

Hydrothermal Reaction Kinetics and Pathways of Phenylalanine Alone and in Binary Mixtures

Shujauddin Changi, Minghan Zhu, and Phillip E. Savage^{*[a]}

We examined the behavior of phenylalanine in high-temperature water (HTW) at 220, 250, 280, and 350 °C. Under these conditions, the major product is phenylethylamine. The minor products include styrene and phenylethanol (1-phenylethanol and 2-phenylethanol), which appear at higher temperatures and longer batch holding times. Phenylethylamine forms via decarboxylation of phenylalanine, styrene forms via deamination of phenylethylamine, and phenylethanol forms via hydration of styrene. We quantified the molar yields of each product at the four temperatures, and the carbon recovery was between 80–100% for most cases. Phenylalanine disappearance follows first-order kinetics with an activation energy of $144 \pm 14 \text{ kJ mol}^{-1}$ and a pre-exponential factor of $10^{12.4 \pm 1.4} \text{ min}^{-1}$. A kinetics model based on the proposed pathways was consistent with the experimental data. Effects of five different salts (NaCl, NaNO₃, Na₂SO₄, KCl, K₂HPO₄) and boric acid (H₃BO₃) on

phenylalanine behavior at 250 °C have also been elucidated. These additives increase phenylalanine conversion, but decrease the yield of phenylethylamine presumably by promoting formation of high molecular weight compounds. Lastly, binary mixtures of phenylalanine and ethyl oleate have been studied at 350 °C and three different molar concentration ratios. The presence of phenylalanine enhances the conversion of ethyl oleate and molar yields of fatty acid. Higher concentration of ethyl oleate leads to increased deamination of phenylethylamine and hydration of styrene. Amides are also formed due to the interaction of oleic acid/ethyl oleate and phenylethylamine/ammonia and lead to a decrease in the fatty acid yields. Taken collectively, these results provide new insights into the reactions of algae during its hydrothermal liquefaction to produce crude bio-oil.

Introduction

The world's escalating energy demands motivates the production of sustainable liquid fuels. Research is conducted in alternative and renewable sectors, and biofuels obtained from biomass is one such area. Algae are potentially promising biomass candidates. They have a higher ($\approx 20\times$) photosynthetic efficiency than terrestrial plants.^[1] Algae can grow in brackish and saline water, thereby reducing the competition for land with conventional food crops.^[2] Thirdly, algae have higher lipid content per area of land cultivated than other biomass sources such as soybean, sunflower, or palm oil.^[3] Lastly, microalgae cultivation can be coupled with recycling of wastewater and refinery flue gases to make the process more economical and environmentally benign.

Hydrothermal processing of algal biomass has been widely reported in the past, involving conversion of wet algal biomass to crude bio-oils,^[2] carbonized solids,^[4,5] and fuel gases.^[6] Hydrothermal liquefaction of microalgae is a potentially promising route to obtain high-energy-density, fungible advanced biofuels. This process involves treatment of algal biomass in an aqueous medium at elevated temperatures (250–350 °C) and pressures (1.5–17 MPa). Water has distinctly different properties at high temperatures and pressures than at ambient conditions. For example, water at high temperature and high pressure has a lower dielectric constant, higher dissociation constant, and increased solubility of organic compounds as compared to that at room temperature.^[7] Thus, biomacromolecules in algae are more susceptible to hydrolytic attack under these

conditions. Hydrothermal processing is well suited for wet biomass because it obviates the need to dry the feedstock, an energy-intensive pretreatment step that lowers the overall efficiency of the process.

Algae biomass is a complex mixture of biomacromolecules such as polysaccharides, triglycerides, lipids, and proteins.^[2] It can be very challenging to understand the chemistry of hydrothermal liquefaction, if starting directly with algae. Instead, one can represent the complex biomacromolecules with simple model compounds containing the same functional groups. One expects these model compounds to behave similarly to their corresponding biomacromolecules in high temperature water (HTW). The study of biomass model compounds can provide useful insights and understanding into the reaction products, pathways, kinetics, and mechanisms of bio-oil production from actual biomass. However, there have been only a few previous hydrothermal reaction studies for model compounds of algal biomass. We recently reported on phytol and ethyl oleate, model compounds representing chlorophyll

[a] S. Changi, M. Zhu, P. E. Savage
Chemical Engineering Department
University of Michigan
Ann Arbor, MI 48109-2136 (USA)
Fax: (+1) 734-763-0459
E-mail: psavage@umich.edu

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and ester linkages in triglycerides, respectively.^[8–10] The present study uses an amino acid as a model compound to understand the behavior of protein residues in algae during hydrothermal liquefaction.

Different strains of algae have varying composition of proteins ranging from as low as 6 wt% to as high as 71 wt% (on a dry algae basis).^[11] Leucine, valine, lysine, phenylalanine, arginine, aspartic acid, glutamine, alanine, and glycine are the major amino acids constituting various proteins in algae.^[11] During hydrothermal liquefaction, the proteins likely undergo hydrolytic depolymerization to release peptide fragments. Thus, hydrothermal liquefaction of protein-containing algae produces “bio-oil” containing either protein residues with the above amino acids or new products obtained through their reaction with other constituents of algae (e.g., glucose, triglycerides etc.) Researchers have identified indole, pyrazine, pyridine, pyrrole, and their derivatives in the bio-oil obtained from liquefaction of algae from 200–600 °C.^[2,12–15] These products likely arise from different amino acids. For example, Hwang et al.^[16] have identified pyridines, pyrroles, oxazoles, and other N-containing compounds during the reaction of glucose and several amino acids in hot liquid water. To obtain a better understanding of the chemistry of bio-oil production during algae liquefaction, one needs to investigate the reactions of amino acids in HTW. We use this motivation to explore the kinetics of a model amino acid in HTW and elucidate its implications on algae liquefaction.

Several researchers have studied the behavior of different amino acids in HTW.^[17–30] Vallentyne^[17] studied the decomposition of several amino acids under subcritical water conditions ($T < 243$ °C) and modeled the decarboxylation of amino acids using first-order kinetics. Despite the great efforts made in his research, he faced many technological problems due to the breaking of glass reactors used in his work. Others too have mentioned decarboxylation as the main reaction of alanine and glycine in HTW and reported first-order rate constants.^[18–20]

Sato et al.^[22] measured the decomposition of five amino acids (alanine, leucine, phenylalanine, serine, and aspartic acid) at 200–340 °C, 20 MPa, and 20–180 s. They reported that decarboxylation to amines and deamination to organic acids were the two main pathways under these conditions. These authors suggest that alanine and glycine are produced as intermediates from serine. However, a limitation that prevents their work from being more useful for learning about algae liquefaction is the short residence times (< 3 min) used.

Klingler et al.^[23] studied alanine and glycine in hydrothermal conditions at 250–450 °C, 34 and 24 MPa, and a residence time of 2.5–3.5 s. They too found organic acids and amines as the main products of reactions, but they also suggest that glycine could dimerize to diketopiperazine. Other researchers have also reported trimers and tetramers of glycine and alanine at 200–350 °C, 15–40 MPa, and 120 s.^[31–33] Cox and Seward^[24] used a custom-built spectrophotometric cell and in situ observation to report dimerization and subsequent cyclization of alanine and glycine. They also fit the oligomerization kinetics of these amino acids to their experimental data using various

reaction orders and concluded that the best fit was obtained when oligomerization followed second-order kinetics. However, their study was conducted only at low temperatures from 120–165 °C. In a recent study, Otake et al.^[34] reported oligomers up to pentamers for alanine and glycine at high temperatures (180–400 °C), reaction times from 2 to 24 h, and high pressure (1.0–5.5 GPa) without using a catalyst. However, these authors do not report rate constants for these oligomerization reactions starting from amino acids. Otake et al.^[34] also report that under their experimental conditions, deamination of amino acids was favored over decarboxylation. They attribute this difference in the pathways between their work and that of others^[17–20] to the high pressures (several GPa) used in their work. Sakata et al.^[35] studied the effect of pH on the dimerization rate of glycine under hydrothermal conditions. They have shown that the rate of dimerization increases with pH, with a maximum rate occurring at pH of 9.8 and 150 °C.

