Deep eutectic solvents for deacidification of waste biodiesel feedstocks: an experimental study

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Abstract

The key challenge in reaching the goals set by Renewable Energy Directive is developing a sustainable, profitable and environmentally acceptable biodiesel production process. In order to achieve balance between the above criteria, any use of high-quality edible oils as feedstocks needs to be avoided and utilization of waste feedstocks, such as used coffee grounds and waste animal fats, should be encouraged. The main drawback of these waste feedstocks is their high impurity content which usually requires an additional purification step.

It is the purpose of this research to investigate the deacidification of three different waste biodiesel feedstocks by means of liquid-liquid extraction with deep eutectic solvents, in order to identify possible connections between solvent properties and extraction efficiency. Eight deep eutectic solvents were chosen to cover a wide range of different properties and the three used feedstocks varied in free fatty acid content.

The relationship between solvent properties and extraction efficiency was determined by Spearman's rank-order correlation. Strong, statistically significant positive correlation was found for solvent pH values, while a strong negative correlation was observed for polarities and molar volumes. The most effective solvent was potassium carbonate : ethylene glycol (1:10, mol.). Depending on the initial total acid number, solvent to feedstock mass ratios 0.1:1 and 0.25:1 were enough to reduce the acidity of waste animal fat below 2 mg of potassium hydroxide / g fat and the solvent was successfully reused up to four times.

Key words:

deacidification; deep eutectic solvents; free fatty acids; liquid–liquid extraction; sustainable biodiesel feedstock

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1. Introduction

Biodiesel is produced by a transesterification reaction during which the oil or fat reacts with an alcohol in the presence of a catalyst, and the corresponding alkyl esters of the fatty acids present in the feed are obtained. The reaction may be carried out with homogeneous or heterogeneous catalysts, wherein the homogeneous catalyst may be alkaline or acidic, and heterogeneous catalysts may be solid catalysts or enzymes [1]. However, such a reaction mechanism is very sensitive to the purity of raw materials, or the presence of water and free fatty acids. The use of high-quality edible oils as feedstock for biodiesel production results in ethical doubts as well as in an increase in the price of biodiesel. The increase in global demands for the production of biodiesel results in increasing interest for the use of waste materials of plant and animal origin or non-edible oils [2].

The quality of biodiesel largely depends on the quality of feedstock. When produced from high quality vegetable oil, it can easily satisfy the requirements of standard quality. On the other hand, when low grade feedstock is used, it needs to be purified in order to fulfil the conditions necessary for base catalyzed transesterification reaction. Based on the type of impurity present in feedstock, different purification methods can be utilized. Solid particles found in low grade animal fats or used frying oil can be easily removed by filtration. Waste frying oil may also contain water that can be separated by evaporation. Moreover, different types of oils and fats may contain too high concentrations of metals that can be removed by adsorption or extraction [3]. However, the most common reason for purification of oils and fats is too high concentration of free fatty acids (FFA). For base-catalyzed transesterification, concentration of FFAs in the feedstock should be less than 1% in order to avoid saponification [4]. Several methods for the lowering of the FFA concentration are known and used on the industrial scale. These methods can be classified in three major groups: physical, chemical and miscella deacidification [5]. Chemical deacidification is an alkali neutralization in which sodium hydroxide reacts with FFA to produce soaps [6]. Major disadvantages of this method are high oil loses, the need for further separation step for removal of soapstock, high quantity of wastewater produced and lower oxidative stability of oil. In physical deacidification, FFAs are removed from oils by steam distillation under vacuum. Even though the process is environmentally more acceptable than chemical deacidification since it is carried out without use of chemicals, formation of polymers or transisomers and decomposition of oil may occur. The process is also economically unfavorable due to high operating cost of production of steam. Miscella deacidification involves mixing oil with hexane and then neutralization with sodium hydroxide followed by reaction with phosphatides. This method also has its drawbacks, such as high equipment and operating costs and the necessity to remove the solvent in several steps. The selection of method depends on the FFA concentration. Oils and fats with high concentration of FFA have to be refined by physical or miscella deacidification. The disadvantages of commercial methods have motivated researchers to investigate some alternatives. Alternative methods proposed in the literature are: supercritical fluid extraction, membrane processes, liquid-liquid extraction, biological deacidification (selective removal of FFA with microorganisms or enzymatic deacidification/reesterification) and reesterification [7].

Among the proposed alternative methods, liquid-liquid extraction has very high potential due to its mild operating conditions (room temperature and atmospheric pressure). The most important step here is a proper selection of solvent used for extraction, which needs to satisfy several requirements. A highly selective non-toxic solvent with low viscosity and volatility, which can be regenerated, would be the best choice. Deep eutectic solvents emerge as a promising alternative to conventional organic solvents. They are easily prepared from two or three environmentally acceptable and inexpensive compounds bound together by hydrogen bonds [8]. The use of deep eutectic solvents (DESs) was investigated in various processes because of their outstanding properties, the most important of which are: simple preparation from environmentally friendly raw materials, non-volatility, thermal and chemical stability, non-toxicity, biodegradability and non-flammability, as well as capability to dissolve different types of organic, inorganic and polymeric compounds [9]. It is important to emphasize the possibility of adjusting the properties by simple combination of different salts and hydrogen bond donors. Due to negligible environmental impact and simple and inexpensive preparation, DESs are interesting both on the laboratory research and industrial level [10]. Their utilization for biomass pretreatment, namely for dissolution of cellulose, lignin and chitosan, as well as for extraction of proteins, phenols, aldehydes and ketones was explored [11]. In the process of biodiesel production, DESs were investigated as catalysts for transesterification and for extraction of glycerol, glycerides and leftover catalyst from crude biodiesel [12].

The research on extractive deacidification with deep eutectic solvents is still in its infancy. So far, deacidification of palm oil using betaine monohydrate-based deep eutectic solvents has been explored by Zahrina et al. [13]. The group did experimental and computational research regarding molecular interactions in the betaine monohydrate-polyol DES [14]. They also investigated molecular interactions between betaine monohydrate-glycerol deep eutectic solvents and palmitic acid [15]. Deacidification of rice bran oil by choline chloride and betaine monohydrate based DESs was done by Zullaikah et al. [16]. In previously published work, Sander et al. used potassium carbonate based DES for deacidification of different coffee ground oils at room temperature and low DES to oil mass ratio (0.1:1) for 30 minutes, after which they synthesized biodiesel from deacidified oils and purified it with a choline chloride based DES [17].

The goal of this paper is to investigate, for the first time, deacidification of three different waste biodiesel feedstocks with eight deep eutectic solvents and to determine if (and which) solvent properties affect deacidification efficiency. The working hypothesis was that deacidification was occurring due to the extraction of free fatty acids from feedstocks and that there could be a solvent property (one or more) that would expedite the process of screening for a selective solvent for this and similar purposes. The solvents were chosen to cover a wide range of different properties while also bearing in mind their price.

2. Experimental methods

2.1.Material

Waste animal fats (WAF1 and WAF2) were obtained from the local company Agroproteinka d.d.. Waste animal fats were melted by heating up at 60 °C and residual solid particles were filtered. Waste coffee

grounds were collected from the local coffee shop. Wet coffee waste was firstly dried in a cabin convective dryer until all water was removed. The moisture content in waste coffee was measured by Kern MLS IR Moisture analyzer at 105 °C to check if the drying was over. The oil (waste coffee grounds oil, WCGO) was then extracted from dry coffee waste in a Soxhlet extractor with *n*-hexane. 200 g of dry coffee waste were put in an extraction thimble and 2000 mL of solvent was heated to boiling temperature. The extraction lasted for 2 hours. Solvent (*n*-hexane) was removed by evaporation under vacuum.

2.2.Chemicals

D-fructose p.a., urea p.a., glycerol anhydrous p.a. and potassium carbonate anhydrous p.a. were purchased from Lach-ner. High purity choline chloride was purchased from VWR Life Science AMRESCO; ethylene glycol p.a. from T.T.T. d.o.o.; citric acid 1-hydrate p.a. was purchased from Gram mol; potassium hydroxide 0.1 mol/l (0.1N) in ethanol from VWR Chemicals; DL-malic acid was purchased from ACROS ORGANICS and diethyl ether p.a. from Honeywell. Chemicals used for preparation of deep eutectic solvents (D-fructose, urea, glycerol anhydrous, potassium carbonate anhydrous, choline chloride, ethylene glycol, DL-malic acid and citric acid 1-hydrate) were vacuum dried at 60 °C for 8 hours before use.

2.3.Preparation of deep eutectic solvents

After vacuum drying, hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) were directly weighed in a round bottom flask and placed on a rotary vacuum evaporator (IKA RV 10, rotary evaporator, basic), equipped with vacuum pump (KNF LAB LABOPORT) (10 mbar and 60 °C) until clear homogeneous liquid was obtained. Prepared DESs are presented in Table 1. Eight DESs used in this work were chosen to cover a wide range of different properties.

