomes weaker in the IR spectra of eutectic mixtures, which also supports the assumption of the formation of complex compounds of menthol and amine due to the formed H-bond between their functional groups.

1.2. DES mentholamine as an extractant for L-lactic acid

The extraction efficiency (Figure 5(a)) and the distribution coefficient (Figure 5(b)) between the phases were calculated for all 12 DESs.



(a) ***(б)***

Fig. 5. *Extraction of L-lactic acid:*
(a) *extraction efficiency;* ***(б)*** *distribution ratio*

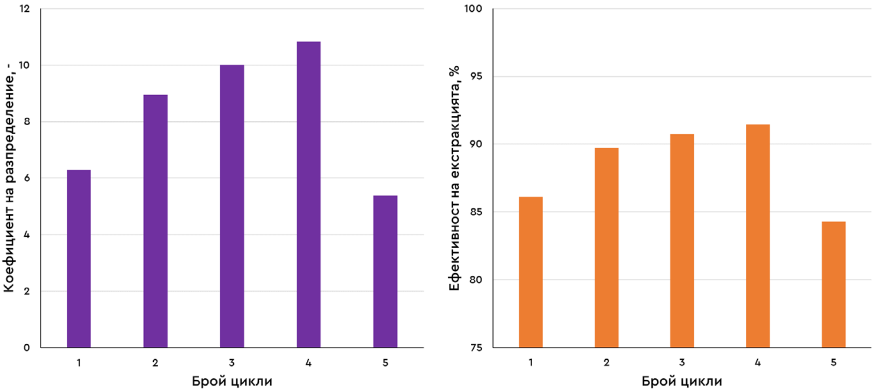
A variation in extraction efficiency (Figure 5 (a)) from 43% (for M-THA 1:2) to 86% (for M-DOA 1:2) was observed. The DES with the best results, formed from DOA and menthol, achieved efficiencies of 82-86%. DES which achieved the best K_D values (around six) – M-DOA 2:1 and M-THA 2:1. Most K_D values are between 1.5 and 4.5.

The lactic acid re-extraction of is performed with NaOH. The re-extraction efficiency is between 70 and 95%. The second re-extraction can separate only about 1–2% more. The data for the first and second re-extractions are presented in Table 2, which shows the most effective DESs.

Таблица 2. Re-extraction of L-lactic acid from the most effective DES.

ДЕР	**Съотношение на компонентите**	**Първа реекстракция, %**	**Първа и втора реекстракция, %**
M-ДОА	2:1	95	97
M-TXA	1:2	86	86
M-TXA	2:1	94	95

To study the stability of repeated use of DES as an extractant, five consecutive cycles of extraction and re-extraction with DES M-DOA 2:1 were performed. The same portion of DES was used for extraction after re-extraction and washing. Figure 6 shows that DES is stable and that the distribution coefficient increases from the first to the fourth cycle. Explanation of the statement is the additional sites for bonding between the LA molecule and those of the already extracted acid..



(a) ***(b)***

Fig. 6*. Consecutive extraction of L-lactic acid with DES M-DOA 1:2:*
(a) *distribution coefficient;* ***(б)*** *extraction efficiency.*

The results presented so far show that the 12 DESs synthesised and characterised from the menthol-amine system were successfully used to extract L-lactic acid from aqueous solution.

The results obtained with the 2:1 menthol-dioctylamine DES show the highest Kd of 6.2 and 86% extraction of the initial amount of the substance. The Lactic Acid-loaded DES can be successfully regenerated with NaOH. The eutecitc solvent is stable and can be used in at least five consecutive cycles of extraction and re-extraction without any change in efficiency. These results may serve as a basis for further research into the use of DES in the extraction of lactic acid from real fermentation broth.

1.3. DES menthol-salicylic acid as an extractant for separating the isomers of butenedioic acid.

The results from L-lactic acid extraction served as an impetus for the study of new DESs for carboxylic acid extraction.

The literature review showed that a hDES based on menthol and salicylic acid in a 4:1 molar ratio has been used as an extractant for heavy metals in various cases reported in four scientific publications. Figure 7 shows the stable DESs obtained with menthol and salicylic acid in ratios of 4:1, 5:1, and 6:1.

***Fig.** 7. Photo of the synthesised DESs with menthol and salicylic acid in ratios of 4:1, 5:1, and 6:1.*

Density

Examinations of the density of the menthol-salicylic acid (M-SA) system as a function of temperature showed that it varies linearly, as with other known liquids. The density decreases with increasing temperature over the range of 20 to 50°C in the studied DESs. Figure 8 presents the linear dependencies obtained.

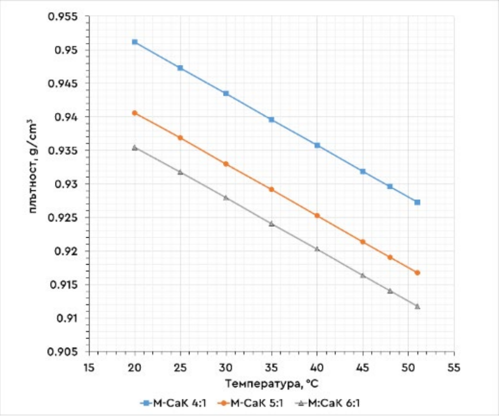


Fig. 8. *Density of the DES mentol-salicylic acid (M-Sac) with temperature.*

According to the literature, the density of the synthesised DES, menthol-salicylic acid (4:1), is *0.9511 g/cm3* at 25°C. The density of the synthesized DES M-SAc 4:1 for this work at a temperature of 25°C is *0.9473 g/cm3*. Comparing the obtained data, the difference between them is slight – 0.4%. The literature confirms the reliability of the data obtained and presented here for DES M-SAc 4:1.