Abdelmoez et al.^[25,26] studied the kinetics of 17 amino acids in saturated subcritical water (230–290 °C), batch holding times ranging from 2.5–40 min, both individually and as mixtures. Glycine, alanine, valine, and proline were produced as intermediate products from other amino acids in their study. For phenylalanine in HTW, the authors proposed a deamination pathway due to the formation of formic acid, carbonic acid, and ammonia. However, these authors may have overlooked the possibility of carbonic acid formation due to a decarboxylation pathway. They also reported that amino acids in mixtures were less stable and that their activation energies decreased. Furthermore, they conducted a pH study and found most amino acids to be labile at acidic and near-neutral pH values but more stable at basic pH 10. Li and co-workers^[28–30] have studied the kinetics of decarboxylation of aliphatic amino acids, effects of different side chain constituents, and effect of pH on the hydrothermal stability of these amino acids at 270–340 °C using an FTIR spectroscopy flow reactor. They are also the only research group, to the best of our knowledge, to have studied the effect of addition of salt (KCl) on hydrothermal decarboxylation.^[28] They found that the decarboxylation rate for α -alanine decreases with addition of KCl. They hypothesized that either the transition state was less polar than the zwitterionic reactant due to solvent association on addition of salt or that K^+ or Cl^- ions formed complexes with the ionic sites of α -alanine thereby reducing its activity. However, the authors presented no detailed work to support their hypothesis.

Although a number of studies exist on the behavior of amino acids in HTW, there is a gap in the literature because no detailed characterization of products, pathways, and kinetics at high temperatures and longer batch holding times have been carried out in the past. To the best of our knowledge, no study has been reported for amino acids above 340 °C and at times longer than 3 min. These longer times are very relevant for hydrothermal liquefaction of algae because they lead to maximum yields of bio-oil. There has been no previous detailed exploration of all the possible products and pathways that may arise from amino acids under these conditions. Furthermore, different pathways, decarboxylation and deamination, have

been reported previously for reaction of amino acids in HTW but only on the basis of measuring carbon dioxide product yields. Measuring carbon dioxide evolution alone is not sufficient. Other products from amino acid reaction in and with HTW should be thoroughly identified to obtain a proper understanding of the reaction pathways. Furthermore, most of these previous studies are devoted to understanding primarily the behavior of simple amino acids such as alanine and glycine and not the more complicated amino acids.

It is interesting to note that dried algal biomass contains salts in varying amounts (5–20 wt% ash content).^[2,36] To the best of our understanding, only Li et al.^[28] has previously studied the effect of salts on amino acid reaction pathways and kinetics in water at high temperatures. It is possible that addition of salts could accelerate the decomposition of amino acids as is evident during hydrothermal gasification reactions of amino acids or other carbon containing compounds.^[37–40]

Lastly, given the broad motivation to better understand reactions occurring during hydrothermal liquefaction of algae, mixtures of organic compounds need to be studied in HTW. It is quite possible that a mixture of organic compounds may give new products that are otherwise not observed when working with individual model compounds. To the best of our knowledge, glucose is the only organic compound that has been studied along with amino acids in high-temperature, high-pressure water.^[41–43] Peterson et al.^[41] showed that a binary mixture of glucose and glycine at 250 °C and 10 MPa undergoes the Maillard reaction, where the sugar and amino acid combine to form the so-called Amadori compound. This product then undergoes a number of reactions to form polymeric compounds referred to as melanoidins. The authors also showed that the presence of glucose accelerated glycine disappearance, whereas the presence of glycine either increased or decreased glucose decomposition (depending on the initial glycine concentration). Clearly, the binary mixture behaves differently than either of the starting model compounds. Kruse et al.^[42,43] have studied the influence of alanine on glucose in the presence of potassium salts but for hydrothermal gasification carried from 460–550 °C at 25 MPa for reaction times between 5–9 s. They found that the gas yields decrease in the presence of amino acids and the dissolved organic carbon content increases, which they also attributed to the Maillard reaction.

To sum up, this critical assessment of the literature shows that information about the behavior of amino acids at hydrothermal liquefaction conditions, the influence of salts, and the influence of organic mixtures is not readily available. Such information would provide a better understanding of hydrothermal liquefaction of algae. Therefore, we report herein on the kinetics of phenylalanine in high temperature water from 220–350 °C, including a thorough product identification, quantification, and examination of possible pathways operative under these conditions. We also report on the effects of various inorganic compounds commonly used in the culture media for algae (NaCl, NaNO₃, Na₂SO₄, KCl, K₂HPO₄), and boric acid (H₃BO₃), on phenylalanine behavior in HTW. Finally, we report

on the reactions of a binary mixture of ethyl oleate and phenylalanine at 350 °C from 10–60 min.

We selected phenylalanine as the model amino acid because 1) phenylalanine is one of the most abundant amino acids in algae; 2) phenylalanine has an aryl substituent on a β-alanine backbone, which might lead to previously unexplored steric or electronic effects; 3) phenylalanine has not been previously studied in great detail in HTW; and 4) phenylalanine and its products have better sensitivity and ease of detection by HPLC and GC. The binary system of ethyl oleate and phenylalanine was chosen because of the work reported for these individual model compounds previously and herein.^[8,9]

Results and Discussion

In this section, we first describe preliminary work that was performed to validate the experimental methods. We then report the product distribution, elucidate the reaction pathways, and develop a corresponding quantitative phenomenological kinetics model. Lastly, we note the results pertaining to the binary mixtures of phenylalanine with salts (or boric acid) and with ethyl oleate.

Control experiments were performed to assess our ability to measure accurately the amount of phenylalanine in the reactor. These experiments, which were essentially a run performed at room temperature, resulted in a phenylalanine recovery of 98 and 95 % without and with K₂HPO₄, respectively, thereby verifying the suitability of the methods used for quantifying the amount of phenylalanine.

Reaction products

The product molar yields and phenylalanine conversion at different times and temperatures are presented in Table 1. Phenylethylamine was the major product at all but the most severe conditions examined. Small amounts of styrene and phenylethanol formed as minor products when conversion was < 95 %. Both 1- and 2-phenylethanol appeared in these experiments, but we summed their yields and report that sum hereafter as phenylethanol. To the best of our knowledge, neither styrene nor phenylethanol has been reported previously as a product from hydrothermal treatment of phenylalanine.

The yields of phenylethylamine increased with increasing batch holding times for runs at 220, 250, and 280 °C. The highest yield for phenylethylamine was about 0.68 at 280 °C and 60 min. At 350 °C, the yield of phenylethylamine increased from 0.36 at 5 min to 0.62 at 10 min and then decreased thereafter. This behavior suggests that phenylethylamine decomposes to secondary products under severe conditions. The yields of styrene were low (< 0.01) at 220, 250, and 280 °C (until 30 min). However, the yield of styrene increased to 0.044 at 280 °C and 60 min. At 350 °C the yield of styrene increased from 0.001 at 5 min to 0.38 at 60 min. At this condition, the yield of styrene exceeded that of phenylethylamine, and styrene was the major product.

