Research Article

Hydroxide-Catalyzed Ellagic Acid Synthesis from Biobased Ethyl Gallate Using Deep Eutectic Solvents

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ABSTRACT: Dilactone ellagic acid (EA) is a polyphenolic compound in various fruits, vegetables, and other biomass structures that has many health-promoting properties. We directly synthesized EA from a gallic acid derivative, ethyl gallate. Previously reported methods were utilized, including synthesis with *N,N*-dimethylammonium *N′,N′*-dimethylcarbamate (DIMCARB) ionic liquid and 3 different hydroxides with aeration, and in addition, we tested a novel method with 5 different choline chloride (ChCl)-based deep eutectic solvents (DESs) in combination with hydroxide and aeration. Despite our optimization attempts, DIMCARB performed very poorly (<2% yield of EA). On the other hand, the reactions with a 1% NH_4OH catalyst resulted in a 50.3% yield of EA with an 85% purity. When 10% DES was added to 1% NH_4OH, conversions increased from 50.3% up to 77.5% with an 82.9% purity. It was confirmed that neutral DES reduces the solubility of EA, which importantly decreases the rate of the reverse reaction, partly explaining the drastic increase in the yield. A scale-up of the most efficient DES/NH_4OH system was also assessed, reaching a 61.8% yield and a purity of over 80%, respectively, indicating importance of O_2 aeration. Finally, a quantitative model of the engineered synthesis process was developed, showing that pH is the most important design factor influencing equilibrium, mechanistic rate kinetics, and the solubility of the product and why the combination of NH_4OH and neutral DES works.

KEYWORDS: ellagic acid, ethyl gallate, hydroxide, deep eutectic solvents, kinetic model
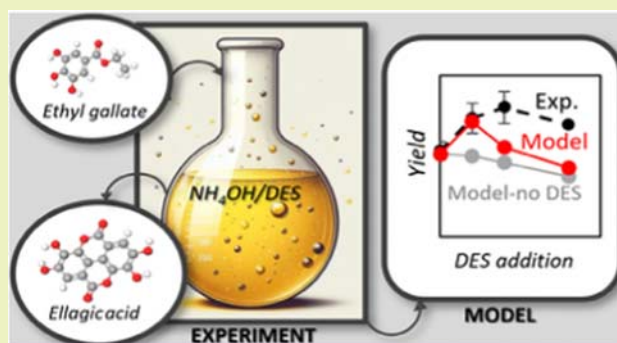
INTRODUCTION

Ellagic acid (EA), a natural polyphenolic compound, is found in various fruits and vegetables and other plant sources and has antioxidant,[1,2] antimutagenic,[3] anticarcinogenic,[1,4−7] anti-inflammatory,[8] and antimicrobial properties.[9] Due to these numerous beneficial effects on human health, developing new and efficient ways of EA production is of utmost importance.

EA is a dimeric derivative of gallic acid. Most of EA is found in the form of hydrolyzable tannins called ellagitannins, which are esters of hexahydroxydiphenic acid and glucose.[10−13] EA is mostly produced by hydrolysis of ellagitannins.[14−16] Ellagitannins are first extracted from sources like waste pomegranate husk and then hydrolyzed using different chemical methods,[15] fungal fermentation,[17] or isolated enzymes.[16] However, if ellagitannin-rich biomass is not available or the extraction of EA would come at the expense of other valuable products from the same biomass source, EA can also be directly synthesized from gallic acid or its esters (e.g., waste biomass gallotannin).[18,19] This direct synthesis approach is also favorable for the production of EA derivates (symmetric and asymmetric), which have been shown to have increased activity.[20,21] There are a few different reaction mechanisms of direct EA synthesis found in the literature (Figure 1).

Chowdhury et al.[19] proposed a mechanism of EA synthesis from ethyl gallate (EtGal) in an ionic liquid that involves transesterification of two EtGal molecules and subsequent oxidative reactions to produce the intramolecular C−C bond (aryl−aryl bond) and thus EA. Others described different varieties of oxidative coupling where esterified gallic acid is first dimerized through aryl−aryl bond formation, followed by formation of the lactone ring (esterification).[18,21−23] One group even described a mechanism where EA and its derivate were synthesized from gallic acid ester through an ellagitannin analogue and its subsequent hydrolysis.[20]

In our study, we aimed at synthesizing EA from ethyl gallate (EtGal). We used simple reaction protocols from the literature, one with an ionic liquid[19] and another with a hydroxide and aeration.[18] We attempted to increase the EA yields and purity by varying the reaction parameters and by adding deep eutectic
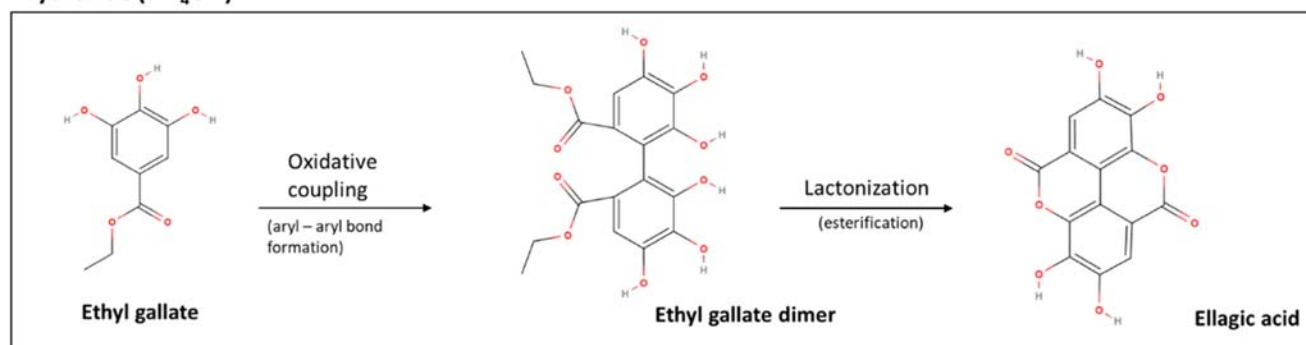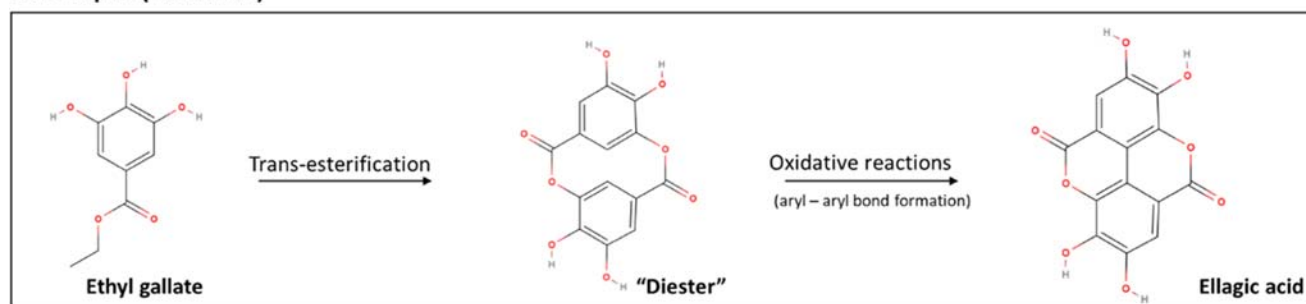
Figure 1. Proposed mechanism of ellagic acid synthesis from ethyl gallate with hydroxides[18] or ionic liquids.[19]