Table 1 – Composition of prepared DESs

DES	HBA	HBD	Molar ratio	Molar mass, g/mol
DES 1	Choline chloride	Malic acid	1:1	136.86
DES 2	Choline chloride	Citric acid	2:1	163.13
DES 3	Choline chloride	Fructose	1.5:1	155.84
DES 4	Choline chloride	Glycerol	1:2	107.93
DES 5	Choline chloride	Ethylene glycol	1:2.5	84.23
DES 6	Choline chloride	Urea	1:2	86.58
DES 7	Potassium carbonate	Glycerol	1:6	98.68
DES 8	Potassium carbonate	Ethylene glycol	1:10	68.99

Molar mass of DESs was calculated according to the following equation [18]:

$$M = \frac{X(\mathrm{HBA}) \cdot M(\mathrm{HBA}) + X(\mathrm{HBD}) \cdot M(\mathrm{HBD})}{X(\mathrm{HBA}) + X(\mathrm{HBD})} \quad (1)$$

where: *X*(HBA) and *X*(HBD) are molar ratios of HBA and HBD respectively; *M*(HBA) and *M*(HBD) are molar masses of HBA and HBD respectively.

2.4.Extraction of FFA

Extraction of FFA from oil and fats was performed in a batch extractor equipped with temperature controlled magnetic stirrer (LLG-uniSTIRRER 3, LLG-Labware). All experiments were carried out at mass ratios of DES and oil, *m*(DES)/*m*(oil) = 1 for three hours at 60 °C. After phase separation, purified fats and oils were characterized.

Based on the initial and final total acid number (*TAN*), extraction efficiency was calculated:

$$\varepsilon = \frac{TAN_i - TAN_f}{TAN_i} \quad (2)$$

where ε is extraction efficiency, TAN_i and TAN_f are initial and final total acid numbers of feedstock.

To reduce the amount of solvent used, a set of experiments was conducted with WAF1 where mass ratio of DES to WAF1 was 0.1, 0.25 and 0.5; for 3 hours at 60 °C and 500 rpm. Additionally, experiments were conducted with K_2CO_3, ethylene glycol, glycerol and with DES 5 (choline chloride/ ethylene glycol), for 3 hours, at 60 °C, 500 rpm and at mass ratio of solvent : WAF1 0.1:1, as control experiments, in order to check if the deacidification effects were due to DES or one of its constituents and to check if some other effects would appear at lower mass ratio with DES5.

2.5.Characterization of deep eutectic solvents, oils and fats

Samples were characterized by Fourier-transform infrared spectroscopy (FTIR) and proton nuclear magnetic resonance spectroscopy (^{1}H NMR). FTIR measurements of samples before and after extraction were conducted with a Bruker Vertex 70 spectrometer, in FTIR ATR mode with a range of 4000-400 cm^{-1} (MIR). ^{1}H NMR spectra were recorded in deuterated chloroform on a Bruker Avance 300 spectrometer. ^{13}C APT spectrum was acquired on Bruker Avance Neo 300 spectrometer using a C/H dual 5 mm probe. The spectra were recorded in $CDCl_3$ and TMS was used as the internal standard. Spectra with spectral widths of 18,000 Hz were collected with 256 scans. Digital resolution was 0.27 Hz per point.

Total acid number was determined titrimetrically, according to the standard method ISO 660:2009.

For all prepared deep eutectic solvents, conductivity, pH-value, refractive index and viscosity were measured in the temperature range from 20 to 60 °C, with 10 °C intervals. All instruments were thermostated with JULABO CF41 Cryo-Compact Circulator.

Conductivity and pH-value were measured with WTW inoLab pH/Cond 740 multi-meter. Refractive indices were measured with Exacta Optech RMI Abbe refractometer, with a precision of ±0.0001. Dynamic viscosity was measured with Brookfield DV-III Ultra programmable rheometer. Triplicate measurements were performed for all properties and the average values are reported here. Density data for all used solvents was collected from the literature listed in Table 2.

Table 2. Literature density data

Deep eutectic solvent	Reference
DES 1	[19]
DES 2	[20]
DES 3	[21]
DES 4	[22]
DES 5	[23]
DES 6	[24]
DES 7	[25]
DES 8	[18]

Polarity of deep eutectic solvents was measured with solvatochromic dye Nile Red, with slight modifications [26]. Stock solution was prepared containing 1.0 g/L of Nile Red in ethanol. It was then diluted 100 times in ethanol and mixed with DES or referent solvent in a flask (DES : Nile Red solution volume ratio was 1 : 2). The absorption spectra of the dye were obtained using UV-1280 Shimadzu spectrophotometer. Five referent solvents (cyclohexane, acetone, acetonitrile, methanol and water) were measured and the results were compared to the literature [27]. Measured values were then recalculated to fit the $E_T(30)$ scale [28]. The following equation was used to calculate the $E_T(30)$ values from λ_{max} values obtained with Nile Red dye:

$$E_T(30) = \exp\left(4.23637 - \frac{2.28659}{\lambda_{\max,NR} - 542.85}\right) \quad (3)$$

3. Results and discussion

When liquid–liquid extraction is considered as a purification method of liquid feed, the selection of an appropriate solvent is a crucial step to design an efficient separation. Except the phase equilibrium data (distribution coefficients, selectivity, and mutual solubility of solvents), knowledge of physical properties of phases engaged in mass transfer is of great importance. In order to investigate the influence of physical properties of solvent on its ability to extract FFA from different types of waste feedstocks, six DESs based on choline chloride and two DESs based on potassium carbonate were prepared. Physical properties measured for the selected DESs are presented in the Appendix A, Fig. 13. Experimental data was fitted with the appropriate mathematical model and the corresponding model parameters are given in Table A.1. Mutual solubility of DES and feedstock were analyzed by means of NMR and FTIR spectroscopy. Representative spectra are shown in Figures. Results obtained by spectroscopic methods provided a deeper insight into the nature of the deacidification process – whether the process is physical, due to interfacial mass transfer, or a chemical reaction. Finally, reusability of the most efficient DES was tested.

3.1. Influence of DESs' properties on extraction efficiency

In order to see how the properties of DESs and feedstocks influence the extraction of FFA from waste oils and fats, liquid-liquid extraction experiments were performed at the same process conditions. Since waste animal fats are solids, extraction was conducted at elevated temperature (60 °C). Measured properties of WCGO, WAF1 and WAF2 are: viscosity at 60 °C: 0.0209, 0.0190 and 0.0204 Pa s; density

at 15 °C: 952.7, 908.3 and 922.7 kg/m^3; TAN: 4.34, 28.39 and 18.45, respectively. All feedstocks have viscosities and densities lower than selected DESs.

Low viscosity means low resistances to momentum, heat and mass transfer, while low density means there is a significant difference in densities of both phases (oil/fat and DES) necessary for phase separation. The influence of the measured DESs' properties at 60 °C on the extraction efficiency is presented in Table 3.

Table 3 – Influence of DESs' properties (at 60 °C) on extraction efficiency

T, K	ρ, kg/m^3	V_m, m^3/kg	η, Pa s	k, S/m	n_D	pH	$E_T(30)$, kcal/mol	ε, %		
								WCGO	WAF1	WAF2
DES 1	1.2539	0.1091	2.027	0.0187	1.4853	0.48	61.01	-1.0	-9.0	-8.6
DES 2	1.3391	0.1218	3.947	0.0189	1.4906	0.63	59.81	-2.5	-6.2	-8.2
DES 3	1.2842	0.1214	4.267	0.0157	1.5093	6.84	-	8.9	-1.5	-2.7
DES 4	1.1708	0.0922	0.056	0.3210	1.4778	6.55	59.70	4.7	2.2	1.0
DES 5	1.0962	0.0768	0.010	1.3040	1.4579	5.76	59.03	26.2	5.9	4.4
DES 6	0.9839	0.0880	0.086	0.3570	1.4979	8.17	59.16	17.6	7.4	8.9
DES 7	1.4003	0.0705	0.747	0.0395	1.4775	12.28	-	100.0	99.6	100.0
DES 8	1.2327	0.0448	0.026	0.5670	1.4403	12.94	56.83	90.2	100.0	100.0

From the density, ρ, and molar mass of DES, M, molar volumes of DESs, V_m, were calculated:

$$V_m = \frac{M}{\rho} \tag{4}$$

High molar volume means higher free volume and consequently lower mobility of HBDs and HBAs, so it can be expected that DESs with lower molar volume will have higher ability to participate in interphase mass transfer. The influence of DESs' molar volume on the extraction efficiency is presented in Table 3. It can be seen that the highest efficiencies were obtained with low molar volume solvents (DES 7 and DES 8).

It was expected that low viscosity DESs will have the highest extraction efficiency. However, the lowest viscosity DES (DES 5) extracted only a small amount of FFA. The highest efficiency was achieved with low *TAN* feedstock - WCGO. On the other hand, the highest extraction efficiency was observed with more viscous DES 7. It is obvious that viscosity is not the major factor that influences the extraction of FFA from waste oil and fats. The same conclusion can be drawn from the influence of DESs' conductivity on the extraction efficiency, Table 3. High ionic mobility did not enhance mass transfer from oil/fat to DES. Here it must be pointed out that solubility of FFA in DES is a very important thermodynamic property. Viscosity and conductivity of solvent basically only influence the rate of mass transfer and the time necessary to reach an equilibrium at given process conditions.