Phenylethanol was not formed in yields exceeding 0.003 except at longer batch holding times at 350 °C. At this temper-

Table 1. Phenylalanine conversion and molar yields of products.

<i>T</i> [°C]	<i>t</i> [min]	Phenylalanine conversion	Yield phenylethylamine	styrene	phenylethanol	Carbon balance [%]
220	60	0.12	0.10	0.0012	0	99
	120	0.20	0.10	0.0012	0	90
	150	0.23 ± 0.04	0.11 ± 0.01	0.0012 ± 0.0001	0	89 ± 4
	180	0.25	0.12	0.0012	0.0011	87
	240	0.27	0.13	0.0013	0.0018	88
250	30	0.33 ± 0.03	0.20 ± 0.04	0.0006 ± 0.0005	0.0018 ± 0.0010	88 ± 8
	60	0.53 ± 0.04	0.28 ± 0.04	0.0016 ± 0.0010	0.0017 ± 0.0010	77 ± 11
	90	0.60 ± 0.02	0.38 ± 0.01	0.0029 ± 0.0010	0.0010 ± 0.0010	86 ± 3
	120	0.66	0.41	0.0045	0.0014	84
	150	0.70	0.41	0.0053	0.0016	80
280	180	0.73	0.44	0.0070	0.0017	81
	10	0.62	0.43	0.0018	0	85
	15	0.80	0.55	0.0033	0	80
	20	0.84	0.59	0.0052	0	82
	30	0.91 ± 0.01	0.64 ± 0.01	0.0135 ± 0.0005	0.0014 ± 0.0004	81 ± 1
	40	0.95	0.67	0.0273	0.0022	80
	60	0.97	0.68	0.0448	0.0030	81
	5	0.63	0.36	0.0009	0.0015	76
	10	1.00 ± 0.003	0.62 ± 0.03	0.0902 ± 0.0200	0.0016 ± 0.0003	68 ± 9
	15	1.00	0.60	0.0703	0.0033	70
350	20	1.00	0.45 ± 0.05	0.2006 ± 0.0500	0.0124 ± 0.0020	64 ± 3
	30	1.00	0.33 ± 0.04	0.2704 ± 0.0400	0.0244 ± 0.0040	61 ± 5
	40	1.00	0.30	0.2802	0.0242	57
	60	1.00	0.23	0.3806	0.0342	60

The residual for minimization was the summation of squared errors (SSE) between the calculated (C_{mod}) and experimental (C_{exp}) phenylalanine concentrations [Eq. (2)].

$$\text{SSE} = \sum_j [C_{j,\text{mod}} - C_{j,\text{exp}}]^2 \quad (2)$$

j is the number of discrete reaction times at any particular temperature. A non-linear regression was carried out using the SOLVER add-in in Excel 2007, to obtain the values of k_n and n , as displayed in Table 2. The reaction order for phenylalanine is almost unity at all temperatures under consideration, which is consistent with previous work. Hence, we reanalyzed the data using first-order kinetics to obtain the corresponding first-order rate constants (k_{first}) at different temperatures (Table 2).

ature the yield of phenylethanol increased from about 0.002 at 5 min to nearly 0.034 at 60 min.

The conversion of phenylalanine was below 30% at mild conditions [e.g., 220 °C (<240 min), 250 °C (<30 min)]. In general, the conversion increased with both temperature and time. Nearly complete conversion was observed at 280 °C at a batch holding time of 60 min and at 350 °C for batch holding times greater than 10 min.

The carbon balance was 90% ± 10% in most cases, and fell below 80% only for experiments at 350 °C. The conversion of phenylalanine was very high under these conditions. It is likely that the more severe conditions led to the formation of higher molecular weight products via oligomerization, as will be explained in the next section. It should be noted that there was considerable gas formation (most likely carbon dioxide), especially under severe conditions. Although we have not quantified CO₂, we account for it in the carbon balance by assuming its yield was equal to the yield of phenylethylamine, since CO₂ would form in parallel with phenylethylamine during the decarboxylation of phenylalanine.

To compare the present rate data for the disappearance of phenylalanine (PA) with previous work,^[17,25,29] which indicated first-order kinetics, we fit the phenylalanine conversion data of Table 1 to Equation (1), which is the integrated form of the batch reactor design equation coupled with an n^{th} -order rate equation, and k_n is the n^{th} -order rate constant.

$$C_{\text{PA}} = \left[C_{\text{PA,initial}}^{(1-n)} - (1-n)k_n t \right]^{\frac{1}{1-n}} \quad (1)$$

Table 2. Rate constant and order for phenylalanine disappearance.

<i>T</i> [°C]	Reaction order (<i>n</i>)	k_n [mol, min, L]	k_{first} [min ⁻¹]
220	0.94	0.0008	0.0015
250	0.97	0.0065	0.0083
280	0.98	0.0600	0.0692

An Arrhenius plot (based on first-order rate constants) gave rise to an activation energy of 144 ± 14 kJ mol⁻¹ and a pre-exponential factor of 10^{12.4 ± 1.4} min⁻¹. Table 3 compares these Ar-

Table 3. Arrhenius Parameters for the hydrothermal reaction of phenylalanine.

Source	A_0 [min ⁻¹]	E_a [kJ mol ⁻¹]	$k_{250^\circ\text{C}}$ [min ⁻¹]
this work	10 ^{12.4 ± 1.4}	144 ± 14	8.3 · 10 ⁻³
Li and Brill ^[29]	10 ^{15.1 ± 2.5}	171 ± 8	9.5 · 10 ⁻³
Abdelmoez ^[25]	10 ^{10.5}	126	9.2 · 10 ⁻³
Vallentyne ^[17]	10 ^{10.1}	129	1.4 · 10 ⁻³

rhennius parameters with those reported previously for phenylalanine in HTW. Although there is some variability in the Arrhenius parameters, the rate constants calculated at a given temperature (250 °C is used in Table 3) in this work compare very well with those calculated using the Arrhenius parameters reported by Li and Brill^[29] and Abdelmoez et al.^[25] The small differences between these parameter values can arise due to the differences in operating temperatures, pressures, process

times, and procedures by which the data is collected. These rate constants, however, are six to seven times higher than that calculated using the Arrhenius parameters reported by Vallentyne.^[17] Li and Brill^[30] had also noted discrepancies between their rate constants and those computed by Vallentyne^[17] for other amino acids, such as glycine. They attribute these discrepancies to differences in the reactor surface materials. Li and Brill used a titanium flow cell, whereas Vallentyne used glass reactors. The analytical procedures used by Vallentyne were not as advanced as the current methods for quantification, which could also result in some discrepancies between the rate constants.

Reaction pathways

The previous section showed that phenylalanine in HTW produced phenylethylamine as its main product, whereas styrene and phenylethanol were produced in minor amounts at most of the conditions under consideration. However, at 350 °C and 60 min, the yield of styrene becomes greater than that of phenylethylamine. We desired to discover the complete set of reaction pathways for phenylalanine and these products.

Phenylethylamine can form via decarboxylation of phenylalanine. Decarboxylation mechanisms for amino acids can be complicated and several have been proposed. Direct decarboxylation of the zwitterions of carboxylic acid and carboxylate ion, such as occurs in amino acids, has been reported.^[30,44–46] Li and Brill^[30] mentioned that an aromatic ring at the β -carbon can act as an electron sink that dissipates the developing negative charge on the α -carbon atom during the decarboxylation reaction, as would be applicable for phenylalanine. Researchers have also proposed a general water-molecule-catalyzed transition state involving a six-membered ring for decarboxylation.^[30,47,48]

Styrene or a corresponding alkene has not been reported in the past as a product from amino acid conversion in HTW. We hypothesize that phenylethylamine undergoes deamination at severe conditions to form styrene because the yield of phenylethylamine decreases and the yield of styrene increases. To test this hypothesis, we reacted phenylethylamine (20 μ L; or 0.16 mmol equivalent to the amount of phenylethylamine formed at complete conversion of phenylalanine) in water at 350 °C and 30 min. The phenylethylamine conversion was 0.51 ± 0.04 , and the yield of styrene was 0.31 ± 0.02 . The above results indicate formation of styrene as a secondary product (from phenylethylamine) during treatment of phenylalanine in HTW.