solvents (DESs) to the hydroxide solution. The reactions with the highest yields were also tested in a simple scale-up process. To interlink the knowledge gained experimentally, a kinetic model was developed that explains the relations between experimental yields and reaction conditions, providing additional insights into the process mechanisms.

■ EXPERIMENTAL SECTION

Materials. Ellagic acid (EA; Thermo Scientific), ethyl gallate (EtGal; Thermo Scientific), *N,N*-dimethylammonium *N′,N′*-dimethylcarbamate (DIMCARB; Acros Organics), NH₄OH (25% solution; Roth), KOH (Honneywell), NaOH (Supelco), choline chloride (ChCl; Sigma-Aldrich), glycerol (Sigma-Aldrich), urea (Acros Organics), p-toluene sulfonic acid (p-TSA; Fluka), acetic acid (CH₃COOH; Emsure), and oxalic acid (Sigma-Aldrich) were used in the experiments.

Synthesis of EA with Ionic Liquids. The synthesis of EA using ionic liquids was carried out based on the method by Chowdhury et al.[19] The ionic liquid used was DIMCARB. In the initial experiment, 660 mg of EtGal was dissolved in 4.43 g of DIMCARB and 0.12 g of water and left for 6 h at room temperature (RT) and 150 rpm using a magnetic stirrer. Afterward, 2.3 g of water was added to the mixture and left for EA to precipitate overnight. The original protocol by Chowdhury et al.[19] was then modified by using either 330 or 660 mg of EtGal; reaction times 6 or 9 h; addition of 2.3 (1×), 4.6 (2×), or 6.8 (3×) g of water; and precipitation times up to 14 days. After the reaction, the mixture was transferred to a 14 mL centrifuge tube and centrifuged at 6800 rcf (relative centrifugal field) for 10 min. The supernatant was removed, and the pellet was thoroughly mixed (washed) with 14 mL of lukewarm distilled water (18.2 MOhm) and centrifuged again. This washing procedure was repeated 6 times. After the final washing and centrifugation, the remaining pellet was dried for 24 h at 75 °C.

Synthesis of EA Using Hydroxides. The initial protocol for synthesis of EA in NH₄OH with aeration (circulating air above or through the solution) was based on the method by Zeng et al.,[18] and other hydroxides, KOH and NaOH, were selected based on the literature where they were used in (trans)esterification reactions.[24−27]

A typical reaction consisted of 200 mg of EtGal dissolved in 4 mL of a hydroxide solution in a glass bottle with a cap, left at RT, and mixed at 150 rpm. The cap was not screwed on tight to let in air (aeration). Afterward, the mixture was transferred to a 14 mL centrifuge tube and centrifuged at 6800 rcf for 10 min. The supernatant was removed, and the pellet was thoroughly mixed (washed) with 14 mL of lukewarm distilled water (18.2 MOhm) and centrifuged again. This washing procedure was repeated 6 times. After the final washing and centrifugation, the remaining pellet was dried for 24 h at 75 °C. First 1 (v/v) % NH₄OH was used in 24, 48, and 72 h reactions to determine the optimal reaction time. Then, 0.5, 1, 3, and 5 (v/v) % NH₄OH, KOH, and NaOH were tested in 48 h reactions. The pH values of the hydroxide solutions are listed in Table 1. A control reaction was also prepared where EA instead of EtGal was mixed with 1 (v/v) % NH₄OH and left for 48 h at room temperature. Standard deviation was calculated based on 2−4 separate experiments.

Synthesis of EA Using Deep Eutectic Solvents and NH₄OH. The DES used was selected based on the literature where it was used in esterification and transesterification reactions.[25,26,28] The selected DES were: ChCl/glycerol 1:2 (DES1), ChCl/urea 1:2 (DES2), ChCl:p-TSA 1:3 (DES3), ChCl/acetic acid 1:2 (DES4), and ChCl/oxalic acid 1:2 (DES5). DES was used in 5, 10, and 20 (w/v) % concentrations and was diluted by either water (DES/water) or 1% NH₄OH (DES/NH₄OH). A typical reaction mixture for 10% DES consisted of 200 mg of EtGal, 400 mg of DES, and 3.6 mL of 1% NH₄OH or water. The mixture of DES and NH₄OH or water was prepared first and mixed until a homogeneous liquid was formed, and afterward, EtGal was added. The reaction time was 48 h, and all other reaction and preparation steps were the same as for reactions with hydroxides. The pH values for all of the DES/NH₄OH solutions are presented in Table 1. A control reaction was also prepared, where EA instead of EtGal was mixed with 10% DES/NH₄OH and left for 48 h at room temperature. Standard deviation was calculated based on 2−4 separate experiments.