DESs' pH influences the extraction efficiency as presented in Table 3. In general, an increase in DESs' pH results in increasing capability of solvent to extract FFA. DES 3 does not follow this rule. It is possible that very high viscosity reduces mass transfer. Secondly, FFA solubility can be the lowest in this solvent. Based on the obtained results, basic DESs were efficient for lowering FFA content in all types of waste feedstocks, probably because acids become deprotonated in basic medium, and their carboxyl groups

are then available for hydrogen bonding with hydroxyl groups in ethylene glycol (or glycerol, in the case of DES 7). In contrast, extraction with acidic DESs resulted in the increase of TAN, so efficiency was given as a negative number in Table 3. It may have occurred because some glycerides were extracted from the feedstock which led to the increase of free fatty acid fraction [29]. It is also possible that those two DESs, or citric and malic acids as their constituents, are soluble in the feedstocks leading to the increase in the feedstock TAN. Furthermore, in low pH medium carboxylic acids are in their protonated state, forming dimers and not readily forming hydrogen bonds with the solvent, which might explain their low solubility in acidic solvents.

It is also observed that the quality of feedstock influences the extraction capability of DESs, especially in case of DESs with pH value in the range from 6.55 to 8.17 (at 60 °C). Extraction efficiency is higher in case of higher quality feedstock with lower concentration of FFA.

The trend between polarity and extraction efficiency can also be observed, Table 3. Less polar solvents are better for deacidification of waste animal fats and waste coffee grounds oil. This result can be explained with the rule “like dissolves like”. Long chain fatty acids are basically nonpolar due to the nonpolar hydrocarbon chain, so it is obvious that their solubility in less polar solvent is higher.

Statistical analysis was run using the software package *Statistica 64* and the results are shown in Table 4. Spearman’s *R* indicates the existence of strong monotonic relationship between three of the tested properties and extraction efficiencies. A positive correlation was found for pH values, while polarities and molar volumes demonstrated a negative trend. Pearson’s correlation was statistically significant ($p<0.05$) for these same properties but its coefficient (*r*) was somewhat lower, proving that the relationship between tested variables was not strictly linear.

Table 4 – Spearman rank-order correlation and Pearson product-moment correlation results

Pair of Variables		Valid N	Spearman R	p-value	Pearson r	p-value
	ε(WCGO)	**8**	**0.88095**	**0.00385**	**0.88658**	**0.00334**
pH value	**ε(WAF2)**	**8**	**0.92217**	**0.00111**	**0.86905**	**0.00508**
	ε(WAF1)	**8**	**0.92857**	**0.00086**	**0.86905**	**0.00550**
	ε(WCGO)	8	0.54762	0.16003	0.12901	0.76077
conductivity	ε(WAF2)	8	0.65869	0.07569	0.03429	0.93576
	ε(WAF1)	8	0.69048	0.05799	0.04070	0.92377
	ε(WCGO)	8	-0.52381	0.18272	-0.45334	0.25927
viscosity	ε(WAF2)	8	-0.58684	0.12620	-0.42601	0.29261
	ε(WAF1)	8	-0.61905	0.10173	-0.42065	0.29938
	ε(WCGO)	8	-0.57143	0.13896	-0.59654	0.11851
refractive index	ε(WAF2)	8	-0.55091	0.15702	-0.59008	0.12360
	ε(WAF1)	8	-0.57143	0.13896	-0.59822	0.11721
	ε(WCGO)	**6**	**-0.94286**	**0.00480**	**-0.94268**	**0.00483**
polarity	**ε(WAF2)**	**6**	**-0.94286**	**0.00480**	**-0.91840**	**0.00972**
	ε(WAF1)	**6**	**-0.94286**	**0.00480**	**-0.92246**	**0.00879**
density	ε(WCGO)	8	-0.07143	0.86653	0.30522	0.46226

	ε(WAF2)	8	-0.16767	0.69147	0.34403	0.40403
	ε(WAF1)	8	-0.21429	0.61034	0.35371	0.39003
	ε(WCGO)	**8**	**-0.90476**	**0.00201**	**-0.82361**	**0.01197**
molar volume	**ε(WAF2)**	**8**	**-0.89822**	**0.00244**	**-0.79664**	**0.01795**
	ε(WAF1)	**8**	**-0.90476**	**0.00201**	**-0.79532**	**0.01828**

3.2. ^{1}H NMR analysis

In order to see if DESs are soluble in waste fats and oil, ^{1}H NMR spectroscopy was applied. Selected spectra of feedstocks before and after extraction are presented in Figs. 1, 2 and 3. Spectral regions with the observed differences are enlarged. Signals typical for lipids can be observed in WCGO spectrum before treatment (A and B: -C**H**$_3$, C: -(C**H**$_2$)$_n$, D: -OCO-CH$_2$-C**H**$_2$-, E: -C**H**$_2$-CH=CH, G: -OCO-C**H**$_2$, H: CH=CH-C**H**$_2$-CH=CH-, O: ROC**H**$_2$-CH(OR')-C**H**$_2$OR", S: ROCH$_2$-C**H**(OR')-CH$_2$OR", T: -C**H**=C**H**). Spectra of WCGO before and after treatment with the most efficient DESs (DES7 and DES8) are shown in Fig. 1. Signals characteristic for triglycerides (TGs) (O, S) diminish in spectrum of WCGO treated with DES7, while signals of monoglycerides (MGs) (K: HOC**H**$_2$-CH(OR)-C**H**$_2$OH, L: ROCH$_2$-C**H**OH-CH$_2$OH, N: ROC**H**$_2$-CHOH-CH$_2$OH, Q: HOCH$_2$-C**H**(OR)-CH$_2$OH) and diglycerides (DGs) (M: ROC**H**$_2$-C**H**OH-C**H**$_2$OR′, R: ROCH$_2$-C**H**(OR′)-CH$_2$OH) evolved as well as some new signals in the range 3.43 to 3.82 ppm. Additionally, signal D' at 1.52 ppm appeared. This signal probably belongs to H$_2$O [30]. Since MG and DG signals are not visible in the spectrum of untreated WCGO, it is obvious that neutralization rather than extraction is responsible for deacidification. Similarly, after treatment with DES8, disappearance of TGs (S, O) signals and appearance of MGs signals (K, N) indicates transformation of TGs to MGs resulting from chemical reaction. Chemical shift and multiplicity of signal D implies differences in the composition of samples. Down field shift of signal D from 1.61 (characteristic for TG) to 1.63 ppm indicates presence of 1,3-DG, 1-MG and FA [31]. Additionally, signals characteristic for DES8 are clearly visible in spectrum of WCGO. In both spectra (after treatment with DES7 and DES8 at 60 °C) low intensity signal F at 2.13 and 2.15 ppm, respectively, appeared. This may be attributed to 1,3 diolein or FFA [32, 33]. Since WCGO is liquid at room conditions, the experiment was conducted at 25 °C. No evidence of chemical reaction can be seen in this spectrum, so it can be concluded that reduction of TAN resulted from extractive deacidification.

Fig. 1 ^{1}H NMR spectra of WCGO before and after treatment with DES7 and DES8

Spectra of WAF2 before and after treatment with three DESs that exhibit different deacidification efficiencies are given in Fig 2. Besides signals characteristic for TGs, signals of MGs (I: ROCH$_2$-CHOH-C**H**$_2$OH; K; L; Q) and DGs (J: ROCH$_2$-CH(OR′)-C**H**$_2$OH; R) can be observed in the enlarged spectrum of WAF2 before treatment. Solubility of DES 4 and DES6 in acidic animal fat is clearly visible. Methylene proton of choline chloride, being the most intensive signal in DES4 and DES 6, is positioned slightly downfield compared to pure DESs (at 3.14 ppm in DES4 and 3.22 ppm in DES6), due to the change in magnetic environment solution of DESs in WAF2. In spectrum of WAF2 after treatment with DES 6 a low intensity broad signal at 5.02 ppm can be assigned to -N**H**$_2$ protons from urea in DES6. Evolution of

multiple signals in the range between 3.45 and 3.70 ppm indicates a possible chemical reaction (neutralization with basic DES7). Similar results are obtained with DES8.

Fig. 2 ^{1}H NMR spectra of WAF2 before and after treatment with DES4, DES6 and DES7

Due to its low viscosity and high TAN reducing efficiency, DES 8 was chosen as the best solvent for deacidification. Since it was shown that at the specific process condition, DES8 may act as solvent for extraction of FFA from WCGO, further experiments were devoted to examine the possibility if lower amount of solvent will result in extractive deacidification rather than neutralization and WAF1 was chosen for those experiments because it had the highest TAN. A set of experiments was conducted with WAF1 where mass ratio of DES to WAF1 was 0.1, 0.25 and 0.5; for 3 hours at 60 °C and 500 rpm. Additionally, experiments were conducted with K_2CO_3, ethylene glycol, glycerol and with DES 5 (choline chloride/ ethylene glycol), for 3 hours, at 60 °C, 500 rpm and at mass ratio of solvent : WAF1 0.1:1. Deacidification efficiencies after experiments with different mass ratios of DES to WAF1 (0.1, 0.25, 0.5 and 1:1) were 98.37, 98.49%, 67.26% and 100%, respectively, and after experiments with K_2CO_3, ethylene glycol, glycerol and with DES 5 were 29.16%, 1.48%, 0.15% and 1.18%, respectively. It was found that at high DES to WAF1 ratios neutralization was indeed occurring, which was not the case at low ratios.