Phenylethanol isomers, which were minor products, could be obtained via hydration of styrene in HTW. 1-phenylethanol, which can form via Markovnikov's rule, was more abundant than 2-phenylethanol, the anti-Markovnikov product. These observations are consistent with An et al.,^[49] who report hydration of some olefins without any added catalysts at 250 °C in high temperature water and also mention that the alcohol formed via Markovnikov's rule is the more abundant alcohol. We tested whether this pathway would be operative for styrene by reacting styrene (20 μ L, 0.15 mmol) in HTW at 350 °C

and 30 min. Styrene conversion under these conditions was 0.35, forming phenylethanol in a yield of 0.05. It is interesting to note that under these conditions styrene mostly formed several high molecular weight products, including 1,3-diphenylbutane, a styrene dimer. Styrene oligomerization under these conditions is reasonable. Erdmenger et al.^[50] reported polymerization of styrene under near-critical-water conditions to produce polystyrene, with a number-average molecular weight (M_n) of 25 790 Da, in yields of 0.31. They mentioned that polystyrene has a high decomposition temperature (≈ 310 °C) and thus can be polymerized up to 300 °C. Furthermore, the polymerization of styrene is auto-initiating at high temperatures.^[51]

We also expect that phenylethanol can dehydrate in HTW to form styrene. There is ample evidence in the literature for alcohol dehydration in HTW.^[52–54]

Similar to styrene, phenylalanine could also dimerize at high temperatures and longer batch holding times, as it has been mentioned for other amino acids.^[24,31–34] LC-MS chromatograms of the products from phenylalanine at 250 °C and 30 min and at 350 °C and 60 min (Figure 1) indicate the presence of not only phenylethylamine (main decarboxylation product), but also oligomers of phenylalanine (up to 706 amu).

Comparing Figure 1 a and 1 b shows that more of the higher molecular weight oligomers are obtained at the higher temperature and longer batch holding time. Since we did not quantify the yields of the many oligomers of phenylalanine and styrene, the carbon balances shown in Table 1 are not 100%. The propensity for more oligomerization at the higher temperatures and longer times is consistent with the carbon balance being lower at more severe conditions.

Based on the reasoning provided in this section, we propose the reaction network in Scheme 1 to describe the reactions of phenylalanine in high temperature water. The reactions include decarboxylation, hydration/dehydration, and oligomerization, all of which have been previously documented in HTW.

Kinetics model

The reaction network in Scheme 1 provides different pathways that interconnect the observed reaction products. Our focus now turns to constructing a chemical kinetics model based on the reaction network. All reactions in the network were treated as being irreversible and proceeding through first-order kinetics, except oligomerization of phenylalanine, which was taken to follow second-order kinetics, as suggested by Cox and Seward^[24] for alanine and glycine. The model can be represented by differential equations [given through Eqs. (3)–(6)], which apply to reactions in a constant-volume batch reactor.

$$\frac{dC_{PA}}{dt} = -k_1 C_{PA} - k_4 C_{PA}^2 \quad (3)$$

$$\frac{dC_{PEA}}{dt} = k_1 C_{PA} - k_2 C_{PEA} \quad (4)$$

$$\frac{dC_{STY}}{dt} = k_2 C_{PEA} - k_3 C_{STY} - k_5 C_{STY} + k_6 C_{PEOH} \quad (5)$$

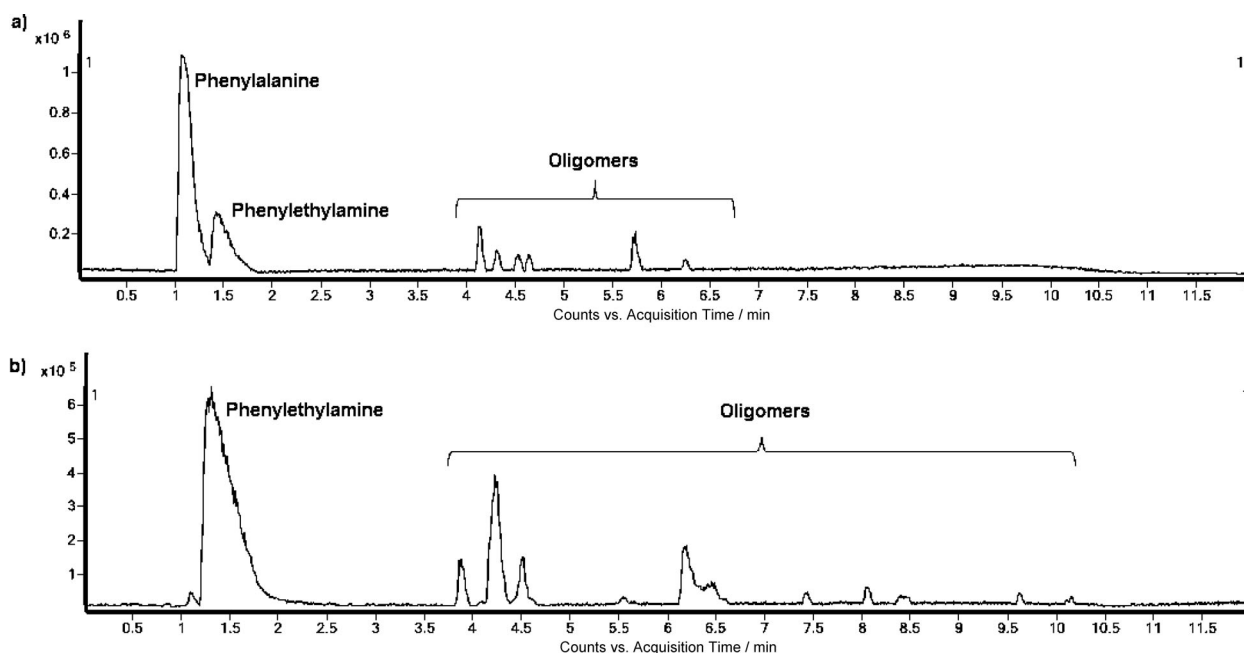
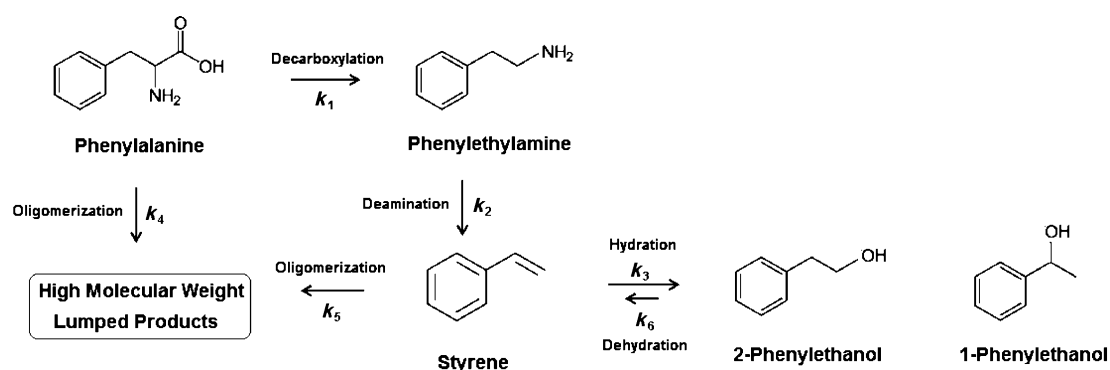


Figure 1. LC-MS chromatograms for phenylalanine reaction products at a) 250 °C and 30 min, and b) 350 °C and 60 min.