Dynamic viscosity of the DES

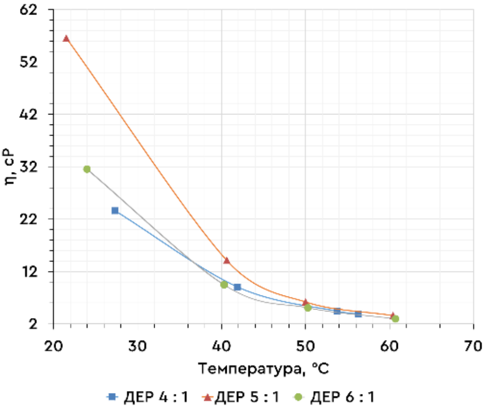


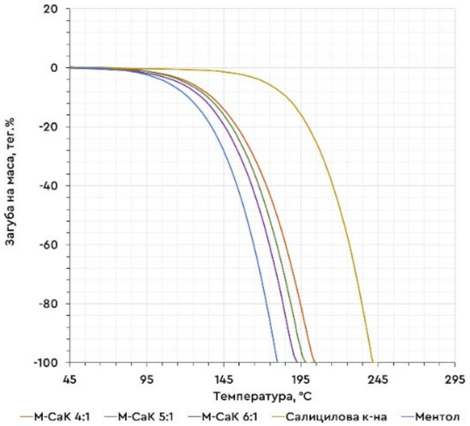
Fig. 9. *Dynamic viscosity (η) of DES M-SAc with temperature*

Figure 9 presents the curves of dynamic viscosity for the obtained eutectic mixtures, which show an unusual distribution. The 5:1 solution has the highest viscosity index, which, with increasing temperature, approaches the other two eutectic systems. The dynamic viscosity readings in the 4:1 and 6:1 systems are quite close – the eutectic mixture with a molar ratio of 5:1 has the highest viscosity. Below is the DES 4:1, and the lowest viscosity is found in the 6:1 system. However, as shown in Figure 9that, no pattern can be found according to which the curve for the 5:1 system is as shown in . An increase in the solutions's menthol content increases viscosity.

The information about the viscosity of menthol allows us to form a hypothesis that could explain the sharp change in the dynamic viscosity readings for the 6:1 system: due to the imperfection in the thermoregulation system of the rheometer used, it is possible that a solid phase was formed, which would increase the viscosity in the temperature range of 20-25°C.

Thermogravimetric analysis of the M-SAc DES.

The thermal decomposition of individual substances and the three DESs (4:1, 5:1, 6:1) were studied by thermogravimetric analysis (TGA). Figure 10 illustrates the results for the dependence of mass loss (in weight %) on increasing temperature at a constant rate.



Фиг. 10. *Curves of the thermal decomposition of substances from TGA analysis of DES M-SAc (4:1 in orange; 5:1 in green; 6:1 in purple).*

The thermal decomposition curves of the individual substances show that the decomposition of menthol and salicylic acid differs significantly. At 180°C, menthol decomposes completely, whereas for salicylic acid complete decomposition occurs at 242°C. In eutectic solutions, a shift in the decomposition temperature with increasing menthol concentration is clearly observed. Increasing the amount of menthol in the solution brings the decomposition temperature closer to that of the same individual substance.

Figure 10 shows that the decomposition of the individual components: at 100°C, menthol starts to decompose and ends around 180°C, while for salicylic acid it begins above 145°C and ends at 240°C. Table 3 presents the decomposition temperature (Tdeg) and degree of decomposition of the eutectic solutions.

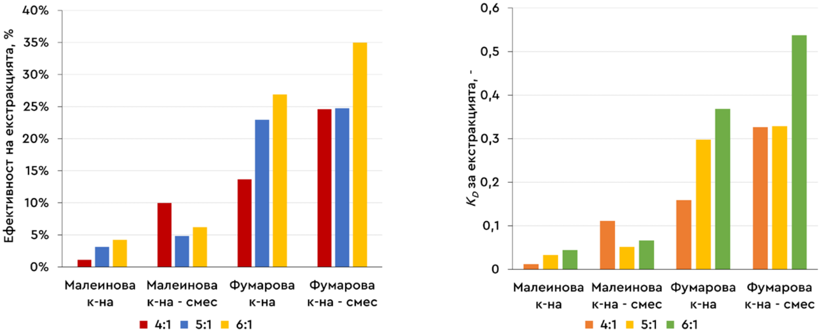
Table 3. Thermal properties of the individual components and their eutectic mixtures.