Fig. 3 shows ^{1}H NMR spectra of WAF1 treated with different mass ratios of DES8, K_2CO_3, DES5, glycerol and ethylene glycol. In spectra of samples S1, S3, S4, S7 and S8 evolution of new signals indicates mutual solubility of WAF1 and applied solvents. Signals at about 3.66 and 3.73 ppm are attributed to CH_2 of Ethylene Glycol, while signals at around 4.50 and 4.77 ppm correspond to OH group. Due to the low solubility of Ethylene Glycol and DES5 in WAF1 other solvent signals can't be detected. As mentioned previously, chemical shift and multiplicity of signal D implies differences in the composition of samples. The shift of this signal from 1.61 (characteristic for TG) to 1.63 ppm indicates presence of 1,3-DG, 1-MG and FA [31]. This is observed in spectrum of sample S4 treated with the highest mass ratio of DES8:WAF1 as a probable result of conversion of TG to partial glycerides. In the spectrum of sample S4 signal D' centered at 1.54 ppm appears. This signal is usually attributed to H_2O [30]. Since this spectrum also exhibits a signal at 3.66 ppm it is possible that FFAs are partially esterified. Besides that, multiplicity of signal at 1.61 is more pronounced in mixtures without FFA [31]. Additionally, in spectrum S4 evolution of new triplet centered at 2.15 may be a result of formation of 1,3 diolein or may belong to FFA [32]. According to Brovč et al., at lower FFA concentration, FFA signal can be separated from acyl groups of TG, MG and esters [33]. Evolution of signal F observed in spectra S1 and S3 centered at around 2.24 ppm may also belong to FFA. Significant differences are also observed in the spectral region between 2.38 and 2.26 ppm. Due to the overlapping of signals in a complex mixture of glycerides and acids, it is hard to evaluate the degree of deacidification from ^{1}H NMR spectra. However, FFA signals at 2.33 and 2.35 ppm, clearly visible on signal G on WAF1 spectrum (S1), disappeared in spectra after extraction with DES8 at mass ratios up to 0.5:1 (S2, S3), glycerol (S6) and DES5 (S8) as well as after treatment with K_2CO_3. The shape of signal G in spectrum of sample S4 is specific for 1,2-DG. The quarterlike shape of this signal can also be explained by formation of esters [34]. However,

production of glycol esters from TG with large excess of diethylene glycol and K_2CO_3 as catalyst requires high temperatures (210 °C), so it can be assumed that this reaction did not take place [35].

Signals in the spectral region between 4.05 and 4.45 ppm (M, N, O, P: ROC**H**$_2$-CH(OR′)-CH_2OH) indicate that all samples, except sample S4, are composed of TG, DG and MG. Completely different signals can be observed in WAF after treatment with DES8 at the mass ratio 1:1 (sample S4). Due to the absence of signal O it can be concluded that TGs were converted to MG and DG. Signal R (Glyceryl group in 2-MG) is clearly visible in all spectra. On the other hand, signal Q is overlapped with signal that belongs to O**H** from solvents (samples S3 and S4). Finally, in the spectral region between 5.40 and 5.20 ppm, the intensity of signal S of glyceryl group in TG is very low, meaning that the concentration of TG in sample S4 is negligible.

The spectrum of sample S4 indicates that when WAF1 is treated with the highest amount of DES8, composition of WAF1 is changed as a result of chemical reaction. Explanation of what may have happened is as follows. According to the literature, neutralization of FFA with K_2CO_3 can occur at elevated temperatures (99-150 °C) and high amount of K_2CO_3 [36]. With sufficient amount of K_2CO_3 neutralization of FFA occurs producing potassium carboxylate salt, carbon dioxide and water. Carbon dioxide is dissolved in solvent and it usually forms ethylene carbonate [37]. Characteristic signal of ethylene carbonate is at around 4.54 ppm. This signal is overlapped with broad OH signal centered at 4.76 ppm. Water signal can be observed at 1.54 ppm as mentioned previously. Due to the presence of water, hydrolysis of glycerides starts and consequently concentration of TG, DG and MG changes along with generation of FFA (after every reaction step) and glycerol (if hydrolysis of MG occurs).

The spectrum of sample S1 indicates miscibility of solvent in WAF1 (solvent signals at 3.68 and 4.50 ppm). When this spectrum is compared with spectrum of WAF1 after extraction with Ethylene Glycol (sample S7), it can be concluded that a small amount of K_2CO_3 increases solubility of DES8 in WAF1 (sample 7 exhibits lower intensity signal at 3.72 ppm). When WAF1 is treated only with K_2CO_3, signal characteristic for FFA at 2.35 ppm disappears. Based on the above discussion, it can be concluded that the amount of solvent (DES8) added affects whether a physical process or a chemical reaction will occur. At 1:1 mass ratio, total deacidification was due to neutralization, while at 0.1 and 0.25:1 ratios the reduction was due to extraction. The deacidification at 0.5:1 ratio appears to be a result of partial neutralization.

Fig. 3 ^{1}H NMR spectra of WAF1 before (S0) and after treatment with: DES8 (S1, S2, S3, S4; mass ratios: 0.1:1, 0.25:1, 0.5:1 and 1:1, respectively), K_2CO_3 (S5), Glycerol (S6), Ethylene glycol (S7) and DES5 (S8)

Oxidation products may be formed from main oil components, like glycerides or FFA, and minor oil components. The oxidation can be induced by oxygen in the presence of metal ions, light, heat and free radicals [38]. Since experiments in this study were performed at elevated temperature in a glass reactor, oxidation can be initiated by heat and/or light. Waste animal fats contain a certain amount of metals that can also initiate oxidation. If the concentration of minor oil components is very low, their presence may

not be detected by ^{1}H NMR [39]. Oxidation can be detected by a decrease of the intensity of signals at 2.019, 2.768 and 5.337 ppm and evolution of new signals between 8 and 10 ppm that correspond to hydroperoxides and their decomposition products (aldehydes) [40].

Intensities of signals at 2.768, 2.019 and 5.337 ppm compared to the intensities of signals at 2.309 and 1.610 ppm are the same for experiments performed at mass ratio of DES and WAF up to 0.5:1. At the highest mass ratio, differences of intensities can be observed indicating degradation of unsaturated acyl groups [41].

Fig. 4 ^{1}H NMR spectra of WAF1 before (S0) and after treatment with: DES8 (S1, S2, S3, S4), K_2CO_3 (S5), Glycerol (S6), Ethylene glycol (S7) and DES5 (S8) – spectral range from 5.00 to 7.20 ppm

Fig. 5 ^{1}H NMR spectra of WAF1 before (S0) and after treatment with: DES8 (S1, S2, S3, S4), K_2CO_3 (S5), Glycerol (S6), Ethylene glycol (S7) and DES5 (S8) – spectral range from 7.90 to 10.30 ppm

In all ^{1}H NMR spectra of WAF1 before and after extraction, low intensity signals that belong to conjugated linoleic acids (CLAs), primary and secondary oxidation products can be observed. In the spectral range from 5.50 to 8.90 ppm signals that belong to CLA (5.5 to 6.9 ppm) and primary oxidation products (hydroperoxides) (5.5 to 8.9 ppm) are observed [42–44]. Signals in the spectral range between 9.30 and 10.26 belong to secondary oxidation products (aldehydes and carboxylates) [40, 42, 43, 45].

As seen in Fig. 4, in spectra of samples S5 to S8 evolution of broad signals between 6.3 and 7.0 ppm can be observed. For samples treated with Glycerol, Ethylene glycol and DES5 these signals can be attributed to OH due to the mutual solubility of WAF1 and solvent. High intensity signal in spectrum of sample treated with K_2CO_3 may be assigned to glycerol OH, assuming that MGs also hydrolyses. Additional signals of primary (8.52 ppm) and secondary oxidation (9.82 and 10.26 ppm) products evolved in spectrum of sample S4, Fig. 5. This may be a result of thermal oxidation of WAF1 which can be enhanced by the presence of K_2SO_4 and ethylene glycol. Formation of hydroperoxides at lower temperatures has been previously confirmed by Guillén and Uriarte [46]. Low intensity signal centered at 3.43 ppm (secondary alcohol) and sharp signal at 8.15 ppm (ketones) in the spectrum of the same sample may be attributed to the formation of secondary oxidization products [42].

In order to confirm that FFAs are really transferred to solvents, ^{1}H NMR spectra of solvents after extraction were measured and the obtained spectra are presented in Fig. 6 and Fig. 7. Besides solvent signals, the signals characteristic for FFA (A, B, C, D, E, F, G, H, T) are visible in all spectra after extraction, except when signals are overlapped with solvent signal (H in spectra SS1 and SS4; T in spectrum SS1), Fig. 6. Due to the presence of signals J, K, L, M and N in spectra of samples SS1 to SS3 it can be concluded that monoglycerides (1-MG, 2-MG) and diglycerides (1,2-DG, 1,3-DG) were transferred from WAF1 to DES8. Signals of TGs (O, S) are visible only on sample SS3 (DES8:WAF1 mass ratio = 0.5:1) which means that higher amount of solvent is necessary to allow dissolution of TG. The solubility of MGs and DGs is higher than TGs so these smaller molecules are likely to be dissolved.

When solvent is saturated with MGs and DGs, TGs will not dissolve. Due to the absence of signal M in spectrum SS4 it can be concluded that 1,3-DGs are not present in this sample. Having in mind that when WAF1 was treated with the highest amount of solvent (DES8) neutralization of FFA and hydrolysis of glycerides occurred, it is obvious that TGs cannot be expected in solvent.