Scheme 1. Reaction pathways for phenylalanine in HTW.

$$\frac{dC_{\text{PEOH}}}{dt} = k_3 C_{\text{STY}} - k_6 C_{\text{PEOH}} \quad (6)$$

where, C is the concentration and the subscripts PA, PEA, STY, and PEOH represent phenylalanine, phenylethylamine, styrene, and phenylethanol, respectively. Each of the rate constants k_i can be expressed in Arrhenius form given by Equation (7).

$$k_i = A_i \exp\left(-\frac{E_{a_i}}{RT}\right) \quad (7)$$

We used MATLAB 2010 to solve simultaneously the set of ordinary differential equations above and perform parameter estimation to obtain the values of A_i and E_{a_i} (12 parameters total). We used *fmincon* to perform the optimization using the *optimtool* GUI. The minimization algorithm uses a sequential quadratic programming active set optimization method.^[55] The objective function for minimization was the summation of squared relative error (SSRE) between the calculated and ex-

perimental product concentrations at a given reaction temperature. This quantity was calculated using Equation (8).

$$\text{SSRE} = \sum_j \sum_{i=1}^4 \left[\frac{C_{j,i,\text{mod}} - C_{j,i,\text{exp}}}{(C_{j,i,\text{mod}} + C_{j,i,\text{exp}})/2} \right]^2 \quad (8)$$

j is the number of discrete reaction times at a particular temperature and i is the number of components. The rate constants at each temperature were then fit to the linearized form of the Arrhenius equation to obtain A_i and E_{a_i} along with the associated standard errors for each rate constant.

Table 4 displays the Arrhenius parameters that provided the best description of the experimental data. It also displays the values of the rate constants at 250 °C so that the relative rates of the different paths can be easily discerned. The rate constant for decarboxylation, k_1 , is the largest of the first-order rate constants at all temperatures. It is also larger than the pseudo-first-order rate constant for oligomer formation from

Table 4. Arrhenius parameters for phenylalanine reaction network.				
Rate constant (k) in Scheme 1	Units of A_i , k_i	A_i	E_a [kJ mol ⁻¹]	k_i , 250 °C
1	min ⁻¹	$10^{12.5 \pm 0.6}$	146 ± 6	$7.49 \cdot 10^{-3}$
2	min ⁻¹	$10^{7.7 \pm 1.1}$	113 ± 11	$2.77 \cdot 10^{-4}$
3	min ⁻¹	$10^{10.6 \pm 0.3}$	155 ± 4	$1.12 \cdot 10^{-5}$
4	L mol ⁻¹ min ⁻¹	$10^{13.9 \pm 4.3}$	153 ± 12	$4.17 \cdot 10^{-2}$
5	min ⁻¹	$10^{7.3 \pm 0.4}$	132 ± 4	$1.25 \cdot 10^{-6}$
6	min ⁻¹	$10^{8.5 \pm 0.5}$	140 ± 6	$3.23 \cdot 10^{-6}$

phenylalanine ($k_4 C_{PA,initial} \approx 3 \cdot 10^{-3} \text{ min}^{-1}$). These observations indicate that decarboxylation of phenylalanine to phenylethylamine is always the main pathway. The rate constants k_2 and k_3 , which govern the formation of styrene and phenylethanol, are one and two orders of magnitude lower than k_1 at 250 °C. This result is consistent with the products being in low yields at this temperature. The rate constants k_5 and k_6 are three orders of magnitude lower than that for decarboxylation of phenylalanine, consistent with the pathways for styrene oligomerization and dehydration of phenylethanol being the minor ones.

Figure 2 shows the experimental concentrations and those calculated from the model for phenylalanine, phenylethyl-

amine, styrene, and phenylethanol at all four temperatures investigated. The model accurately describes the trends in the data and typically provides the species concentrations within experimental errors.

Binary mixtures of inorganics and phenylalanine

The previous section showed the behavior of phenylalanine in pure HTW. As mentioned in the introduction, one of the objectives in this article is to discover the influence of various inorganic compounds present in algae and the culture medium on the behavior of phenylalanine in HTW. This section presents these results.

Figure 3a shows that addition of salts and boric acid generally increases the phenylalanine conversion. Comparing results from addition of the three sodium salts shows that NaNO₃ has the greatest influence on phenylalanine conversion ($\approx 66\%$ increase). Based on this observation, we speculate that perhaps nitrate has a more favorable interaction with phenylalanine or stabilizes the transition state better than chloride or sulfate. KCl increases the phenylalanine conversion less than NaCl although the uncertainty in the KCl result is higher. Mishima and Matsuyama^[56] attributed the higher activity of sodium salts