HPLC Analysis. The amount of EA (purity) in the samples was determined using HPLC. Each sample was dissolved in 100% methanol with 0.1% formic acid (protonation of the EA OH groups) to a final concentration of 50 mg/L, sonicated for 3 min, and filtered through a 0.22 μm syringe filter. The samples were then analyzed

Table 1. pH Values of Reaction Solutions without Ethyl Gallate

solution	[%]	pH
NaOH	0.5	13.2
	1	13.5
	3	13.8
	5	13.8
DES1/NH₄OH	5	11.4
	10	11.3
	20	11.2
KOH	0.5	12.9
	1	13.2
	3	13.7
	5	13.9
DES2/NH₄OH	5	11.4
	10	11.3
	20	11.1
NH₄OH	0.5	11.3
	1	11.4
	3	11.8
	5	12.0
DES3/NH₄OH	10	2.3
DES4/NH₄OH	10	4.7
DES5/NH₄OH	10	3.9

using an HPLC system (Dionex Ultimate 3000, Thermo Scientific) with a Kinetex C18 column (5 μm, 100 Å, 100 × 4.6 mm, Sigma-Aldrich). The mobile phase consisted of distilled water with 0.1% formic acid (A) and 100% methanol with 0.1% formic acid (B). The column temperature was set to 40 °C, the flow rate at 1 mL min⁻¹, the injection volume at 10 μL, and detection at a UV−vis absorption wavelength of 366 nm for EA and 275 nm for EtGal. The mobile-phase gradient was as follows: 0−5 min 40% A and 60% B, 5−7 min 60−90% B, 7−12 min 10% A and 90% B, 12−14 min 90−40% mobile phase B, and 14−16 min 40% A and 60% B. Ellagic acid in the samples was identified and quantified by the external calibration standard (retention time and UV−vis spectra). The retention times were 1.35 min for EA and 1.17 min for EtGal. The UV−vis spectra of the ellagic acid standard showed signature peaks at 254 and 367 nm and EtGal at 217 and 273 nm. The chromatograms and UV−vis spectra of EA, EtGal, and selected samples are presented in the Supporting Information (Figures S1−S8).

Scanning Electron Microscopy and Elemental Analysis. The surface morphology of the samples was analyzed by scanning electron microscopy coupled with energy dispersive spectroscopy (SEM; SUPRA35 VP, Carl Zeiss, EDS: SDD Ultim max 100, Oxford Laica). The samples were mounted on aluminum stubs with double-sided carbon tape and sputtered with a 2.3 nm thick Au coating before observation to ensure sufficient conductivity. SEM-EDS was performed at 20 kV at three different locations. SEM-EDS mapping was also performed to determine the distribution of the impurities.

Scale-Up Experiment. The scale-up experiment was carried out only for 10% DES1/NH₄OH and DES2/NH₄OH reactions. The reaction mixtures were prepared in 500 mL beakers and consisted of 10 g of EtGal, 20 g of selected DES, and 180 mL of 1% NH₄OH. The mixture of DES and NH₄OH was prepared first and mixed until a homogeneous liquid was formed, and afterward, EtGal was added. In the first experiment, the reaction mixture was only stirred at 150 rpm, and in the second, we also introduced air bubbling into the solution. The mixtures were left for 48 h, and the resulting product was centrifuged and washed 6 times in lukewarm water and dried at 75 °C. Each experiment was performed only one time with one parallel.

Theoretical Analysis. Since a large influence of pH on the product yield was observed, hydroxyl groups were included as reaction catalysts in the kinetic modeling. Transesterification can occur under basic conditions,[29] while the biphenyl bond is typically

formed by an acid catalyst and an oxidant.[30] For this reason, we chose the first or second transesterification in the gallate dimer molecule as the rate-determining step (eq 1) (the kinetic expression is the same in both cases). We assume that the concentrations in the reactor are uniform. The byproducts are not part of the model.

$$2\frac{d[EA]}{dt} = k_f[EtGal]^2[OH^-] - k_b[EA][OH^-] \tag{1}$$

Here, k_f and k_b stand for the lumped kinetic coefficients of the forward and reverse reactions and [OH−] for the molar concentration of the hydroxyl groups in the solution, which correlates with a NH₃ pK_b of 4.75. The pK_a of the deprotonation of the phenolic hydroxyl groups present in EtGal is 8.7, 11.4, and above 13,[31] resulting in an initial pH decrease. For simplicity of the model, we assumed single deprotonation for EtGal and double deprotonation for ellagic acid, resulting in a constant pH during the reaction. The deprotonation of ellagic acid is explained below. Therefore, the initial pH was corrected for the addition of EtGal by subtracting the initial NH₄OH concentration.

Since ellagic acid is poorly soluble in water, it precipitates during the reaction. However, the solubility is pH-dependent, with deprotonation of the phenolic hydroxyl groups leading to increased solubility under basic conditions. The literature indicates that ellagic acid undergoes at least 2 stages of deprotonation (at pH 5.6 and 8.5[32]), with solubility increasing with increasing pH. At pH 12.4 and above, hydrolysis of the lactone moiety was observed, resulting in a carboxyl derivative.[33] We have compiled all available data on the solubility of ellagic acid in the range of pH 0.6−10 and temperatures between 20 and 170 °C from various sources,[32−35] from which trends can be derived that correlate with the Henderson−Hasselbach equation, as shown in the work of Bala et al.[35] (Supporting Information Figure S12). However, the typical pH used here is 11.4, which was measured separately in our work (3.1 ± 0.4 g/L) using 40 mg of ellagic acid in 0.5 mL of 5 wt % NH₄OH (1 h at 25 °C), centrifugation, and precipitation with 10% HCOOH to achieve a pH of 3, followed by centrifugation and drying of the precipitate at 75 °C overnight. The Henderson−Hasselbach coefficients of the second deprotonation of ellagic acid to determine solubility were estimated for 25 °C (pK_a = 8.5, S_0 = 3 mg/L) (Table S1). The [EA], the ellagic acid concentration, was limited by the solubility, which was determined with the following equation

$$S(EA) = S_0 \cdot (10^{pH-pK_a} + 1) \tag{2}$$

■ RESULTS AND DISCUSSION

Synthesis of EA with DIMCARB. Our first attempt at EA synthesis was based on a study by Chowdhury et al.,[19] where they used ionic liquid DIMCARB as a solvent/catalyst to produce EA from EtGal. We used the original protocol as well as adapted versions with various amounts of EtGal, reaction and precipitation times, and amounts of added water for EA product precipitation. Although Chowdhury et al.[19] reported a 70% EA yield, we obtained no visible product when using their original protocol. Only after the reaction time was extended from 6 to 9 h, the amount of water increased from 1× to 3×, and the precipitation time extended to more than 24 h did we obtain some EA product in the form of light-yellow crystals (Supporting Information, Figure S9a). However, despite these protocol adaptations, the highest yield of EA was only 1.7%, with a 29.4% purity. The yields were also very variable, ranging from a maximum of 1.7% to as low as 0.2% and the purity from 26.2 to 31.8% (Figure 2). Due to these low and variable yields, the synthesis of EA with DIMCARB was not pursued further.