Чисто вещество	T, °C	ДЕР	T_{deg}, °C	Първичен етап на разлагане, %
L-ментол	180	М/СаК 4:1	204,5	Не се наблюдава
Салицилова киселина	242	М/СаК 5:1	198	
		М/СаК 6:1	193	

Analysis of the thermal curves of the eutectic mixtures of menthol/salicylic acid reveals that the decomposition process occurs at much higher temperatures compared to the decomposition of menthol, but at lower temperatures than the decomposition of salicylic acid. The above supports the hypothesis of the formation of a eutectic solution between menthol and salicylic acid.

1.4. DES M-SAc as an extraction solvent for separating the butenedioic acid cis-trans isomers.

The extraction and re-extraction of cis-trans butenedioic acid isomers — maleic and fumaric acids — is not as efficient as with DES menthol-amine. However, Figure 11(a) shows that the extraction efficiency ranges from 1% to 35%. The highest efficiency is achieved by DES 6:1 when extracting fumaric acid from the total solution with maleic acid. The results show that the other two eutectic systems (4:1 and 5:1) in the same case have extremely close values: 24.6% for 4:1 and 24.73% for 5:1.



(a) ***(b)***

Фиг. 11*. DES M-SAc extraction of maleic and fumaric acids:*
(a) *extraction efficiency;* ***(b)*** *distribution ratio*

The efficiency of fumaric acid extraction from its individual solution is lower compared to its extraction from the solution of both acids. Extraction efficiency increases with increasing menthol fraction in the eutectic mixture. Here, again, DES 6:1 shows the highest extraction efficiency (26.9%) (Fig. 16(a))..

Maleic acid is poorly extracted (1-4%, figure 11(a)) from its individual solution with all the M-SAc DES. There is a tendency for extraction to increase with increasing menthol proportion in the eutectic mixture, as shown in Figure 11(a). When the maleic acid is extracted from the mixed solution with fumaric acid doesnt follow any trends. The results show that the extraction of maleic acid from DES 4:1 is 9.97%, from DES 5:1 it drops to 4.85%, and from DES 6:1 it increases again to 6.2%. There is currently no suitable explanation for the variation in maleic acid extraction from the mixed solution.

The distribution coefficient (*KD*) varies from 0.01 to 0.5 depending on the ratio of the components in the eutectic solvent (Fig. 16 (b)). The best Kd values were obtained for the extraction of fumaric acid from the mixed solution using DES – 6:1. The DES 5:1 and 4:1 have similar *KD* values: for 5:1 – 0.328; for 4:1 – 0.326. For *the KD* of fumaric acid from an individual solution, the distribution between the phases increases with an increase in the proportion of menthol in the eutectic system: from 0.158 for 4:1, through 0.297 for 5:1, to 0.367 for 6:1.

The distribution of maleic acid from the mixed solution between the phases, expressed by KD, shows the same trend as in the case of process efficiency. KD is highest in the 4:1 system (0.11), then drops in the 5:1 system (0.051) and rises again in the 6:1 system (0.066). Meanwhile, in the individual solution of maleic acid, there is a tendency for KD to increase with an increase in the molar ratio of menthol in the eutectic system.

With a single extraction of the butanedioic acid solution, with pH = 1.8, the extraction coefficient *D* for:

- $D_{(fumaric\ acid)} = 0{,}51$, $K_D = 0{,}54$
- $D_{(maleic\ acid)} = 0{,}038$, $K_D = 0{,}066$

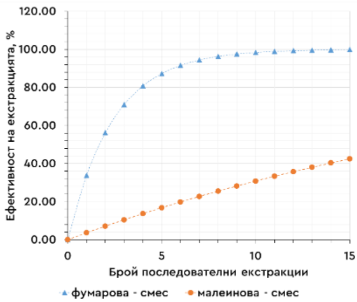
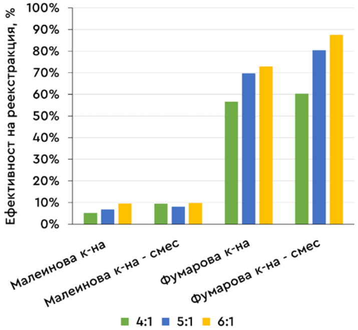


Fig. 12. *Theoretical efficiency of consecutive extraction of maleic and fumaric with DES M-SAc 6:1*

The *D* values obtained are close to those from the experimental data.

Figure 12 shows theoretical efficiency of concsecutive extractions for the 6:1 DES, according to the organic phase. With 11 consecutive extractions, fumaric acid would pass completely into the organic phase, and maleic acid – 33.32%

Figure 13 and Table 7 present the efficiency of re-extraction process. Significantly higher efficiency is observed in the re-extraction process compared to extraction. Once again, fumaric acid shows better results when extracted from the extractant. When extracting the individual solution of fumaric acid from DES, the efficiency is between 56-72%, and the indicator increases with the increase in the menthol content in the eutectic mixture.



Фиг. 13. *Reextraction efficiency of maleic and fumaric acid from the studied DES M-SAc*

The re-extraction of fumaric acid from the mixed solution has a higher efficiency (60-87%) and increases further with increasing menthol content in the DES. For maleic acid, the extraction efficiency from its individual solution varies from 5-9%, and from the mixed solution it ranges from 8-9.8%. When extracting maleic acid, it is observed that the extraction capacity from the individual solution increases with the increase in the proportion of menthol in the DES, while the extraction capacity of maleic acid from the mixed solution is apparently slightly affected by the amount of menthol in the DES.