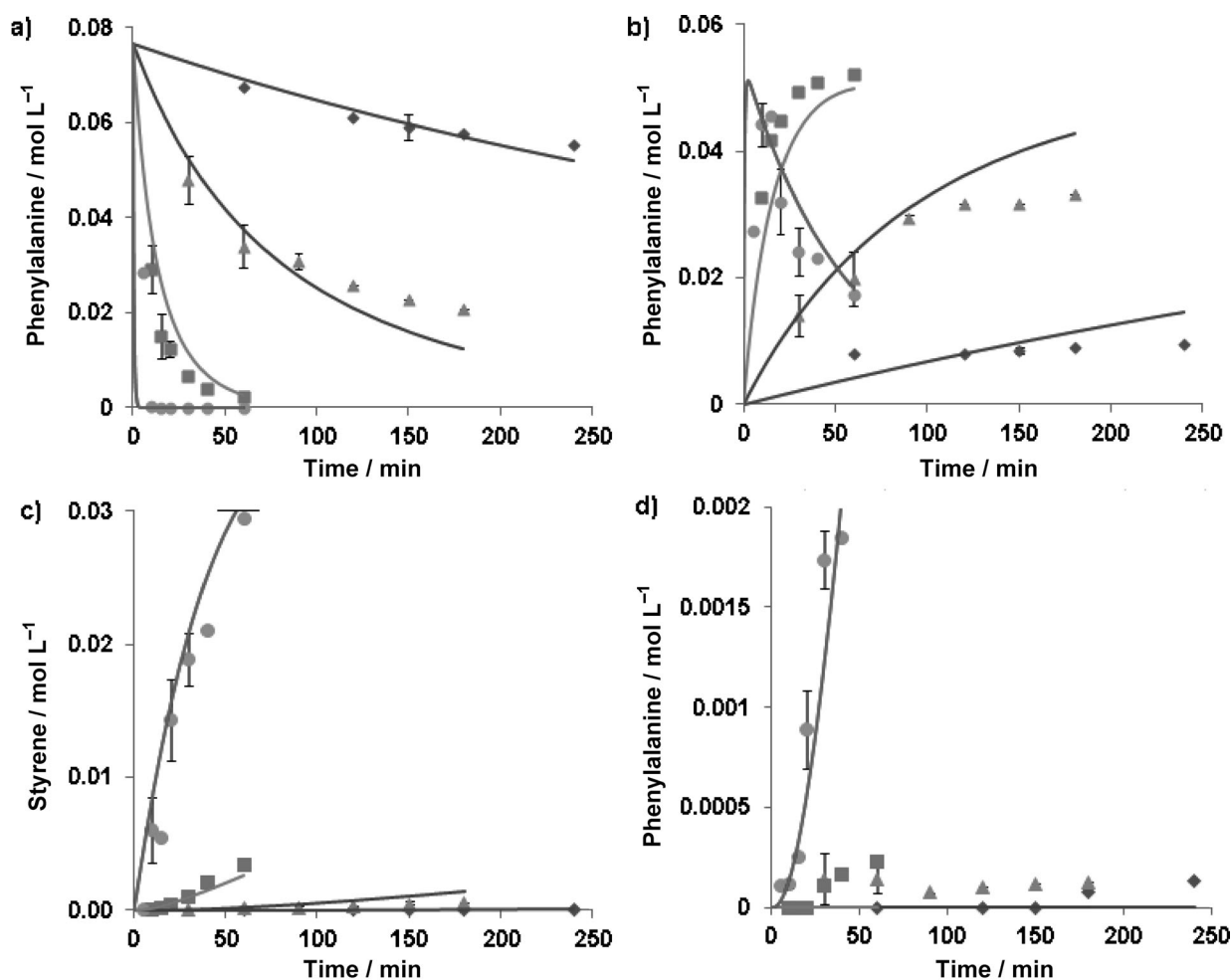


Figure 2. Comparison of experimental (discrete points) and model (smooth curves) results for a) phenylalanine, b) phenylethylamine, c) styrene, and d) phenylethanol at \blacklozenge , \blacktriangle , \blacksquare , \bullet 220, 250, 280, and 350 °C.

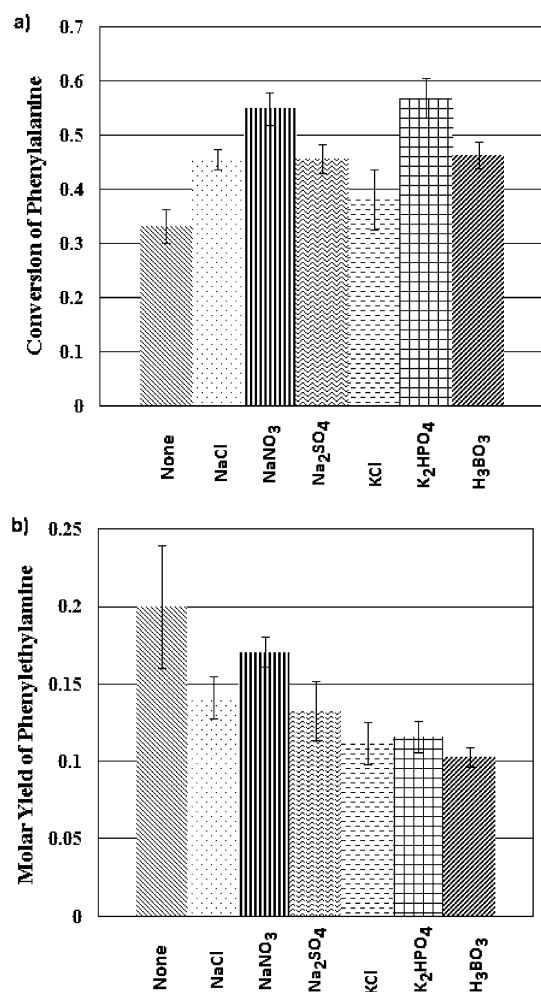


Figure 3. a) Phenylalanine conversion and b) phenylethylamine yield in the presence of salts and boric acid at 250 °C and 30 min in HTW.

over potassium salts to the higher hydration enthalpy of Na⁺. The same phenomenon might be at work in the present system as well. K₂HPO₄ shows the most pronounced effect on phenylalanine conversion (increase of about 71%), probably because of two divalent K⁺ ions leading to greater interactions between the salt and phenylalanine. Lastly, H₃BO₃ increased the phenylalanine conversion only moderately (by around 40%).

One might expect that an increase in phenylalanine conversion would lead to a corresponding increase in the rate of decarboxylation, the major reaction path for phenylalanine reacting alone, thus increasing the yield of phenylethylamine. Figure 3b, however, shows that the yield of phenylethylamine decreases in the presence of each of the additives. H₃BO₃ and KCl provide the greatest decreases (about 49 and 45%, respectively) compared to the case with no additive. NaNO₃ induces the least decrease of about 15%. These observations are in accord with the addition of KCl reportedly reducing the decarboxylation rate for α -alanine.^[28] Those authors proposed the decrease to be due to either, 1) formation of a transition state less polar than the starting zwitterion of α -alanine, or 2) formation of complexes of K⁺ or Cl⁻ with ionic sites of α -alanine

zwitterions, thereby reducing its ability to undergo decarboxylation.

The reaction network in Scheme 1 shows that phenylalanine can undergo two parallel primary reactions—decarboxylation or oligomerization. As the rate of decarboxylation decreases in the presence of salts (and boric acid), there must be an increase in the rate of the oligomer formation pathway. The carbon balances from the experiments with added salts were always lower than 70% (see the Supporting Information, Table 1), whereas without salt the carbon balance was around 90% under the same conditions. This result is consistent with an increase in the rate of formation of oligomers in presence of salts. LC-MS analysis of a sample from the reaction of phenylalanine in the presence of added NaNO₃ confirmed the presence of high molecular weight compounds.

From the results and discussion above, we conclude that the salts examined here and boric acid inhibit decarboxylation but lead to an increase in high molecular weight compound formation from phenylalanine, thereby increasing phenylalanine conversion. This work is, to our knowledge, the first to report this effect of salts (or boric acid) on oligomerization of amino acids in HTW. It also reveals that these inorganic compounds could influence the relative rates of different reaction paths during hydrothermal liquefaction of algae.

Binary mixtures of ethyl oleate and phenylalanine

The previous sections characterized the products from phenylalanine alone in HTW and the effect of added salts and boric acid on the behavior of phenylalanine. This section examines the effect of an added organic molecule (ethyl oleate, which is a model compound for triglycerides in algae). The concentrations of ethyl oleate and phenylalanine were varied to mimic the broad range of triglyceride-to-protein ratios in different strains of algae. We carried out the reactions at 350 °C as the yield of algal bio-oil is maximum at this temperature.^[2] The conversion of phenylalanine was always complete at this temperature, however, so we focus this discussion on the influence of added ethyl oleate on the yields and identities of products from phenylalanine. The effect of phenylalanine on ethyl oleate conversion and oleic acid yield is also explored. Note that a 0.2 molar ratio of ethyl oleate to phenylalanine would correspond to a 5.0 molar ratio of phenylalanine to ethyl oleate. The former will be considered to study the effects of ethyl oleate on phenylalanine and the later to explore the effects of phenylalanine on ethyl oleate.

As a control experiment, we examined phenylalanine alone in HTW (no ester added) at concentrations corresponding to those used in the mixtures (4000, 15000, and 22000 ppm). Similar yields of phenylethylamine and styrene were obtained for all three cases (see the Supporting Information, Table 2). These results indicate that the yields of phenylethylamine and styrene are independent of the phenylalanine concentration (as expected for a first-order reaction) and that any effects observed in the mixtures arise solely from the addition of ethyl oleate.