Synthesis of EA with Hydroxides. Based on the proposed reaction mechanisms,[18,21−23] the synthesis of EA from EtGal involves oxidation reactions (oxidative coupling) and esterification (lactonization) of two EtGal molecules. The
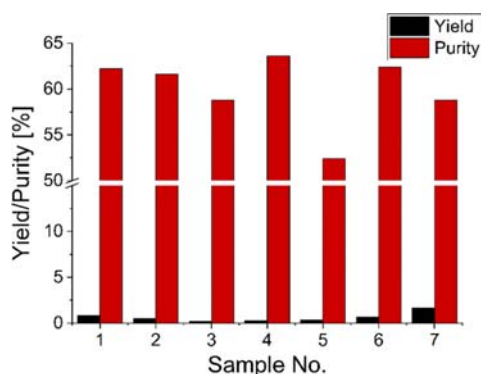
Figure 2. Yield and purity of 7 different ellagic acid samples synthesized with DIMCARB.

oxidant in these reactions is provided by air (oxygen), and the catalyst for esterification is a hydroxide. Hydroxides like KOH and NaOH have been previously used to catalyze these types of reactions;[24−27] however, we first focused on NH₄OH since it has already been used in a similar EA synthesis reaction with good results.[18] Reactions of EtGal in 1% NH₄OH were prepared and left for 24, 48, and 72 h in order to determine the best reaction times. As shown in Figure 3a, the EA yield after 24 h was 41.1%, and the yields after 48 and 72 h were 50.3 and 51.5%, respectively. The purity of all of the EA products was between 80 and 85%, with the highest being that of 48 h samples (84.7%). Since the difference in EA yields between 48 and 72 h reaction time was minimal and the purity of 48 h samples seemed to be the highest, the 48 h reaction time was used in all further reactions. In the next step, different concentrations (0.5, 1, 3 and 5%) of NH₄OH as well as KOH and NaOH were tested (Figure 3b−d). The results in Figure 3b show that the most optimal concentration of NH₄OH was

1% with a 50.3% yield, and the least optimal concentration was 5% with a 33.9% yield. The purity of all samples was again between 80 and 85%. In reactions with KOH, a product was obtained only in 0.5 and 1% solutions with 3.2 and 27.8% yields and 76.6 and 83.9% purities, respectively (Figure 3c). The yields were the lowest with NaOH, where we obtained a 19.5% yield (82.2% purity) with a 1% solution and no product with other concentrations (Figure 3d). The low yield when using KOH and NaOH could be explained by the large pH change when EtGal is introduced into the solution. As mentioned earlier in the Theoretical Analysis section, the phenolic hydroxide of gallic acid deprotonates at about 8.7,[32] which means that almost all of the hydroxide is neutralized at a concentration of 0.5 and 1% NaOH and KOH, resulting in low conversion activity. At concentrations of 3% and more, the pH value on addition is above 12.4, favoring lactone ring-opening (also considering the neutralization of the third proton at pK_b = 11.4). No EtGal was detected in any of the samples, and all of the samples were in the form of a fine light brown powder (Supporting Information, Figure S9b). When EA instead of EtGal was used in the reaction with 1% NH₄OH (control), the resulting product was green in color with a yield and a purity of 36.1 and 78.8%, respectively (Supporting Information, Figure S10), additionally pointing to EA solubility in basic conditions.

Research on the direct synthesis of EA or its derivates from gallic acid or its esters is relatively scarce. Researchers used different one-step methods like direct oxidative coupling with hydroxides or oxidants[18,23] and enzymatic coupling[18] or multistep methods involving Ullman coupling[22] or a combination of oxidants and esterifying agents (methanol).[23] An 83% yield of EA was obtained in 24 h using a one-step reaction of methyl gallate and the oxidant phenyliodine bis(trifluoroacetate) (PIFA).[23] The same group also used a two-step reaction. There, methyl gallate was first dimerized by
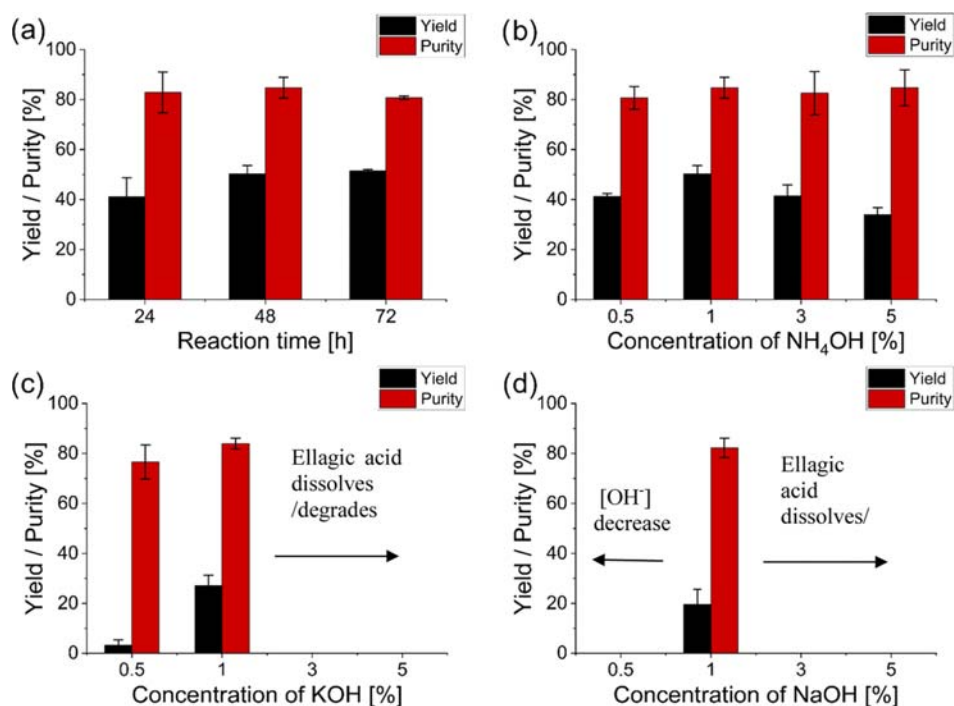