Table 4. Reextraction of the cis-trans isomers of the butenedioic acid with the studied DES M-SAc.

DES	M : SAc	Reextraction of the individual components, %	Reextraction from the mixed, %
M-SAc	4:1	Maleic acid – 5,17	9,45
		Fumaric acid – 56,61	60,34
	5:1	Maleic aicd – 6,74	8,11
		Fumaric acid – 69,74	80,42
	6:1	Maleic acid – 9,55	9,85
		Fumaric acid – 72,9	87,5

The physical properties of the synthesised deep eutectic solvent (DES) composed of menthol and salicylic acid have been detailed. The extraction results demonstrate that DESs with ratios of 4:1, 5:1, and 6:1 effectively extract the isomers of butenedioic acid. Among these, DES 6:1 exhibits the highest extraction efficiency for fumaric acid, achieving a 35% extraction rate from a mixed solution of butenedioic acid isomers. Additionally, DES 6:1 displays the highest extraction capacity, with a distribution coefficient (KD) of 0.5. In contrast, maleic acid is extracted poorly by all tested DESs, with extraction efficiencies remaining below 5%. However, DES 4:1 stands out by extracting maleic acid from the total solution with a 10% efficiency. All DESs can be successfully regenerated using NaOH, but DES 6:1 is particularly effective during re-extraction, yielding the most significant proportion of fumaric acid. Notably, there is a clear trend showing that the efficiency of the extracted acids decreases as the molar fraction of menthol decreases during re-extraction.

These results are encouraging for the future application and practical significance of DES menthol-salicylic acid in the extraction of heavy metals. The possible separation of the butenedioic acid isomers complements the already published results. Based on the obtained results, the study of the applied aspects of the DES menthol-SAc solvent as extractants or membrane phase should continue.

2. Changes in the content of carboxylic acids and sugars during nanomembrane filtration for the dealcoholization of red wine from the Mavrud variety

2.1. Concentration mode of operation

The process efficiency during the experimental work was assessed by analysing the amount of substances retained by the membrane. Therefore, it is possible to describe the effect of pressure and the applied operating regime during the process on the change in the composition of Mavrud wine — for the identified carboxylic acids and sugars.

The retention of the various groups of compounds in Mavrud wine for each of the membrane brands used is shown in Figures 14 (Alfa Laval NF99HF); Figure 15 (Nadir NP030P); Figure 16 (Alfa Laval RO99).

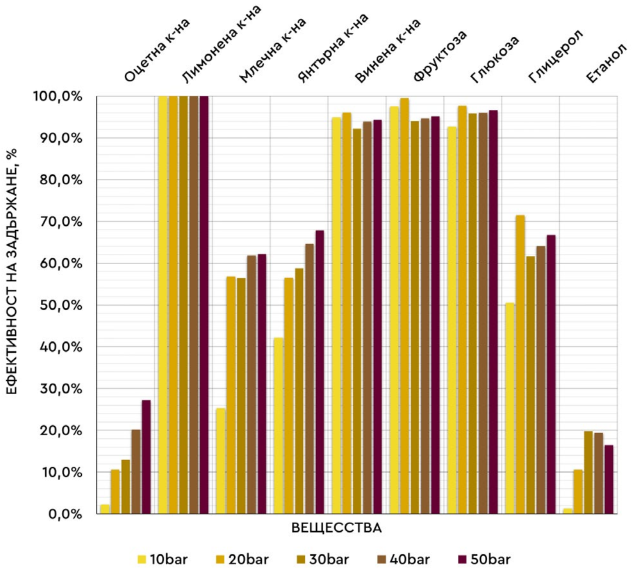


Fig. 14. *Retention (%) of carboxylic acids and sugars from Mavrud red wine during nanofiltration in concentration mode, varying the transmembrane pressure. Alfa Laval NF99HF membrane.*

As shown in Figure 14, the range of transmembrane pressure (TMP) applied to the Alfa Laval NF99HF membrane is 10 bar, determined by the membrane's morphology. The NP030P and RO99 membranes were also tested at pressures of 10 and 20 bar, but no permeate flow was observed. This fact suggests the absence of similar results for these membranes. Regardless, Fig. 20 shows the results of NF99HF membrane retention at a TMP of 10 bar.

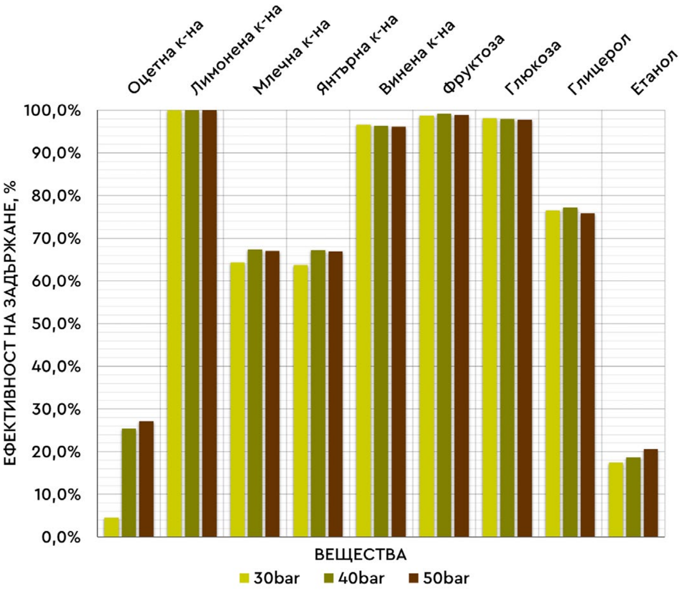


Fig. 15. *Retention (%) of carboxylic acids and sugars from Mavrud red wine during nanofiltration in concentration mode, varying the TMP. Membrane: Nadir NP030P.*