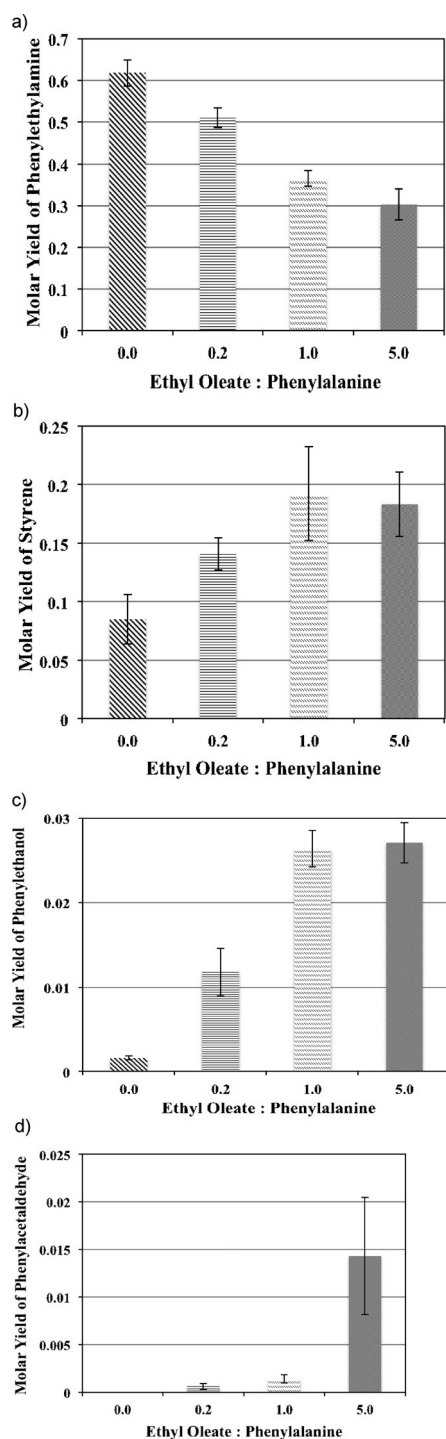


Figure 4. Molar yields of a) phenylethylamine, b) styrene, c) phenylethanol, and d) phenylacetaldehyde at 350 °C and 10 min for different initial molar ratios of ethyl oleate to phenylalanine.

Tables 3 and 4 in the Supporting Information provide the yields of the various products at different batch holding times and the different initial molar ratios of ethyl oleate to phenylalanine. We present just a representative subset of that data in Figures 4–7. We remind the reader that the yields of phenylethylamine, styrene, and phenylethanol (Figure 4) are based on initial mols of phenylalanine loaded into the reactor, the yields of oleic acid (Figure 6) are based on initial mols of ethyl oleate

loaded, and the yields of oleamides (Figure 7) are based on the initial mols of the limiting reactant loaded (either phenylalanine or ethyl oleate).

Effect of ethyl oleate on phenylalanine

First, we report and discuss the effect of added ethyl oleate on the products obtained from phenylalanine. Figure 4a shows that the molar yield of phenylethylamine decreases from 0.62 without added ester to 0.30 as the initial ratio of ethyl oleate to phenylalanine in the binary mixture increases from 0.0 to 5.0. This behavior suggests faster reaction of phenylethylamine to form secondary products in the presence of ethyl oleate. To test the hypothesis of accelerated disappearance of phenylethylamine, we performed an experiment with 20 μL of phenylethylamine, with and without 40 μL of ethyl oleate, in water at 350 °C for 10, 20, and 30 min. This amount corresponds to a molar ratio of ethyl oleate to phenylethylamine equal to 0.7.

Figure 5 shows that the conversion of phenylethylamine was always higher in the presence of ethyl oleate, thereby confirming that the disappearance of phenylethylamine is enhanced

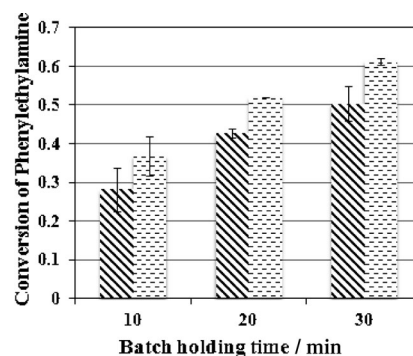


Figure 5. Conversion of phenylethylamine at 350 °C and different batch holding times ⊘ without, ⊚ with 40 μL ethyl oleate (0.7:1 molar ratio ethyl oleate to phenylalanine).

in the presence of ethyl oleate. For example, phenylethylamine conversion increased from 0.28 (without added ester) to 0.37 (in the presence of ester) at 10 min, which is a net increase of 32%. This increase in the conversion of phenylethylamine with added ester implies a corresponding decrease in its molar yield ($\approx 30\%$) when it is formed from phenylalanine with and without ethyl oleate (0.0 and 0.7 molar ratio). Interpolation of the data in Figure 4a leads to an estimated molar yield of phenylethylamine of about 0.4 at an initial ethyl-oleate-to-phenylethylamine molar ratio of 0.7. This value (0.4) is about 30% lower than the yield obtained without added ester, a relative decrease that is consistent with the results in Figure 5.

The increased conversion of phenylethylamine in the presence of ethyl oleate suggests an accompanying increase in the molar yield of styrene on increasing the amount of ester. Figure 4b confirms this expectation. The molar yield of styrene at 10 min is 0.09 without any ester and around 0.19 when the molar ratio ethyl oleate to phenylalanine is 5.0. Similarly, Figure 4c shows that the molar yield of phenylethanol increases

from 0.002 to 0.027 as the ethyl-oleate-to-phenylalanine ratio increases from 0.0 to 5.0. Thus, the addition of ethyl oleate enhances both deamination of phenylethylamine to styrene and hydration of styrene to phenylethanol.

These paths are accelerated upon addition of ethyl oleate because both deamination of organic compounds and hydration of styrene and other alkenes in an aqueous medium are acid-catalyzed reactions.^[57–60] The addition of ethyl oleate reduces the pH of the system due to the production of oleic acid from its hydrolysis,^[8,9] thereby catalyzing both the deamination and hydration reactions in the binary mixture.

In addition to altering the yields of products identified from the reaction of phenylalanine alone, addition of ethyl oleate also permitted quantification of a new product, phenylacetaldehyde. Figure 4d shows that the molar yield of phenylacetaldehyde increases with an increase in the molar ratio of ethyl oleate to phenylalanine. The yield of phenylacetaldehyde also increases with an increase in the batch holding time at a given concentration ratio (see the Supporting Information Table 3). Klingler et al.^[23] have previously reported the formation of acetaldehyde from alanine via a lactic acid intermediate. A similar pathway could be at work here, wherein phenylalanine first forms phenyllactic acid via deamination, which then decarboxylates to form phenylacetaldehyde. The highest yield of phenylacetaldehyde was 0.04, obtained from the reaction at the longest time (60 min) and most added ester (ethyl-oleate-to-phenylalanine molar ratio of 5.0). Once again, it is likely that ethyl oleate catalyzes deamination of phenylalanine to form phenyllactic acid, subsequently forming phenylacetaldehyde under these conditions.

Effect of phenylalanine on ethyl oleate

Having discussed the results from these binary mixture experiments in terms of the influence of added ester on phenylalanine, we now examine the other side of the coin and discuss the influence of added phenylalanine on ethyl oleate. Figure 6a shows that the ethyl oleate conversion at 10 min is 0.49 without phenylalanine and 0.96 at a phenylalanine-to-ethyl-oleate molar ratio of 5.0. An increase in the amount of phenylalanine increases the conversion of ethyl oleate. This behavior is expected because ethyl oleate conversion is acid-catalyzed^[8,9] and the presence of phenylalanine would reduce the initial pH of the solution [isoelectric point of phenylalanine is 5.3 at room temperature^[29] and an aqueous solution of phenylalanine (15000 ppm) has a pH of 5.9 at room temperature].