Due to the different solvent concentrations, hydrogen bonding network was changed, what is observed in Fig. 6. ^{1}H NMR spectra of the lower layer (developed after treatment with K_2CO_3 or solvent: glycerol, ethylene glycol and DES5) are presented in Fig. 7. When WAF1 was treated with K_2CO_3, lower layer after mixing was basically sediment of the same color as WAF1. Composition of this layer is slightly different from the upper layer (WAF1 after treatment, sample S5) as a result of neutralization of FFA and hydrolysis of glycerides. Up-field shift of signal G indicates higher concentration of FFA. In spectrum of sample SS5 FFA signals can be observed (2.35 and 2.33 ppm). Since deacidification with DES5, ethylene glycol and glycerol was of very low efficiency, signals of FFA were not observed in solvents' spectra. Low intensity signals of glycerides are detected in spectra of glycerol and ethylene glycol. It was previously reported that glycerides and FFAs are slightly soluble in glycerol [47]. Signals of glycerides are not present in DES5 spectrum after extraction. Obtained spectra confirm low extractive deacidification with glycerol, ethylene glycol and DES 5.

Fig. 6 ^{1}H NMR spectra of DES8 after extraction: SS0 – pure solvent; SS1 – mass ratio 0.10:1; SS2 – mass ratio 0.25:1; SS3 – mass ratio 0.50:1; SS4 – mass ratio 1:1

Fig. 7 ^{1}H NMR spectra of lower phase after extraction: SS5 – lower layer after treatment with K_2CO_3; SS6 – glycerol; SS7 – ethylene glycol; SS8 – DES5

3.3. ^{13}C NMR analysis

To further investigate the claim that neutralization occurred at higher mass ratios, ^{13}C NMR spectra were taken and are given in Fig. 8. The obtained samples were characterized by NMR spectroscopy. Proton and carbon chemical shifts in CDCl3 were assigned by a combined use of one dimensional ^{1}H and ^{13}C APT experiments. Chemical shift of COOH group of all samples was observed in the range between 172 and 180 ppm. Double bond region was assigned from 127-131 ppm. The signals of -COO-CH3 carbons were found between 60 and 72.10 ppm. The signals of $-CH_3$ carbons were assigned near 14 ppm and signals of -CH and $-CH_2$ were found from 22 to 35 ppm. The analysis of ^{1}H and ^{13}C NMR spectra has shown that some changes in chemical shifts of sample after extraction at 1:1 ratio occurred. Up-field chemical shifts were observed for the COOH carbons in carbon spectrum and down-field chemical shifts for the OH protons in proton spectrum which confirmed the existence of the weaker hydrogen bond interaction. Also, some changes in chemical shifts in -COO-CH3 region were observed.

The signal at 24.64 ppm that can be attributed to linoleic acid is visible in the spectrum of WAF1 [48]. The carboxyl carbon of FFA was found between 178.68 and 179.48 ppm, except in samples S2 and S4 [42]. This result confirms almost complete deacidification of WAF1 by means of extraction (S2) or

neutralization (S4). Appearance of carboxyl carbon signals at 174.24 ppm, which is characteristic for esters and at 182.44 ppm, which is characteristic for carboxylate anion clearly confirms neutralization of FFA when WAF1 is treated with the equal amount of DES8 [42, 49]. Signals at 26.54 and 38.27 ppm can also be attributed to carboxylate salts [49]. In the spectrum of the same sample (S4) only signals of 1,2-DGs, 1-MGs and glycerol can be observed. An increased intensity of signals at 60.94 ppm (1,2-DG) and 65.74 ppm (1-MG) indicates higher concentration of lower glycerides. Absence of TG signals (in the spectral range from 172.67 to 173.23 ppm; 68,80 ppm; 33.97 ppm) and appearance of lower glycerides, esters, carboxylate anion and glycerol confirm chemical reactions proposed. Since carboxylate anion carbon signal did not appear in the spectrum of WAF1 treated with K_2CO_3 and signals of TG, DG and MG are detected, no evidence of a chemical reaction is provided. Signal at around 63.24 ppm, found in spectra of samples S4 and S3 can be attributed to ethylene glycol. No significant difference between spectra of samples S0 and S2 (except the absence of FFA on S2) confirms that deacidification is a result of extraction.

Fig. 8 ^{13}C NMR spectra of WAF1 before (S0) and after treatment with: DES8 (S1, S2, S3, S4) and K_2CO_3 (S5)

3.4. FTIR analysis

FTIR spectra of all three used feedstocks are presented in Fig. 9. Characteristic peaks of fats and oils can be observed: at 2919 and 2851 cm^{-1} corresponding to CH absorption of bending vibrations of CH_2 and CH_3 bands; at 1743 cm^{-1} C=O stretch of triglyceride ester linkage; at 1712 cm^{-1} characteristic carbonyl stretching band of FFAs C=OH; at 1465 cm^{-1} bending vibrations of CH_2 and CH_3; at 1376 and 1236 cm^{-1} bending vibrations of CH_2; at 1161, 1116 and 1097 cm^{-1} absorption of C-O ester bands and at 719 cm^{-1} CH_2 rocking vibration [50, 51].

Fig. 9 FTIR spectra of the three used feedstocks

Fig. 10 shows the spectra of WAF1 before and after extraction experiments and DES 8. Red shift of the peak at 3282.45 cm^{-1} can be observed with decreasing mass ratio. Disappearance of the peak at around 1715 cm^{-1} indicates deacidification. Evolution of the peak at 1647.74 cm^{-1}, belonging to DES 8, in the spectrum of WAF1 after extraction at the highest mass ratio (1:1) can be a result of possible miscibility. Finally, evolution of the peak at around 1567 cm^{-1} at high mass ratios (0,5:1 and 1:1) is a sign of neutralization, since that peak can be attributed to carboxylate anion of the potassium salt; the intensity of that peak increases with increasing mass ratio [52]. At lower mass ratios (0.1:1 and 0.25:1) there is no sign of that peak, leading to the conclusion that neutralization does not occur when the ratio of DES 8 to feedstock is low enough. Increased intensities of peaks that belong to DES 8 in the range between 1250 and 1450 cm^{-1} could further be caused by possible miscibility; with blue shift of peak from 1163 to 1179 cm^{-1} indicating different environment and evolution of stronger H-bonds. Evolution of peaks at

around 1084 and 1040 cm^{-1} that belong to DES 8 is further evidence of miscibility (C-O stretching of ethylene glycol) at high mass ratios (0.5:1 and 1:1).

Fig. 10 FTIR spectra of DES8 and WAF1 before and after extraction: S0 – WAF1; S1 – mass ratio 0.10:1; S2 – mass ratio 0.25:1; S3 – mass ratio 0.50:1; S4 – mass ratio 1:1

FTIR spectra of WAF1 before and after experiments with K_2CO_3, ethylene glycol, glycerol and with DES 5 are given in Fig. 11. A broad peak can be observed at 3300 cm^{-1} after extraction with DES 5 and ethylene glycol, corresponding to –OH groups and indicating possible slight miscibility of these solvents in WAF1. The peak at around 1714 cm^{-1} corresponding to free fatty acids is present in all spectra except when WAF1 was treated with K_2CO_3, further confirming that extraction efficiencies, were very low and deacidification did not occur. After treatment with K_2CO_3, this peak disappeared. This result confirms deacidification.

Fig. 11 FTIR spectra of WAF1 before and after treatment: S0 – WAF1; S5 – K_2CO_3; S6 – glycerol; SS7 – ethylene glycol; SS8 – DES5

Further experiments were conducted to determine how many times DES 8 could be reused before its efficiency dropped significantly. The goal of this work was to reduce *TAN* to less than 2 mg KOH / g fat, which is equivalent to 1% of FFA. In order to minimize the amount of DES used, the experiments were conducted with DES : WAF1 mass ratio 0.1:1. The initial *TAN* was 28.39 mg KOH / g feedstock and resulting *TAN*s were 0.15, 0.25, 0.41, 0.80 and 2.12 mg KOH / g feedstock. FTIR spectra of the samples before and after extraction are shown in Fig 12. The reduction of the peak at ~1712 cm^{-1} can be observed. As stated above, this peak corresponds to free fatty acids and its disappearance after the first extraction indicates that the extraction was successful. After the fifth extraction a weak peak can be observed and the resulting *TAN* is 2.12 mg KOH / g fat, slightly above the goal of this work meaning that DES 8 can be used successfully up to four times before it becomes too saturated with free fatty acids.

Fig. 12 FTIR spectra of WAF1 before and after extraction

4. Conclusions

The initial concentrations of FFAs in waste animal fats and waste coffee grounds oil were successfully reduced by means of liquid-liquid extraction with deep eutectic solvents. Solvent molar volume, polarity and pH value strongly influence its capability to extract FFA from waste biodiesel feedstocks. DESs with lower molar volume and polarity and higher pH value demonstrated higher extraction efficiency, as confirmed by Spearman rank-order and Pearson product-moment correlations.