As shown in Figure 15, the applied TMP on the Nadir NP030P membrane starts at 30 bar. The NP030P and RO99 membranes were also tested at pressures of 10 and 20 bar, but no permeate flow was observed.

The histograms in Figures 15 and 16 show that for nanofiltration membranes (NFM), the highest retention values observed (above 90%) are for the following substances: citric acid, tartaric acid, glucose, and fructose, with approximately 60% of the remaining low molecular weight compounds being rejected by the membrane. Acetic acid and ethanol show lower retention with nanofiltration membranes.

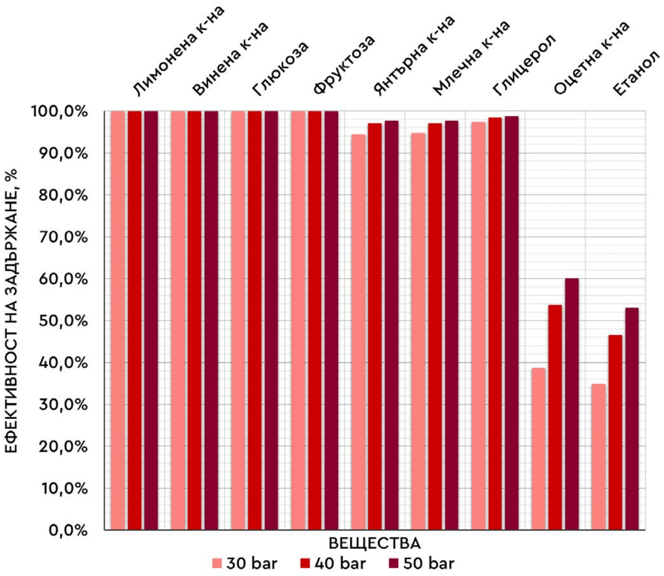


Fig. 16. *Retention (%) of carboxylic acids and sugars from Mavrud red wine during reverse osmosis in concentration mode, varying the TMP. Membrane: Alfa Laval RO99.*

The Alfa Laval RO99 reverse osmosis membrane shows higher retention (Figure 16), exceeding 80% for all compounds except ethanol and acetic acid, which is clearly due to the membrane's pore size. The reverse osmosis membrane has the smallest pores among those used, allowing better retention of most substances in wine.

2.2. Constant volume diafiltration mode of operation followed by a reverse osmosis.

The experimental studies conducted with the exact Mavrud wine show that ethanol and high-molecular bioactive compounds are better retained with diafiltration (dia-NF) than with concentration mode of operation. The results presented (Figure 17) confirm and complement the findings of these studies. The observed retention coefficients for organic acids and sugars present in the studied Mavrud wine are significantly higher when using a single-stage diafiltration regime or a two-stage regime (diafiltration followed by reverse osmosis (dia-NF-RO)).

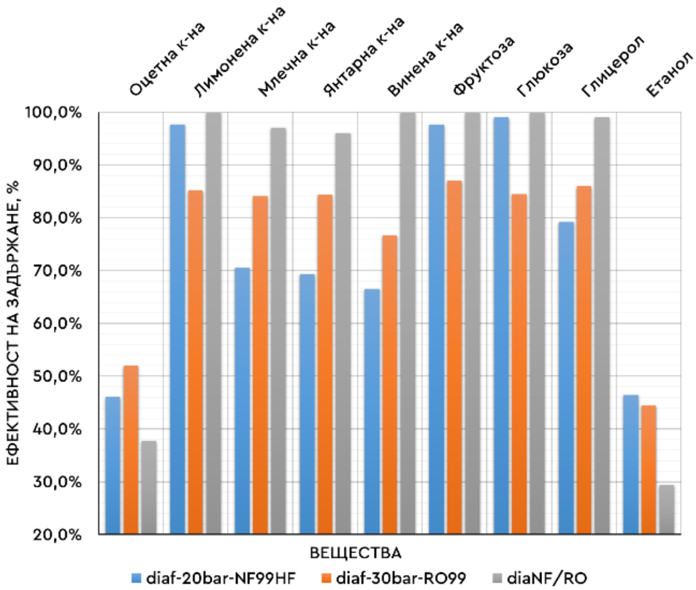


Fig. 17. *Comparison of the retention (%) of substances in Mavrud wine after diaNF – (with NF99HF and RO99 membranes) and dia-NF/RO.*

The histograms (Figure 17) clearly indicate that carboxylic acids and sugars exhibit a high rejection rate (Rej > 80%), except for ethanol and acetic acid. Similar results are found in the filtration of white wine. In terms of high permeate flow and adequate retention, the NF99HF membrane is most suitable for wine dealcoholization in diafiltration mode.