Figure 3. Yield and purity of ellagic acid samples synthesized with 1% NH₄OH at different reaction times (a) or different concentrations of NH₄OH (b), KOH (c), and NaOH (d) after 48 h.
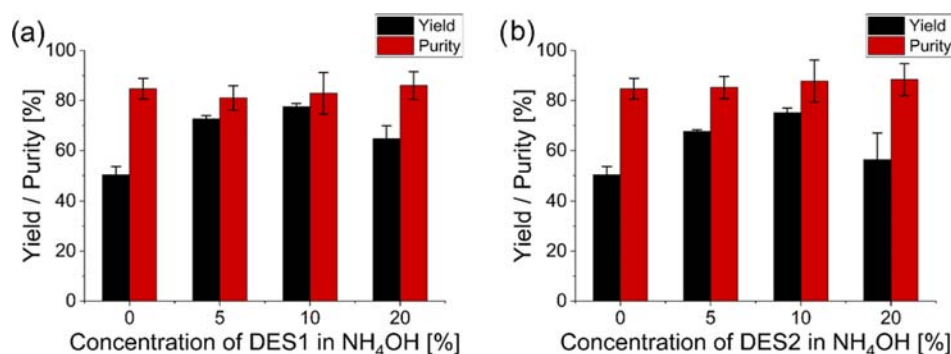
Figure 4. Yield and purity of ellagic acid samples synthesized with different concentrations of DES1 (a) or DES2 (b) in 1% NH$_4$OH after 48 h. DES1—ChCl/glycerol 1:2 and DES2—ChCl/urea 1:2.

oxidative coupling in the presence of the oxidant phenyliodine diacetate (PIDA), yielding 80% of the biaryl in 16 h, which was then quantitatively converted to EA by refluxing with aqueous methanol in 24 h. Our method using hydroxides was based on the method by Zeng et al.,[18] where they used methyl gallate and NH$_4$OH and aeration to produce EA with a yield of 47% and an 88% purity in 24 h. When they used a tyrosinase enzyme instead of NH$_4$OH, the yield and purity increased to 62% and >98%, respectively. Our results, when using the NH$_4$OH/aeration protocol, were in good agreement with this study, with a 50.3% yield and an 84.7% purity of the EA product (Figure 3b).

Compared with NH$_4$OH, the other two hydroxides performed very poorly. Previous research has shown that EA is strongly affected by pH, and strongly alkaline pH results in spontaneous lactone ring-opening in the EA molecule.[33,35,36] Therefore, we considered the pH of the solution as one of the factors influencing the efficiency of the reaction. Table 1 shows that all hydroxide solutions had a strongly alkaline pH (11.3–13.9). Nonetheless, EA synthesis was still very efficient in 1% NH$_4$OH despite the pH of 11.3. However, the EA yield was reduced at higher pH values measured for 3 and 5% NH$_4$OH as well as for all KOH and NaOH solutions. Hasegawa et al.[33] showed that the lactone ring-opening occurs at a pH value of 12.4. This supports our results that show that even 5% NH$_4$OH with pH 12.0 resulted in a higher yield of EA than the lowest concentrations of KOH or NaOH with pH 12.9 and 13.2, respectively. On the other hand, the pH of 0.5% NH$_4$OH was slightly lower than that of a 1% solution, but resulted in lower EA yields. Thus, it seems that in this case, the amount of the hydroxide catalyst was also a limiting factor.

Considering the impurities, the bulk of them are probably reaction intermediates and unspecific products like EA with an open lactone ring, dimers, condensed structures, flavellagic acid, and quinones.[18,33,35,37] However, when analyzed by HPLC, no other peaks were detected. This is because these products either did not bind to the HPLC column or did not absorb at the selected wavelengths. Deprotonated forms of EA also would not be detected since the sample was dissolved in acidified methanol prior to analysis, which would protonate the OH groups.

When the same amount of EA instead of EtGal was used in the reaction with 1% NH$_4$OH, the final product was a green color instead of light brown, and the yield (the remaining EA in the solution) was significantly lower than 100%. A part of the EA was surely lost during the washing process, and some of it was dissolved in the reaction solution, but these losses

should be minimal. More likely, EA reacted with the NH$_4$OH catalyst, which affected its solubility. This reaction between EA and NH$_4$OH is also supported by the reduced purity of this EA. The fact that the reaction with EtGal resulted in higher EA yields compared to the control indicates that reaction byproducts like ethanol could play a role in preventing the interaction between the produced EA and the NH$_4$OH catalyst. Also, the similar purity of the synthesized EA and EA control points to EA−NH$_4$OH interaction products comprising the majority of the impurities.

Enhancing EA Synthesis Using DES Mixtures. Hydroxides have previously been successfully used in combination with DES in (trans)esterification reactions.[25,26,28] We prepared 5 different ChCl-based DES and diluted them to a final concentration of 5, 10, or 20% by either 1% NH$_4$OH (DES/NH$_4$OH) or water (DES/water). Reactions of EtGal in DES/NH$_4$OH or DES/water were prepared and incubated for 48 h. The reaction conditions and subsequent product preparation were identical to those with only 1% NH$_4$OH and other hydroxides. An EA product was formed only with DES1/NH$_4$OH (DES1—ChCl/glycerol 1:2) and DES2/NH$_4$OH (DES2—ChCl/urea 1:2) (Figure 4). Reactions with DES3/NH$_4$OH (DES3—ChCl:p-TSA 1:3), DES4/NH$_4$OH (DES4—ChCl/acetic acid 1:2), and DES5/NH$_4$OH (DES5—ChCl/oxalic acid 1:2) did not yield a product, and neither did reactions with DES1/water and DES2/water (data are not shown). Compared to reactions with only 1% NH$_4$OH (Figure 3b), the addition of either DES1 (Figure 4a) or DES2 (Figure 4b) markedly increased product yields. The highest EA yields were obtained in 10% DES1/NH$_4$OH and DES2/NH$_4$OH mixtures and were 77.5 and 75.1% with 82.9 and 87.8% purities, respectively. The other two DES concentrations (5 and 20%) were less efficient when considering product yields but with similar product purity (between 81.1 and 88.4%). The lowest yields were obtained with 20% DES1/NH$_4$OH and DES2/NH$_4$OH, 64.7 and 56.3%, respectively. No EtGal was detected in any of the samples. As a control, EA instead of EtGal was used in the reaction with 10% DES1/NH$_4$OH, and the resulting product once more displayed a green color and a yield and a purity of 65.2 and 80.6%, respectively (Supporting Information, Figure S10).