Due to the high extraction efficiency and low viscosity, deep eutectic solvent potassium carbonate : ethylene glycol (molar ratio 1:10) was selected as the best solvent for deacidification of waste fats and oils. Further experiments with DES 8 and WAF1 proved that extraction of free fatty acids occurred when mass ratios of solvent to feedstock were 0.1:1 and 0.25:1, while for higher mass ratios neutralization took place. Testing confirmed its reusability in extracting FFAs from waste animal fats of up to four times at the lowest mass ratio (0.1:1). Further investigation will be devoted to the optimization of deacidification process with the selected solvent, as well as to the production of biodiesel from purified advanced feedstocks. By implementing deep eutectic solvents in a cleaner biodiesel production process, this work provides a contribution on the road towards sustainable transport.

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Conflict of interest

The authors declare no competing financial interest.

References

1. Atadashi IM, Aroua MK, Abdul Aziz AR, Sulaiman NMN (2013) The effects of catalysts in biodiesel production: A review. J. Ind. Eng. Chem. 19:14–26
2. Santacesaria E, Tesser R, Di Serio M, et al (2007) Kinetics and Mass Transfer of Free Fatty Acids Esterification with Methanol in a Tubular Packed Bed Reactor: A Key Pretreatment in Biodiesel Production. Ind Eng Chem Res 46:5113–5121. https://doi.org/10.1021/ie061642j
3. Petračić A, Sander A, Cvetnić M (2019) A novel approach for the removal of trace elements from waste fats and oils. Sep Sci Technol. https://doi.org/10.1080/01496395.2019.1706575
4. Jain S (2019) The production of biodiesel using Karanja (Pongamia pinnata) and Jatropha (Jatropha curcas) Oil. In: Biomass, Biopolymer-Based Materials, and Bioenergy. Elsevier, pp 397–408
5. Bhosle BM, Subramanian R (2005) New approaches in deacidification of edible oils - A review. J Food Eng 69:481–494. https://doi.org/10.1016/j.jfoodeng.2004.09.003
6. Vaisali C, Charanyaa S, Belur PD, Regupathi I (2015) Refining of edible oils: A critical appraisal of current and potential technologies. Int J Food Sci Technol 50:13–23. https://doi.org/10.1111/ijfs.12657
7. Dimian AC, Kiss AA (2019) Eco-efficient processes for biodiesel production from waste lipids. J Clean Prod 239:118073. https://doi.org/10.1016/j.jclepro.2019.118073
8. Tang W, Row KH (2020) Design and evaluation of polarity controlled and recyclable deep eutectic solvent based biphasic system for the polarity driven extraction and separation of

compounds. J Clean Prod 268:122306. https://doi.org/10.1016/j.jclepro.2020.122306

9. Verevkin SP, Sazonova AY, Frolkova AK, et al (2015) Separation performance of BioRenewable deep eutectic solvents. Ind Eng Chem Res 54:3498–3504. https://doi.org/10.1021/acs.iecr.5b00357

10. Jeong KM, Ko J, Zhao J, et al (2017) Multi-functioning deep eutectic solvents as extraction and storage media for bioactive natural products that are readily applicable to cosmetic products. J Clean Prod 151:87–95. https://doi.org/10.1016/j.jclepro.2017.03.038

11. Chen Y, Mu T (2019) Application of deep eutectic solvents in biomass pretreatment and conversion. Green Energy Environ 4:95–115. https://doi.org/10.1016/j.gee.2019.01.012

12. Troter DZ, Todorović ZB, Dokić-Stojanović DR, et al (2016) Application of ionic liquids and deep eutectic solvents in biodiesel production: A review. Renew Sustain Energy Rev 61:473–500. https://doi.org/10.1016/j.rser.2016.04.011

13. Zahrina I, Nasikin M, Krisanti E, Mulia K (2018) Deacidification of palm oil using betaine monohydrate-based natural deep eutectic solvents. Food Chem 240:490–495. https://doi.org/10.1016/j.foodchem.2017.07.132

14. Zahrina I, Mulia K, Yanuar A, Nasikin M (2018) Molecular interactions in the betaine monohydrate-polyol deep eutectic solvents: Experimental and computational studies. J Mol Struct 1158:133–138. https://doi.org/10.1016/j.molstruc.2017.11.064

15. Zahrina I, Nasikin M, Mulia K, et al (2018) Molecular interactions between betaine monohydrate-glycerol deep eutectic solvents and palmitic acid: Computational and experimental studies. J Mol Liq 251:28–34. https://doi.org/10.1016/j.molliq.2017.12.016

16. Zullaikah S, Wibowo ND, Wahyudi IMGED, Rachimoellah M (2019) Deacidification of crude rice bran oil (Crbo) to remove ffa and preserve γ-oryzanol using deep eutectic solvents (des). In: Materials Science Forum. Trans Tech Publications Ltd, pp 109–114

17. Sander A, Petračić A, Vuković JP, Husinec L (2020) From coffee to biodiesel—deep eutectic solvents for feedstock and biodiesel purification. Separations 7:. https://doi.org/10.3390/separations7020022

18. Ghaedi H, Ayoub M, Sufian S, et al (2018) Density and refractive index measurements of transition-temperature mixture (deep eutectic analogues) based on potassium carbonate with dual hydrogen bond donors for CO2capture. J Chem Thermodyn 118:147–158. https://doi.org/10.1016/j.jct.2017.11.008

19. Vuksanović JM, Todorović NM, Kijevčanin ML, et al (2017) Experimental investigation and modeling of thermophysical and extraction properties of choline chloride + DL-malic acid based deep eutectic solvent. J Serbian Chem Soc 82:1287–1302. https://doi.org/10.2298/JSC170316054V

20. Popescu AM, Constantin V (2014) Synthesis, characterization and thermophysical properties of

three neoteric solvents-ionic liquids based on choline chloride. Chem Res Chinese Univ 30:119–124. https://doi.org/10.1007/s40242-014-3346-1

21. Hayyan A, Mjalli FS, Alnashef IM, et al (2012) Fruit sugar-based deep eutectic solvents and their physical properties. Thermochim Acta 541:70–75. https://doi.org/10.1016/j.tca.2012.04.030

22. Yadav A, Trivedi S, Rai R, Pandey S (2014) Densities and dynamic viscosities of (choline chloride+glycerol) deep eutectic solvent and its aqueous mixtures in the temperature range (283.15-363.15)K. Fluid Phase Equilib 367:135–142. https://doi.org/10.1016/j.fluid.2014.01.028

23. Shahbaz K, Baroutian S, Mjalli FS, et al (2012) Densities of ammonium and phosphonium based deep eutectic solvents: Prediction using artificial intelligence and group contribution techniques. Thermochim Acta 527:59–66. https://doi.org/10.1016/j.tca.2011.10.010

24. Yadav A, Pandey S (2014) Densities and viscosities of (choline chloride + urea) deep eutectic solvent and its aqueous mixtures in the temperature range 293.15 K to 363.15 K. J Chem Eng Data 59:2221–2229. https://doi.org/10.1021/je5001796

25. Mjalli FS, Naser J, Jibril B, et al (2014) Ionic liquids analogues based on potassium carbonate. Thermochim Acta 575:135–143. https://doi.org/10.1016/j.tca.2013.10.028

26. Ogihara W, Aoyama T, Ohno H (2004) Polarity Measurement for Ionic Liquids Containing Dissociable Protons. Chem Lett 33:1414–1415. https://doi.org/10.1246/cl.2004.1414

27. Harris RC (2009) Physical Properties of Alcohol Based Deep Eutectic Solvents. Thesis Univ Leicester

28. Reichardt C (1994) Solvatochromic Dyes as Solvent Polarity Indicators. Chem Rev 94:2319–2358. https://doi.org/10.1021/cr00032a005

29. Sander A, Antonije Košćak M, Kosir D, et al (2018) The influence of animal fat type and purification conditions on biodiesel quality. Renew Energy 118:752–760. https://doi.org/10.1016/j.renene.2017.11.068

30. Park KS, Kim YJ, Choe EK (2019) Composition Characterization of Fatty Acid Zinc Salts by Chromatographic and NMR Spectroscopic Analyses on Their Fatty Acid Methyl Esters. J Anal Methods Chem 2019:. https://doi.org/10.1155/2019/7594767

31. Nieva-Echevarría B, Goicoechea E, Manzanos MJ, Guillén MD (2014) A method based on 1H NMR spectral data useful to evaluate the hydrolysis level in complex lipid mixtures. Food Res Int 66:379–387. https://doi.org/10.1016/j.foodres.2014.09.031

32. Kumar R, Bansal V, Tiwari AK, et al (2011) Estimation of glycerides and free fatty acid in oils extracted from various seeds from the Indian region by NMR spectroscopy. JAOCS, J Am Oil Chem Soc 88:1675–1685. https://doi.org/10.1007/s11746-011-1846-4

33. Brovč EV, Pajk S, Šink R, Mravljak J (2019) Comparison of the NMR and the acid value determination methods for quality control of input polysorbates. Acta Chim Slov 66:934–943.

https://doi.org/10.17344/acsi.2019.5144

34. Satyarthi JK, Srinivas D, Ratnasamy P (2009) Estimation of free fatty acid content in oils, fats, and biodiesel by 1H NMR spectroscopy. Energy and Fuels 23:2273–2277. https://doi.org/10.1021/ef801011v

35. Suppes GJ, Bockwinkel K, Lucas S, et al (2001) Calcium carbonate catalyzed alcoholysis of fats and oils. JAOCS, J Am Oil Chem Soc 78:139–145. https://doi.org/10.1007/s11746-001-0234-y