A comparative analysis of the individual membranes reveals an increase in retention coefficients when moving from NF to RO, both in single-stage diafiltration with constant volume and in sequential NF/RO processes under identical operating conditions. In the latter arrangement, the first stage employs NF99HF at 20 bar in diafiltration mode, while the second stage utilizes RO99 at 30 bar to process the permeate stream following nanofiltration, effectively separating the ethanol. The composition and concentrations of the inlet stream in the second filtration stage significantly differ from those of the original wine. The retention rates for lactic, succinic, and tartaric acids demonstrate good performance, with rejection rates (Rej) exceeding 80%, while fructose and glucose show even higher retention with Rej above 90%. In contrast, acetic and citric acids exhibit lower retention rates, with Rej falling below 50%. Overall, in the framework of the two-stage filtration system—

where the final products are the retentate from the first stage and the permeate from the second stage—the observed rejection rates range between 90-100% for all compounds, with the exception of acetic acid and ethanol.

The type of nanofiltration membrane directly affects the degree of retention of organic acids and sugars in wine. NP030P and NF99HF nanofiltration membranes show 90% retention of citric acid, tartaric acid, glucose, and fructose. The other wine components studied: lactic acid, succinic acid, and glycerol are retained by the membrane by ≥ 60%. The NF99HF membrane from Alfal Laval retains the highest percentage of glucose, fructose, citric acid, and tartaric acid, which are essential for the taste characteristics of wine.

The reverse osmosis membrane RO99 (by Alfal Laval), showed higher retention levels above 80% for all compounds. An exception is observed in the retention of ethanol and acetic acid. Since ethanol retention is important for dealcoholization, our results show the lowest retention with the NF99HF membrane, followed by the NP030P and RO99 membranes. The latter means that the main difference in the retention coefficients of ethanol and other compounds observed in wine with the NF99HF membrane is a favourable condition for conducting diafiltration to separate ethanol.

The two-stage diafiltration (dia NF/RO) of wine is based on results obtained in concentration and diafiltration modes with the membranes studied, selecting conditions that minimise membrane clogging and provide a comparable permeate flow rate in both stages. This filtration configuration allows the highest retention rates of organic acids and sugars contained in the wine to be observed compared to the final permeate. The results obtained may be of practical value in the production of Mavrud wine with controlled alcohol content. The membrane nanofiltration process preserves the unique content of tannins, anthocyanins, and polyphenols.

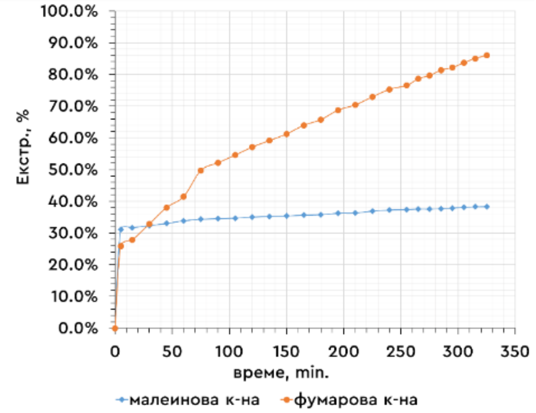
In the ranges examined under experimental conditions utilizing nanomembrane filtration technology in both concentration and diafiltration modes, no substantial differences were found in the membrane retention coefficients. Alongside the consistency of retention values across both filtration modes, transmembrane pressure also influences rejection coefficients. In the majority of cases involving wine filtration, an increase in pressure typically results in a corresponding rise in rejection coefficients and pore clogging, as detailed in the experimental section.

3. Butenedioic acid isomers separation by pertraction with a bulk liquid membrane ina rotary disk pertractor

Пертракцията на изомерите на бутендиовата киселина

The plots in Figure 18 show the results of three experiments on the separation of butenedioic acid isomers by fermentation. The conditions of the experiments were as follows:

- liquid membrane 1-decanol, the feed solution has a pH of 1.8 for brevity – **Experiment I.** Figure 18 (a), colored in blue and orange.;
- dodecanol liquid membrane, the feed solution has a pH of 1.8 – Figure 18 (b), colored gray and green. For brevity – **Experiment II**;



(a)

Fig. 18. (a). *Pertraction separation of the maleic and fumaric acid isomers – kinetics (**Experiment I**).*

- liquid membrane 1-decanol, the feed solution has a pH of 10.65, for brevity – **Experiment III**. Figure 18 (b) – colored in blue and orange.

An analysis of the extraction results presented in Figure 18(a) indicates that in **Experiment I**, fumaric acid is extracted predominantly, with a yield exceeding 80%. In addition, maleic acid is also extracted from the feed phase, starting at approximately 30% and not exceeding 40% by the conclusion of the experiment.