Deep eutectic solvents are mixtures of two or more pure compounds (mostly in solid form), a hydrogen bond donor, and a hydrogen bond acceptor. When combined at specific molar ratios, they form a liquid that exhibits an eutectic point temperature that is far below an ideal liquid mixture and presents low vapor pressure and high thermal stability.[38] DES
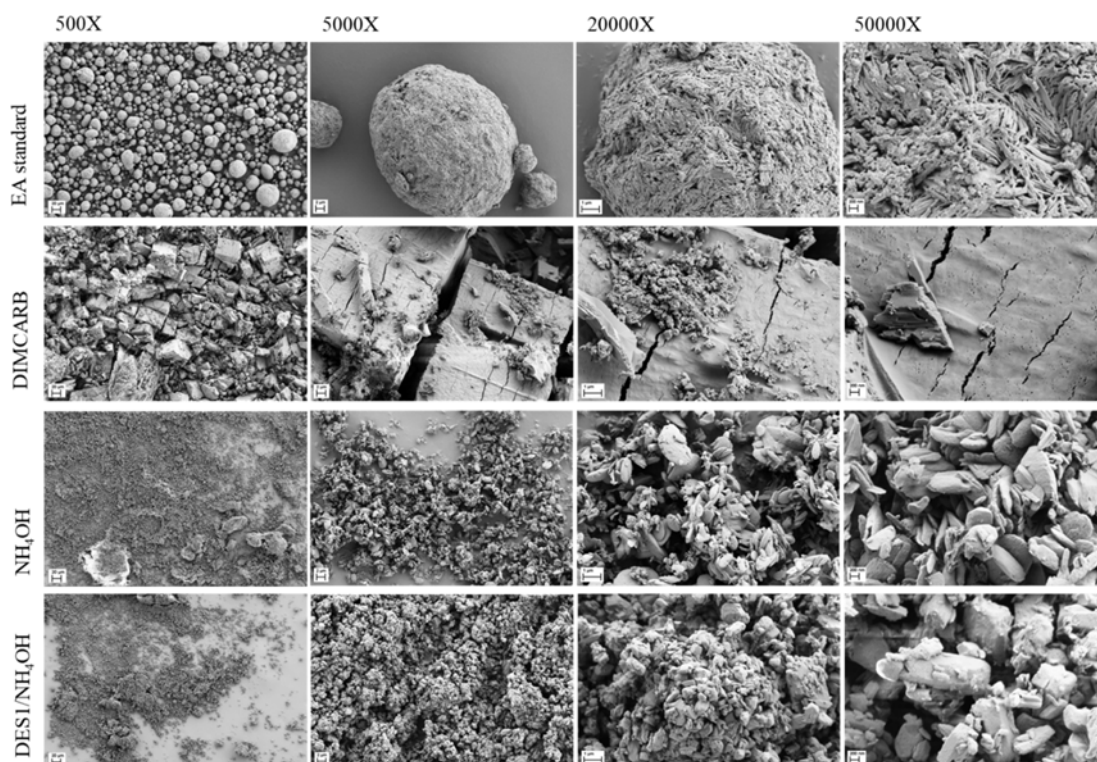
Figure 5. SEM images of the EA standard and ellagic acid samples synthesized with DIMCARB, 1% NH_4OH, and 10% $DES1/NH_4OH$. EA—ellagic acid and DES1—ChCl/glycerol 1:2.

Table 2. EDS Analysis of the Ellagic Acid Standard and Ellagic Acid Samples Synthesized with DIMCARB, 1% NH_4OH, and 10% $DES1/NH_4OH$[a]

name	composition [wt %]						14·O/C at. ratio	14·N/C at. ratio
	C	O	N	Na	Cl	S		
EA standard	58 ± 1	41 ± 1	0.2 ± 0.1	0.3 ± 0	0	0.1 ± 0	7.4 ± 0.2	0 ± 0
DIMCARB	59 ± 2	34 ± 1	7 ± 1	0	0	0	6.1 ± 0.2	1.4 ± 0.2
NH_4OH	57 ± 3	38 ± 2	5 ± 2	0.1 ± 0	0	0	6.9 ± 0.3	1.1 ± 0.5
$DES1/NH_4OH$	51.8 ± 0.1	39.7 ± 0.1	8 ± 0.1	0	0.5 ± 0	0	8.1 ± 0	1.9 ± 0

[a]EA—ellagic acid and DES1—ChCl/glycerol 1:2.

has been shown to modulate (increase) compound solubility, thermal stability, and reaction efficiency.[39−42] These solvents have also been used in (trans)esterification reactions as sole catalysts or in combination with hydroxides.[25,26,28,43,44]

As shown in the previous section, the pH seems to affect EA synthesis. This is again evident here since the only DES solutions that produced EA, i.e., $DES1/NH_4OH$ and $DES2/NH_4OH$ mixtures, were the ones with alkaline pH as opposed to the ones with acidic pH ($DES3−5/NH_4OH$). Furthermore, the fact that reactions in DES/water mixtures did not yield any product indicates that the DESs were not acting as catalysts but rather as solubility modulators. It is possible that DES3−5 increased the solubility of EA, which subsequently did not precipitate from the solution and/or inactivated the alkaline NH_4OH preventing it from catalyzing the reaction. The effectiveness of $DES1/NH_4OH$ and $DES2/NH_4OH$ mixtures is most likely connected to reduced solubility of EA. This is supported by the control reaction where EA instead of EtGal was used (Supporting Information, Figure S10). Although the product was still a different color (green instead of light brown), the yield (remaining EA in solution) in the $DES1/NH_4OH$ mixture was 65.2% compared to only 36.1% in the

only NH_4OH solution. However, the purity of the extracted EA was still reduced (80.6%) and was similar to that of the synthesized EA product, which indicates that the interactions between EA and NH_4OH still took place.

Morphology and Elemental Analyses. The morphological features of EA standard and DIMCARB, 1% NH_4OH, and 10% $DES1/NH_4OH$ samples were analyzed by SEM and are presented in Figure 5. The images were taken at 4 different magnifications. All of the samples showed distinct morphological characteristics. The EA standard showed spherical shapes of different diameters that seem to be composed of mostly smaller rod-like structures. DIMCARB samples exhibited rectangular formations that originated from larger crystals that could be seen in the reaction mixture (Supporting Information, Figure S9). Based on the cracked surface, the crystals were likely very brittle and would break apart into much smaller particles. Another interesting feature that was visualized in certain parts of the sample was a kind of smooth crust on the outside of the large particles with small, ordered, rod-shaped formations on the inside (Supporting Information, Figure S11a,b). NH_4OH and $DES1/NH_4OH$ samples looked similar at 500× magnification, but distinct differences were
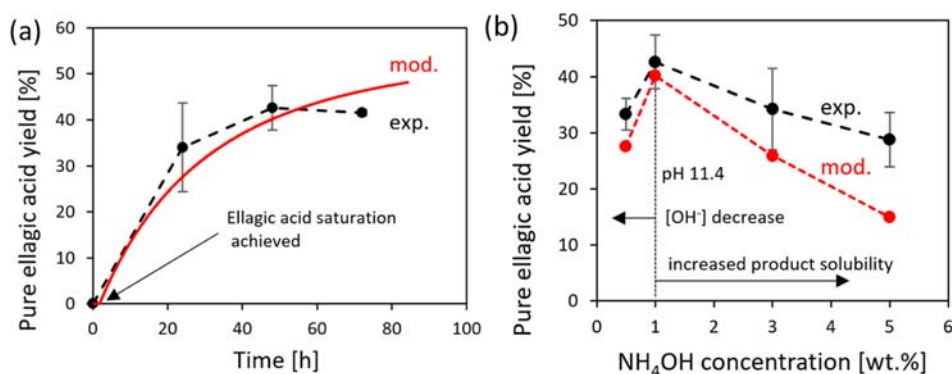
Figure 6. Comparison of model and experimental data of the pure ellagic acid precipitate during the variation of (a) time at 1% wt. NH$_4$OH without DES and (b) NH$_4$OH initial concentration measured at 48 h without DES.