36. Soderholm CG, Younker RS, Albrecht JJ, Wells GE (2015) Partial neutralization of free fatty acid mixtures with potassium, livestock feed compositions including them, and methods of making same. 1–14

37. Ma J, Liu J, Zhang Z, Han B (2012) Mechanisms of ethylene glycol carbonylation with carbon dioxide. Comput Theor Chem 992:103–109. https://doi.org/10.1016/j.comptc.2012.05.010

38. Martínez-Yusta A, Goicoechea E, Guillén MD (2014) A review of thermo-oxidative degradation of food lipids studied by 1H NMR spectroscopy: Influence of degradative conditions and food lipid nature. Compr Rev Food Sci Food Saf 13:838–859. https://doi.org/10.1111/1541-4337.12090

39. Martínez-Yusta A, Guillén MD (2016) Monitoring compositional changes in sunflower oil-derived deep-frying media by ^{1}H Nuclear Magnetic Resonance. Eur J Lipid Sci Technol 118:984–996. https://doi.org/10.1002/ejlt.201500270

40. Yang CM, Grey AA, Archer MC, Bruce WR (1998) Rapid quantitation of thermal oxidation products in fats and oils by 1H-NMR spectroscopy. Nutr Cancer 30:64–68. https://doi.org/10.1080/01635589809514642

41. Guillén MD, Goicoechea E (2007) Detection of primary and secondary oxidation products by Fourier transform infrared spectroscopy (FTIR) and 1H nuclear magnetic resonance (NMR) in sunflower oil during storage. J Agric Food Chem 55:10729–10736. https://doi.org/10.1021/jf071712c

42. Alexandri E, Ahmed R, Siddiqui H, et al (2017) High resolution NMR spectroscopy as a structural and analytical tool for unsaturated lipids in solution. Molecules 22

43. Castejón D, Mateos-Aparicio I, Molero MD, et al (2014) Evaluation and Optimization of the Analysis of Fatty Acid Types in Edible Oils by 1H-NMR. Food Anal Methods 7:1285–1297. https://doi.org/10.1007/s12161-013-9747-9

44. Lankhorst PP, Chang AN (2018) The application of NMR in compositional and quantitative analysis of oils and lipids. In: Modern Magnetic Resonance. Springer International Publishing, pp 1743–1764

45. Kato S, Shimizu N, Hanzawa Y, et al (2018) Determination of triacylglycerol oxidation mechanisms in canola oil using liquid chromatography–tandem mass spectrometry. npj Sci

Food 2:1. https://doi.org/10.1038/s41538-017-0009-x

46. Guillén MD, Uriarte PS (2012) Study by 1H NMR spectroscopy of the evolution of extra virgin olive oil composition submitted to frying temperature in an industrial fryer for a prolonged period of time. Food Chem 134:162–172. https://doi.org/10.1016/j.foodchem.2012.02.083

47. Mamtani K, Shahbaz K, Farid MM (2021) Glycerolysis of free fatty acids: A review. Renew Sustain Energy Rev 137:110501. https://doi.org/10.1016/j.rser.2020.110501

48. Hatzakis E, Agiomyrgianaki A, Kostidis S, Dais P (2011) High-resolution NMR spectroscopy: An alternative fast tool for qualitative and quantitative analysis of diacylglycerol (DAG) oil. JAOCS, J Am Oil Chem Soc 88:1695–1708. https://doi.org/10.1007/s11746-011-1848-2

49. Delaney SP, Nethercott MJ, Mays CJ, et al (2017) Characterization of Synthesized and Commercial Forms of Magnesium Stearate Using Differential Scanning Calorimetry, Thermogravimetric Analysis, Powder X-Ray Diffraction, and Solid-State NMR Spectroscopy. J Pharm Sci 106:338–347. https://doi.org/10.1016/j.xphs.2016.10.004

50. Raba DN, Poiana MA, Borozan AB, et al (2015) Investigation on crude and high-temperature heated coffee oil by ATR-FTIR spectroscopy along with antioxidant and antimicrobial properties. PLoS One 10:e0138080. https://doi.org/10.1371/journal.pone.0138080

51. Gurdeniz G, Ozen B, Tokatli F (2010) Comparison of fatty acid profiles and mid-infrared spectral data for classification of olive oils. Eur J Lipid Sci Technol 112:218–226. https://doi.org/10.1002/ejlt.200800229

52. Ismail AA, van de Voort FR, Emo G, Sedman J (1993) Rapid quantitative determination of free fatty acids in fats and oils by fourier transform infrared spectroscopy. J Am Oil Chem Soc 70:335–341. https://doi.org/10.1007/BF02552703

53. García G, Aparicio S, Ullah R, Atilhan M (2015) Deep Eutectic Solvents: Physicochemical Properties and Gas Separation Applications. Energy & Fuels 29:2616–2644. https://doi.org/10.1021/ef5028873

54. Naser J, Mjalli F, Jibril B, et al (2013) Potassium Carbonate as a Salt for Deep Eutectic Solvents. Int J Chem Eng Appl 4:114–118. https://doi.org/10.7763/IJCEA.2013.V4.275

55. Abbott AP, Harris RC, Ryder KS (2007) Application of hole theory to define ionic liquids by their transport properties. J Phys Chem B 111:4910–4913. https://doi.org/10.1021/jp0671998

56. Bagh FSSG, Shahbaz K, Mjalli FS, et al (2013) Electrical conductivity of ammonium and phosphonium based deep eutectic solvents: Measurements and artificial intelligence-based prediction. Fluid Phase Equilib 356:30–37. https://doi.org/10.1016/j.fluid.2013.07.012

57. Zhang Q, De Oliveira Vigier K, Royer S, Jérôme F (2012) Deep eutectic solvents: Syntheses, properties and applications. Chem Soc Rev 41:7108–7146. https://doi.org/10.1039/c2cs35178a

58. Skulcova A, Russ A, Jablonsky M, Sima J (2018) The pH Behavior of Seventeen Deep Eutectic Solvents. BioResources 13:5042–5051. https://doi.org/10.15376/biores.13.3.5042-5051

59. Brauman JI, Blair LK (1968) Gas-Phase Acidities of Alcohols. Effects of Alkyl Groups. J Am Chem Soc 90:6561–6562. https://doi.org/10.1021/ja01025a083

Appendix A

Fig. 13 The influence of temperature on properties of selected DESs: a) dynamic viscosity; b) conductivity; c) pH; d) refractive index

Table A.1 – Viscosity, conductivity, pH and refractive index model parameters

	Viscosity model parameters			Conductivity model parameters		
DES	η_0, Pas	E_η/R, 1/K	R^2	κ_0, S/m	E_κ/R, 1/K	R^2
DES 1	$5.6\cdot10^{-12}$	8832.8	0.999	$3.126\cdot10^{7}$	-7074.0	0.999
DES 2	$4.9\cdot10^{-11}$	8316.6	0.999	$1.129\cdot10^{6}$	-5956.5	0.990
DES 3	$6.9\cdot10^{-14}$	10480.5	0.999	$9.384\cdot10^{8}$	-8260.9	0.999
DES 4	$3.0\cdot10^{-9}$	5533.2	0.998	$9.725\cdot10^{3}$	-3368.9	0.999
DES 5	$1.4\cdot10^{-7}$	3700.7	0.999	$0.659\cdot10^{2}$	-1302.9	0.995
DES 6	$8.2\cdot10^{-13}$	8434.6	0.999	$8.719\cdot10^{6}$	-5663.9	0.999
DES 7	$4.6\cdot10^{-14}$	10032.4	0.999	$4.626\cdot10^{5}$	-5413.2	0.989
DES 8	$1.2\cdot10^{-8}$	4835.4	0.999	$0.246\cdot10^{3}$	-2020.3	0.993
	pH model parameters			Refractive index model parameters		
DES	a, 1/K	b, -	R^2	a, 1/K	b, -	R^2
DES 1	0.00260	-0.3518	0.303	$-1.8\cdot10^{-4}$	1.5467	0.956
DES 2	0.00394	-0.7989	0.824	$-1.9\cdot10^{-4}$	1.5534	0.996
DES 3	-0.02550	15.2275	0.944	$-2.2\cdot10^{-4}$	1.5835	0.997
DES 4	-0.00075	5.1518	0.969	$-2.5\cdot10^{-4}$	1.5619	0.989
DES 5	-0.01957	12.2344	0.943	$-2.5\cdot10^{-4}$	1.5425	0.992
DES 6	-0.03550	19.4135	0.956	$-1.9\cdot10^{-4}$	1.5600	0.941
DES 7	-0.00877	15.1849	0.980	$-2.6\cdot10^{-4}$	1.5643	0.882
DES 8	-0.00937	16.0667	0.944	$-2.1\cdot10^{-4}$	1.5102	0.992

Viscosity of DESs

The influence of temperature on the DESs' viscosity is presented in Fig. 13 a); (Standard uncertainties u are: for low viscosity DESs, $6.4\cdot10^{-6}$ Pas ≤ $u(\eta)$ ≤ $3.3\cdot10^{-5}$ Pas; for high viscosity DESs, 0.1 Pas ≤ $u(\eta)$ ≤ 1.5 Pas). At room temperature, viscosity of DESs is considerably higher than viscosity of water and organic solvents most frequently used in separation processes. Viscosity is significantly reduced at higher temperatures. This is very important for possible commercial application of this type of solvents. As mentioned previously, low viscosity is one of the most important requirements that a solvent has to fulfil. Lower viscosity means better hydrodynamic conditions for dispersion and lower heat and mass transfer resistances. On the other hand, the most important advantage of liquid–liquid extraction lies in its mild operating conditions (room temperature and atmospheric pressure). However, DESs exhibit other favorable properties, like non-volatility and solvation of different types of compounds, so

conducting experiments at moderately increased temperature can be acceptable. Moreover, when feedstock is in solid state, like waste animal fats, the process must be conducted at an elevated temperature.