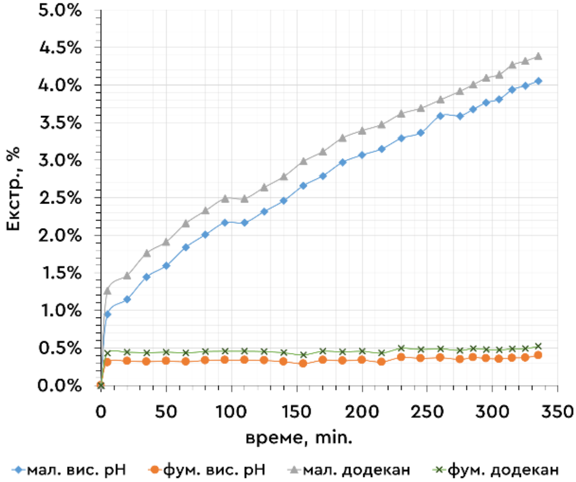
At a pH of 1.8, the degree of electrolyte dissociation in fumaric acid is $\alpha_{fum} < 0.05$, indicating that the majority of its molecules remain undissociated. This suggests a greater formation of hydrogen bonds with the weakly polar solvent 1-decanol. In contrast, maleic acid at the same pH exhibits $\alpha_{mal} > 0.4$, signifying a higher proportion of dissociated molecules, which consequently limits its transport across the membrane.

The receiving solution induces enhanced extraction from the membrane by facilitating the formation of a stronger ionic bond between the alkaline solution and the fumaric acid that has accumulated within the membrane phase. This interaction underscores the significance of the chemical dynamics at play in the extraction process.

Additional information on the initial and final concentration values in the process is presented in Table 5.

Table 5. Initial and final concentrations of the butendioic acid isomers for the according to the phase they are in. **Experiment I**, 1-decanol is used as a membrane.

Solution	Component	Initial conc. – g/L	Final conc. – g/L	E (%)
Feeding	Малеинова киселина	5,0086	4,6093	-
	Фумарова киселина	4,6964	0,8095	
Recieving	Малеинова киселина	0	1,9196	38,33%
	Фумарова киселина	0	4,038	85,98%



(б)

Fig. 18 (b). *Pertraction separation of the maleic and fumaric acid isomers – kinetics (**Experiments II and III**).*

The findings from **Experiments II** and **III** exhibit a striking similarity, as illustrated in Figure 18-b. In both cases, maleic acid is predominantly extracted. Notably, despite variations in membrane type and feed solution pH, the observed curves exhibit a remarkable degree of consistency. This suggests that the extraction process is robust across different experimental conditions.

The kinetic curve for **Experiment II** (Figure 18(b), grey and green) shows that using the nonpolar dodecane membrane does not yield a highly efficient extraction process. However, it should be noted from Figure 18b that maleic acid is predominantly extracted (in grey). Table 6 provides additional information on the concentrations of the substances at the beginning and end of the process, as well as on their extraction into the receiving solution.

Table 6. Initial and final concentrations of the butendioic acid isomers for the according to the phase they are in. **Experiment II**, n-dodecane is used asa membrane.

Solution	Вещество	Initial con. – g/L	Final conc. – g/L	E (%)
Feeding	Maleic ac.	5,1292	5,1292	-
	Fumaric ac.	3,9638	3,9499	
Recieving	Maleic ac.	0	0,2249	4,38%
	Fumaric ac.	0	0,0207	0,52%

At alkaline pH, the two isomers are almost completely dissociated and pass into the membrane phase in minimal quantities. Extraction efficiency is low, and selectivity is practically absent.

The kinetic curves match those from experiment II, even though the membranes are different in experiment III (Figure 18(b), blue and orange). This coincidence clearly demonstrates that the degree of dissociation is the dominant factor:

- at low pH, selectivity is determined by molecular geometry;
- at high pH values, dissociation is almost complete; therefore, mass transfer of ions through the hydrophobic phase is strongly suppressed.

Table 7 presents the initial and final concentrations from **experiment III**.

Table 7. Initial and final concentrations of the butendioic acid isomers for the according to the phase they are in. **Experiment III**, 1-decanol is used asa membrane.

Solution	Вещество	Initial con. – g/L	Final conc. – g/L	E (%)
Feeding	Maleic ac.	5,3161	5,0531	-
	Fumaric ac.	4,7952	4,5587	
Recieving	Maleic ac.	0	0,2135	4,05%
	Fumaric ac.	0	0,01922	0,40%

In all experiments, the rotation speed of the contact devices (discs) was constant. The rotation speed of the discs was set at 10 min-1. Increasing the angular velocity ruptures the water-phase film that forms on the hydrophilic disc. The disruption of this film at higher speeds leads to the formation of an emulsion, which disrupts the process's steady state and changes the device's operating mode.

Two factors are most important in the separation of butenedioic acid isomers: the membrane used and the pH of the feed solution. The weakly polar membrane of 1-decanol and its low pH (1.8) yield the best results for separating maleic acid from fumaric acid. At the end of the process, over 80% of the initial amount of fumaric acid is extracted. The extraction of maleic acid increases slightly until the middle of the time interval and then reaches a steady state.