observed at higher magnifications. The NH$_4$OH sample showed flattened elliptical shapes that came in a wide variety of sizes, whereas the DES1/NH$_4$OH sample showed a variety of different shapes like irregular spheres, rectangular shapes, and rods, also in a wide variety of sizes. Compared to the EA standard, particle size in these samples was significantly smaller.

EDS analysis was also performed on the samples (Table 2). It showed an increased amount of N in EA samples synthesized with DIMCARB, 1% NH$_4$OH, and 10% DES1/NH$_4$OH compared to the EA standard. The amount of N in the EA standard was 0.2 wt %, whereas the amount of N in DIMCARB, 1% NH$_4$OH, and 10% DES1/NH$_4$OH was 6.7, 5.5, and 8 wt %, respectively. 10% DES/NH$_4$OH also contained 0.5 wt % of Cl.

We also observed differences in the O/C and N/C ratios, which could be due to a different particle size of the precipitate, resulting in less decomposition of the ammonium salt during drying or washing. Based on the pK_a values, a diammonium ellagate salt is formed first, and therefore, the theoretical range of nitrogen per ellagic acid (C$_{14}$H$_6$O$_8$) is between 0 and 2, which is shown in Table 2. Furthermore, the SEM-EDS mapping results support this claim, with N evenly distributed across the sample (Supporting Information, Figure S13). The slightly larger measured O/C ratio than expected (7.4 ± 0.2, theoretically 8) could be due to the background contribution of the graphite tape.

Wang et al.[45] analyzed the surface morphology of their crude and purified EA extracted from pomegranate husks. They showed that the crude extract was composed of larger irregular spheres, whereas the purified extract was in the form of smaller block-shaped structures. In our case, the EA standard showed spherical shapes of different sizes, which were all significantly larger compared to the structures in the 1% NH$_4$OH and 10% DES1/NH$_4$OH EA samples. Although the structures in the DIMCARB sample were relatively large, they seemed to readily break down into much smaller ones. This small particle size was likely the cause of some product loss during the washing procedure since the small particles do not settle well during centrifugation compared to larger ones. Although most of the product was gathered in a pellet at the bottom of the centrifuge tube, we could still see a very fine mist in the supernatant (not shown) that we were not able to separate and therefore discarded along with the supernatant. Although minimal, these losses might be a more considerable problem in large-scale

production. Therefore, more efficient separation techniques must be applied.

Considering the impurities, EDS analysis showed the presence of N and Cl in the powdered EA samples. This could indicate the presence of some residual reaction media since DIMCARB, NH$_4$OH, and both DES contain N, and the latter also contains Cl. Therefore, downstream processes should involve removal of ammonia if ellagic acid is specifically required.

Kinetic Modeling. The model was initially based only on experiments with NH$_4$OH. As a weak base, it dissociates poorly, resulting in relatively small changes in pH under relevant reaction conditions when consumed. In contrast, KOH and NaOH dissociate almost completely, leading to large changes in pH and high sensitivity of the system, and were therefore not modeled. From the comparison between the modeling and experimental results of the yield of a pure ellagic acid precipitate (Figure 6), we can see that the trends can be described relatively well with only two reaction constants, where k_f equals 46 m^6/(kmol^2 h) and k_b equals 96 m^3/(kmol h). Compared to the forward reaction constant of glycerol oleate−ethanol transesterification at 298 K (1.7 m^6/(kmol^2 h)),[29] ellagic acid synthesis is relatively fast, although the upper limits of the reaction pH limit the overall reaction rate. In Figure 6b, we see the maximum yield of ellagic acid at 1 wt % (pH 11.4), which is due to a low [OH$^-$] at lower NH$_4$OH concentrations and increased solubility at higher pH. The agreement could be improved by a more accurate solubility measurement, a pH measurement during the experiment, and determination of the byproducts. In addition, we neglect here that the experimental product is the ammonium salt of ellagate, with about one nitrogen per molecule in the dried sample prepared with NH$_4$OH, as shown in the SEM-EDS analysis (Table 2), resulting in a 5% relative lower experimental yield.

Moving to the DES1/NH$_4$OH system, we can observe that pH slightly drops with DES addition (Table 1), which was considered and is the only reason for the differences in simulations with DES amount variation. In Figure 7, we can observe a predicted decrease of the ellagic acid precipitate yield when the DES amount was increased, correlating with the pH decrease. From reference experiments with the ellagic acid standard, we observed a larger recovery of ellagic acid in the case of DES1/NH$_4$OH than with NH$_4$OH only (Supporting Information, Figure S10). Therefore, DES1 probably limits ellagic acid solubility and therefore increases the precipitate yield and limits backward reactions of ellagic acid. To test the
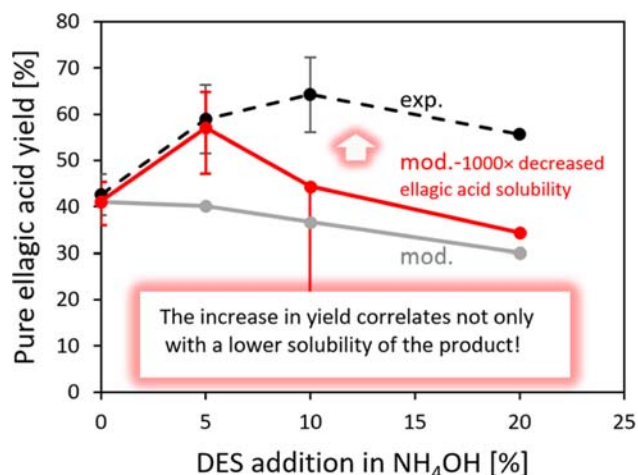
Figure 7. Comparison of the experimental pure EA yield with various amounts of DES1 in 1% NH_4OH (black) with modeling results. The modeling results are presented in grey, without considering the DES1 effect on solubility, and in red, where the solubility of EA is reduced by 1000-fold.

implications to the extreme, we, in the model, divided the ellagic acid solubility by 1000, which resulted in a significantly larger yield at low addition of DES1. However, at high DES1 addition, solubility modification does not have a large effect on the precipitate yield and is more closely correlated with pH. Therefore, some other factor relating to DES addition also influences the yield of the precipitated product.