In general, based on the experimentally obtained viscosities, selected DESs can be divided in two groups: low viscosity DESs (DES 4, DES 5, DES 6 and DES 8) and high viscosity DESs (DES 1, DES 2, DES 3 and DES 7) [53]. Potassium carbonate based DESs are more viscous than choline chloride based DESs, when the same HBD is used. Even though molar ratios are not the same, the obtained results can be compared, since DES viscosity is reduced by the increase of glycerol amount [54]. Similar values for viscosity of DES7 were measured by Mjalli et al. [25] and for DES 6 by Popescu and Constantin [20]. When DESs with the same salt and different HBD are compared, like DES 4 and DES 5, or DES 7 and DES 8, it can be concluded that DESs with less viscous HBD have lower viscosity (viscosity of ethylene glycol is significantly lower than viscosity of glycerol). Sugar based DES has the highest viscosity, and it is followed by acid based DESs. Therefore the type of salt and HBD highly influence the viscosity of DES. From the viscosity point of view, it may be expected that DESs with ethylene glycol (DES 5 and DES 8) will have the lowest resistances to momentum, heat and mass transfer. Low viscosity also means that these solvents can be easily dispersed so they can provide large specific surface.

The viscosity of the selected DESs was fitted with an Arrhenius type equation:

$$\eta = \eta_0 \cdot \exp\left(E_\eta / RT\right) \tag{5}$$

where η is the dynamic viscosity in Pas, η_0 is the pre-exponential constant in Pas, E_η is the activation energy in J/mol, R is the gas constant in J/Kmol, and T is the temperature in K. Evaluated model parameters are given in Table A.1. In general, activation energy follows the viscosity trend: higher viscosity means higher activation energy resulting from the stronger intermolecular forces in the DES [53].

Conductivity of DESs

The influence of temperature on the selected DESs' conductivities is given in Fig. 13 b); (Standard uncertainties u is: $1.0 \cdot 10^{-3}$ S/m ≤ $u(\kappa)$ ≤ $8.3 \cdot 10^{-3}$ S/m). Conductivity is increased at higher temperatures due to the increased ion mobility at elevated temperatures. Electrical conductivity is strongly linked with viscosity, so the two groups of DESs mentioned in the previous section can also be observed. High viscosity DESs exhibit low conductivity. Both properties have been explained by the structure of DES and the hole theory [55]. As in the case of viscosity, salt and HBD influence the conductivity of DESs. The conductivity of choline chloride based DESs is higher than in the case of potassium carbonate based DESs with the same HBD. This can be attributed to higher HBD concentration in potassium carbonate based DESs and consequently strong influence of ions and their interactions which resulted in lower overall mobility of charge carriers [56]. Formation of ion pairs or triplets and their aggregation

reduce the number of free charge carriers thus lowering the conductivity. The structure of HBD influences the conductivity; the more complex the structure is, the lower conductivity is exhibited by DES, as can be seen for DES 1, DES 2 and DES 3. High conductivity is favorable, since it means that DES components are of high mobility. The higher the ionic mobility is, the better hydrodynamic conditions can be achieved and consequently higher mass transfer rates can be accomplished.

The conductivity of the selected DESs was correlated with an Arrhenius type equation:

$$\kappa = \kappa_0 \cdot \exp\left(E_\kappa / RT\right) \tag{6}$$

where κ is the conductivity in S/m, κ_0 is the pre-exponential constant in S/m, E_κ is the activation energy in J/mol, R is the gas constant in J/Kmol, and T is the temperature in K. Evaluated model parameters are given in Table A.1. Activation energy is higher for low conductivity DESs.

pH value of DESs

Based on the pH values of the prepared DESs, it can be seen that almost the entire scale of pH values is covered, Fig. 13 c); (Standard uncertainties u is: $0.023 \leq u(\mathrm{pH}) \leq 0.150$). As expected, the type of salt and HBD strongly influence the pH value of DES [54, 57]. Acid HBDs (citric acid and malic acid) with choline chloride form highly acidic DESs (DES 1 and DES 2). Sugar based DES is neutral, as reported previously by Hayyan et al., while alcohol based DESs are neutral to slightly acidic [21]. Lower pH value of DES with ethylene glycol in comparison to DES with glycerol was also observed by Skulcova et al [58]. Alcohols are acidic in nature due to their acidic hydrogen atom. According to Brauman and Blair, smaller ions are better stabilized by solvation, since the smaller alcoxide ion has a shorter radius of solvation, and consequently larger solvation energy which overcomes the stabilization that results from polarization of the charge [59]. Owing to slightly basic nature of urea, DES 6 is also slightly basic. DES 7 and DES 8 are basic due to the basicity of the salt. From the obtained results it is obvious that both HBD and salt influence pH value of DESs.

Temperature slightly influences pH value of DESs. For highly acidic DESs, pH value is increased by increasing temperature to a small extent. For other examined DESs, pH value is slightly reduced with increasing temperature. The dependence of pH value on temperature was correlated with linear function:

$$pH = \mathrm{a} \cdot T + \mathrm{b} \tag{7}$$

Model parameters are given in Table A.1. For the majority of DESs, extremely low correlation was obtained even though experimentally obtained data followed the linear trend. Experimental data scattering can also be observed for previously published data [21]. The pH value of DES 1 was very low at all tested temperatures, so it is possible that low correlation of measured data resulted from high acidity.

Refractive index of DESs

Refractive index of a solvent enables indirect measurement of concentration if the calibration curve is known. For a given compound, its value depends on concentration and temperature.

The influence of temperature on the examined DESs is presented in Fig. 13 d); (Standard uncertainty *u* is: $3.3 \cdot 10^{-5} \leq u(n_D) \leq 3.7 \cdot 10^{-4}$). At higher temperatures, refractive index is reduced. As for other measured properties, the type of HBD and HBA influence the refractive index of DES. The effect of HBD can be established by comparison of DESs based on the same salt. For choline chloride based DESs with the same molar ratio, DES 4 and DES 6, solvent with urea as HBD exhibits higher refractive index, since the refractive index of urea is higher. Similarly, for DESs based on potassium carbonate, the refractive index of solvent with glycerol is higher, due to the higher refractive index of glycerol in comparison to ethylene glycol. Even though salt/HBD molar ratio in DES 7 and DES 8 is not the same, refractive indices for potassium carbonate : glycerol with molar ratio 1:10 range from 1.4850 to 1.4771 in the temperature range from 293 to 333 K [18] and these values are higher than for DES 8. The lowest values were obtained for DESs with ethylene glycol as HBD. The effect of salt on refractive index can be analyzed by comparing refractive indices of DES 4 to DES 7, and DES 5 to DES 8. Having in mind that choline chloride based DESs have lower concentration of HBD than potassium carbonate based DESs prepared in this article and published data for potassium carbonate DESs [18], it can be concluded that choline chloride based DESs have lower refractive indices. The dependence of refractive index on temperature was correlated with linear function:

$$n_D = \mathrm{a} \cdot T + \mathrm{b} \tag{8}$$

Evaluated model parameters are given in Table A.1.

Polarity of DESs

Fig. 14 Molar transition energy of examined DESs

The polarity of a solvent strongly influences its ability to dissolve solutes. Measured polarities of selected DESs and some selected solvents are presented in Fig. 14. It was not possible to measure the polarities of DES 3 and DES 7, since these two solvents were insoluble in reagent solution. The polarities of DESs are slightly lower than water. The highest $E_T(30)$ were obtained for acidic DESs (solvents with malic and citric acid: DES 1 and DES 2), followed by choline chloride based DESs with glycerol, urea and ethylene glycol, while the lowest polarity was observed for DES 8, based on potassium carbonate. The polarity of DES is usually explained by hydrogen bonding ability of HBDs. Generally speaking, the polarity of functional groups increases in the following order: amide > acid > alcohol. DES 2 exhibits lower polarity than DES 1 due to the higher number of carbons in carboxylic acid. The other possible explanation is that smaller dicarboxylic acid forms stronger hydrogen bonds with choline chloride and in dimers than tricarboxylic acid. Besides that, molar ratio HBA/HBD is higher in DES 2, resulting in formation of a quite

different hydrogen bond network. Hydrogen bonds are divided between one molecule of HBD and two molecules of HBA. Glycerol is more polar than ethylene glycol due to the additional hydroxyl group, so DES 4 exhibits higher polarity than DES 5. The polarity of DES with urea as HBD is between DES 4 and DES 5. Even though polarity of amide group is higher, interactions between HBA and HBD are different. Glycerol has three hydroxyl groups so it can form stronger bonds than urea. All HBDs, as well as choline chloride, can accept and donate hydrogen bonds, so it is not surprising that DES 8 has the lowest polarity, since potassium carbonate can only accept hydrogen bonds on three oxygens.