Conclusions

Based on the experimental studies conducted and the subsequent analysis of the results obtained, along with a comprehensive summary of the influence exerted by various factors on the extraction and membrane processes, several pivotal conclusions can be articulated:

1. The primary correlations identified pertain to the molar ratios in deep eutectic solutions (DES) formulated with menthol and their corresponding physical and thermal properties. These correlations indicate the formation of stable associative structures within the DES, highlighting the influence of compositional ratios on the behavior and characteristics of these solutions.
2. It was proven that DESs menthol–amine exhibit high extraction capacity for L-lactic acid, with dioctylamine (DOA) and trihexylamine (TCA) solvents proving to be the most effective.
3. A selectivity of DER menthol–salicylic acid for fumaric acid (trans-) has been observed, while the extraction of the geometric isomer, maleic acid (cis-), occurred to a limited extent. This indicates a differential interaction between the DER menthol–salicylic acid complex and these two isomeric forms of dicarboxylic acid.
4. The results show that during pertraction, selectivity towards fumaric acid is preserved only under acidic conditions, while under alkaline conditions, dissociation completely limits mass transfer.
5. The impact of various membrane operational modes on the retention of organic acids, sugars, and alcohols in Mavrud wine was evaluated. The study identified that the highest selectivity was achieved through the concurrent implementation of diafiltration and reverse osmosis (designated as dia-NF/RO).

Contributions

This dissertation advances the field of extraction and membrane technologies through the exploration of novel hydrophobic deep eutectic solutions, their extraction capabilities, and the potential of membrane processes for selective separation and dealcoholization. The primary scientific contributions can be outlined as follows:

1. Hydrophobic deep eutectic solvents (DES) based on menthol, combined with organic amines and salicylic acid, were synthesized and characterized from a physicochemical standpoint.
2. The DES thermal, rheological, and structural characteristics relevant to their application as extractants were determined.
3. The extraction capacity of the synthesised menthol-amine DEPs towards L-lactic acid was investigated.
4. The research established correlations between the type of amine, the molar ratios in the DESs, and the extraction efficiency/distribution coefficient.
5. The study determined the re-extraction capacity of the most effective DES menthol-amine. It demonstrated that the M-DOA and M-THA systems achieve high desorption rates and maintain stable parameters during repeated use.
6. The M-DOA and M-THA systems allow a high degree of desorption and maintain stable parameters during repeated use.
7. A theoretical model of sequential extraction has been developed and applied using experimentally determined K_D values, which predicts the behaviour of systems during multiple cycles.
8. The extraction capacity of DES menthol–salicylic acid (M–SAc) towards the geometric isomers of butenedioic acid — maleic and fumaric — was investigated. A distinct selectivity towards the trans isomer (fumaric acid) and dependence on the molar ratios in DES were established.
9. The different distribution mechanism of the two isomers, related to the geometry of the molecules, the possibilities for hydrogen bonding, and the hydrophobic interaction with the organic phase, was confirmed.
10. DES menthol–salicylic acid can serve as a selective extractant for separating geometric isomers, thereby expanding the application of this system beyond its previously documented use for extracting heavy metal ions.
11. The efficiency of the pertraction process for separating butenedioic acid isomers using different membrane phases (1-decanol and dodecane) and varying pH values of the feed phase. The 1-decanol membrane provides the highest selectivity for fumaric acid at pH = 1.8 of the feeding solution, which aligns with the results of liquid-liquid extraction.
12. The research shows that in an alkaline environment (pH = 10.65) the per-traction process is strongly suppressed due to the almost complete

dissociation of the two acids and the limited distribution in the hydrophobic membrane.

13. The kinetic behavior of the per-traction process was studied, and the influence of the membrane phase structure and the degree of dissociation on mass transfer was established.

14. The behavior of industrial nanofiltration and reverse osmosis membranes (NF99HF, NP030P, RO99) during the filtration of Mavrud wine in concentration mode. The retention coefficients of main organic acids, sugars, ethanol, and glycerol were determined.

15. Patterns in membrane selectivity were established, including a clear distinction between high-molecular-weight and low-molecular-weight components, which is an important parameter for the dealcoholization process.

16. The behaviour of the membranes in dia-NF/OO mode was demonstrated. It was proven that this process leads to a significant reduction in alcohol with minimal losses of organic acids and sugars.

17. The influence of transmembrane pressure on retention and mass balance was determined for different membrane configurations in a component-rich matrix such as red wine from the Mavrud variety.

18. Practical guidelines were proposed for the application of membrane processes in the partial dealcoholization of red wines, based on experimental data on the selectivity, flow, and stability of the membranes.

List of the author’s scientific papers part of the dissertation.

1. Ivanova, D.; Apostolov, A.; Tuleshkov, P.; Novakov, C.; Yankov, D. Menthol-Based Hydrophobic Deep Eutectic Solvents as a Tool for Lactic Acid Extraction, New *Appl. Sci.* 2025, *15*, 3564. https://doi.org/10.3390/app15073564 **Q2. Cited by:** 10.1016/j.jece.2025.120147.

2. Apostolov, A.G.; Tsibranska, I.; Yankov, D.; Dencheva-Zarkova, M.; Genova, J. Bulgarian Mavrud Wine Under Nanofiltration and Reverse Osmosis: Evaluating the Composition After the Process. *Chemistry* **2025**, *7*, 134. https://doi.org/10.3390/chemistry7040134, **Q2**

3. Kostanyan, A.E., Milevskii, N.A., Klychevskikh, Y.A. *et al.* On the Operation of a Cascade of Mixing and Settling Extractors in the Modes of Chromatography and in Free Liquid Membranes. *Theor Found Chem Eng* **57**, 764–769 (2023). https://doi.org/10.1134/S004057952304019X, **Q4**