In the DES1/NH_4OH system, we can observe that the pH drops slightly when DES is added (Table 1). This was considered and is the only reason for the differences in the simulations with DES amount variation. In Figure 7 we can observe the predicted decrease in the ellagic acid precipitation yield when the amount of DES was increased, which correlates with the pH decrease. In reference experiments with the ellagic acid standard, we observed a larger yield of ellagic acid in the case of DES1/NH_4OH than with NH_4OH alone (Supporting Information, Figure S10). Therefore, DES1 probably limits the solubility of ellagic acid, thus increasing the yield of the precipitate and limiting the back reactions of ellagic acid. To push the effects to the extreme, we divided the solubility of ellagic acid by 1000 in the model, which resulted in a significantly higher yield with a low addition of DES1. However, at high DES1 addition, the change in solubility

does not have a large effect on the precipitate yield and is more closely correlated with the pH. Therefore, another factor associated with the addition of DES also affects the yield of the precipitated product.

Scale-Up Experiment. In order to test the potential feasibility of the industrial production of EA, a simple scale-up process was also performed. We used only the reactions that yielded the best results, i.e., reactions with 10% DES1/NH_4OH or DES2/NH_4OH. The reactions were performed either with or without air bubbling. The results in Figure 8a,b show that in the absence of air bubbling, the EA yields were only 19.7% for DES1/NH_4OH and 15.8% for DES2/NH_4OH, compared to small-scale reactions where the yields were 77.5% for DES1/NH_4OH and 75.1% for DES2/NH_4OH. However, when air bubbling was introduced, the yields increased to 61.8% and 56.6%, respectively. The purity of all samples was between 82 and 92%. Although the yields were not as good as in small-scale experiments, they were still relatively high and could probably be improved even further with some optimization, such as more efficient air bubbling (better dispersion throughout the reaction solution) or possibly the use of an additional oxidant. In addition, a reactor with (semi) continuous pure solid EtGal addition and precipitate removal and pH and temperature control could be used. In this way, we could increase the product yield and decrease the reactor downtown. However, the feasibility of large-scale production is not dependent only on yields but also on the process of product purification (including the potential toxicity of the impurities), which is not yet developed and, therefore, cannot be evaluated. Nonetheless, the results seem promising and can at least provide a starting point for further research and development.

■ **CONCLUSIONS**

In this study, we synthesized ellagic acid (EA) from ethyl gallate (EtGal) with the use of different catalysts, i.e., ionic liquid, hydroxides/aeration, and DES/hydroxide/aeration. The highest yields were obtained in 10% DES1 (ChCl:glycerol) in 1% NH_4OH and amounted to 77.5% with 82.9% purities. In comparison, only a 50.3% ellagic acid yield was achieved in the DES-free system at a similar product purity. This was partially assigned to a decrease in ellagic acid solubility, as confirmed by additional experiments and kinetic modeling. The impurities were most likely unspecific reaction products and intermediates, products of ellagic acid−NH_4OH interaction, and
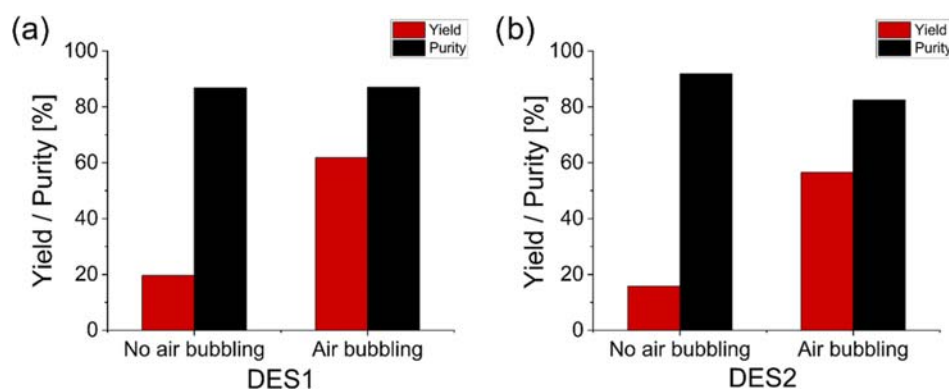

Figure 8. Scale-up process: yield and purity of the ellagic acid product from reactions with 10% DES1/NH_4OH (a) or 10% DES2/NH_4OH (b) with or without air bubbling. DES1 —ChCl/glycerol 1:2 and DES2—ChCl/urea 1:2.

possibly some residual reaction media components. The process was efficient even at a 50-times larger scale with a 61.8% ellagic acid yield, where we also showed that active air introduction is critical when enlarging the process. Using kinetic modeling, we were able to describe the trends in the pure product yield with hydroxides using pH variation and ellagic acid solubility with only two kinetic parameters. Despite the observed reduction in ellagic acid solubility with DES1 (ChCl:glycerol), which we observed in a reference experiment and incorporated into the model, the kinetic modeling predicts that other DES-related factors also increase the ellagic acid yield.

To summarize, the discovery of the positive effect of the inexpensive and safe DES additive represents a very important factor in ellagic acid synthesis. This study opens the field in which pure ellagic acid could be produced by modification of DES and changing the process conditions, while DES could also be introduced into other reactions to influence the solubility of components and thus increase the overall performance.

■ ASSOCIATED CONTENT

ⓈI Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.3c06824.

> Additional details, including HPLC chromatograms of the EtGal and EA product and standard, photographs of the EA product, SEM-EDS mapping results, experimental control reactions, and ellagic acid solubility modeling (PDF)

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Author Contributions

M.M.C. developed the methods, performed the experiments, analyzed the data, and wrote the manuscript. A.P. performed the SEM and EDS analysis, developed the model, and wrote the related parts of the manuscript. B.L. was responsible for funding acquisition, supervised the process, and reviewed the manuscript.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

EA –ellagic acid; EtGal –ethyl gallate; DES –deep eutectic solvents; DIMCARB –N,N-dimethylammonium N',N'-dimethylcarbamate; DES/NH$_4$OH –DES in 1% NH$_4$OH

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