Higher conversion of ethyl oleate implies a corresponding increase in the yields of oleic acid, the major reaction product, which arises from hydrolysis. Figure 6b offers confirmation as the yield of oleic acid at 10 min increases from 0.31 without phenylalanine to 0.78 at a phenylalanine-to-ethyl-oleate molar ratio of 5.0. At batch holding times greater than 10 min, however, the molar yields of oleic acid show the opposite trend at a molar ratio of phenylalanine to ethyl oleate of 5.0 (see the Supporting Information, Tables 4 and 5). That is, at 60 min the oleic acid yield decreases from 0.73 without phenylalanine to 0.63 at a molar ratio of phenylalanine to ethyl oleate of 5.0.

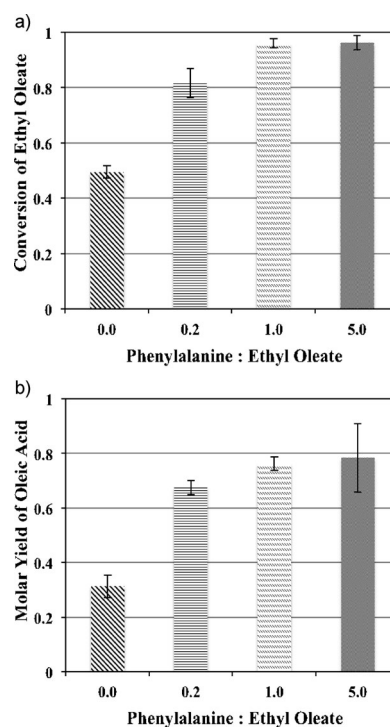


Figure 6. a) Conversion of ethyl oleate and b) yield of oleic acid at 350 °C and 10 min for different initial molar ratios of phenylalanine to ethyl oleate.

As we show below, this observation is consistent with oleic acid undergoing secondary reactions in the presence of phenylalanine to form additional products.

Figure 1 in the Supporting Information, which shows the total ion chromatogram for a reaction at 350 °C and 30 min with a phenylalanine-to-ethyl-oleate molar ratio of 1.0, indicates the presence of three oleamides [9-octadecenamide (ODM), *N*-ethyl-9-octadecenamide (EODM), and *N*-phenylethyl-9-octadecenamide (PEODM)]. Roe et al.^[61] mentioned previously that ODM can be formed in HTW by the reaction of oleic acid or ethyl oleate with ammonia. For the binary system of phenylalanine and ethyl oleate, ammonia is generated in situ during deamination of phenylethylamine to styrene. It is also possible in this environment for ODM to undergo condensation with ethanol (formed in small quantity during formation of ODM from ethyl oleate) and form EODM.^[62] Lastly, phenylethylamine could also react with oleic acid or ethyl oleate in a similar fashion to form PEODM (see reactions in the Supporting Information). These reactions would lead to a decrease in the yield of oleic acid, and this decrease would be expected to be most significant at longer times and in mixtures with more phenylalanine. Both of these expectations are consistent with the experimental results.

Figure 7a shows that the total yield of amides at 10 min is always about 16–20% of the theoretical maximum yield. The yield is not affected by different initial molar ratios of phenylalanine to ethyl oleate considering the experimental uncertainty. Similar results were obtained at other batch holding times (see the Supporting Information, Table 4).

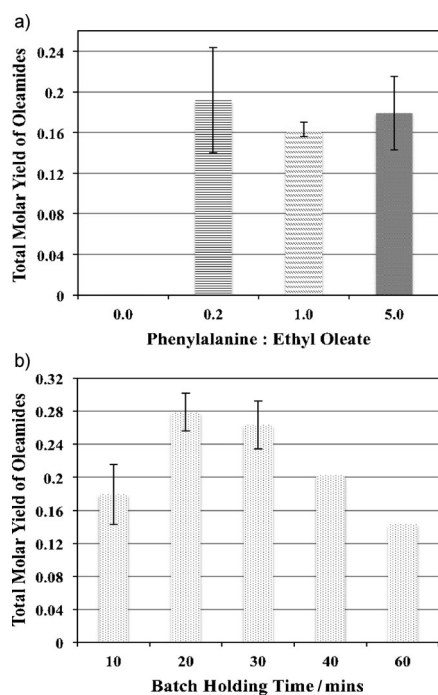
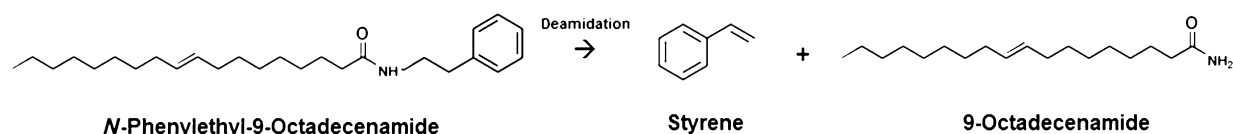


Figure 7. a) Total yield of amides at 350 °C and 10 min for different initial molar ratios of phenylalanine to ethyl oleate and b) total yield of amide at various batch holding times and phenylalanine-to-ethyl-oleate molar ratio of 5.0.

Figure 7b shows that, at a particular initial ratio of phenylalanine to ethyl oleate, the total yield of amides first increases and then decreases as the batch holding time is increased. Results obtained at the other molar ratios showed the same trend. Table 4 in the Supporting Information shows that the yield of PEODM is always higher than the yields of ODM or EODM and that the yield of PEODM first increases with time and then decreases, whereas the yields of ODM or EODM continually increase with time. These trends suggest that PEODM undergoes secondary decomposition. One possibility to account for these observations is deamidation of PEODM to styrene, as shown below. The octadecenamide group on PEODM ($C_{17}H_{33}-CO-NH-$) is a better leaving group than the amino substituent ($-NH_2$) in phenylethylamine because the former anion is stabilized via conjugation with the carbonyl group. Therefore, PEODM can undergo deamidation to form styrene similar to the deamination of phenylethylamine, the latter path having been demonstrated in our work. Deamidation could be another reason why the yields of ODM (see the Supporting Information, Table 4) and styrene (Figure 4b) increase at longer batch holding times for the binary mixture (Scheme 2).

Along with deamidation, hydrolysis is another potential pathway for PEODM degradation. Duan et al.^[63] reported that



Scheme 2. Deamidation of PEODM to styrene and ODM.

N-substituted amides hydrolyze to give the corresponding amine and carboxylic acid. This reaction is reversible and follows first-order kinetics. Thus, there exists a possibility of hydrolysis of PEODM to form phenylethylamine and oleic acid under our conditions. Hydrolysis of PEODM would be favored as more PEODM is formed at higher batch holding times, thus accounting for a net decrease in total yield of amides.

Brown et al.^[2] and Valdez et al.^[64] have characterized and quantified fatty acid amides as products from the hydrothermal liquefaction of algae. Valdez et al.^[64] reported that the yields of palmitic acid amide varied from 0.5 to 3 (mg g^{-1} of dry algae) depending on whether hexane or hexadecane was used, respectively, for recovery of bio-oil. However, these authors have only proposed the reaction of fatty acids with ammonia to account for the amide formation, neglecting the possibilities of amines reacting with fatty acids. The present work offers this new possibility.

Based on the above discussion and results, we propose the modified reaction network in Scheme 3 to account for the reactions that occur in a binary mixture of phenylalanine and ethyl oleate.

We sought to verify that this network could quantitatively account for the effect of addition of ethyl oleate on phenylalanine. Thus, we used the system of differential equations implied by this network [Eqs. (9)–(13)] and the methodology of optimization outlined earlier to fit the data from the mixture experiments.

$$\frac{dC_{PA}}{dt} = k_1 C_{PA} - k_4 C_{PA}^2 \quad (9)$$

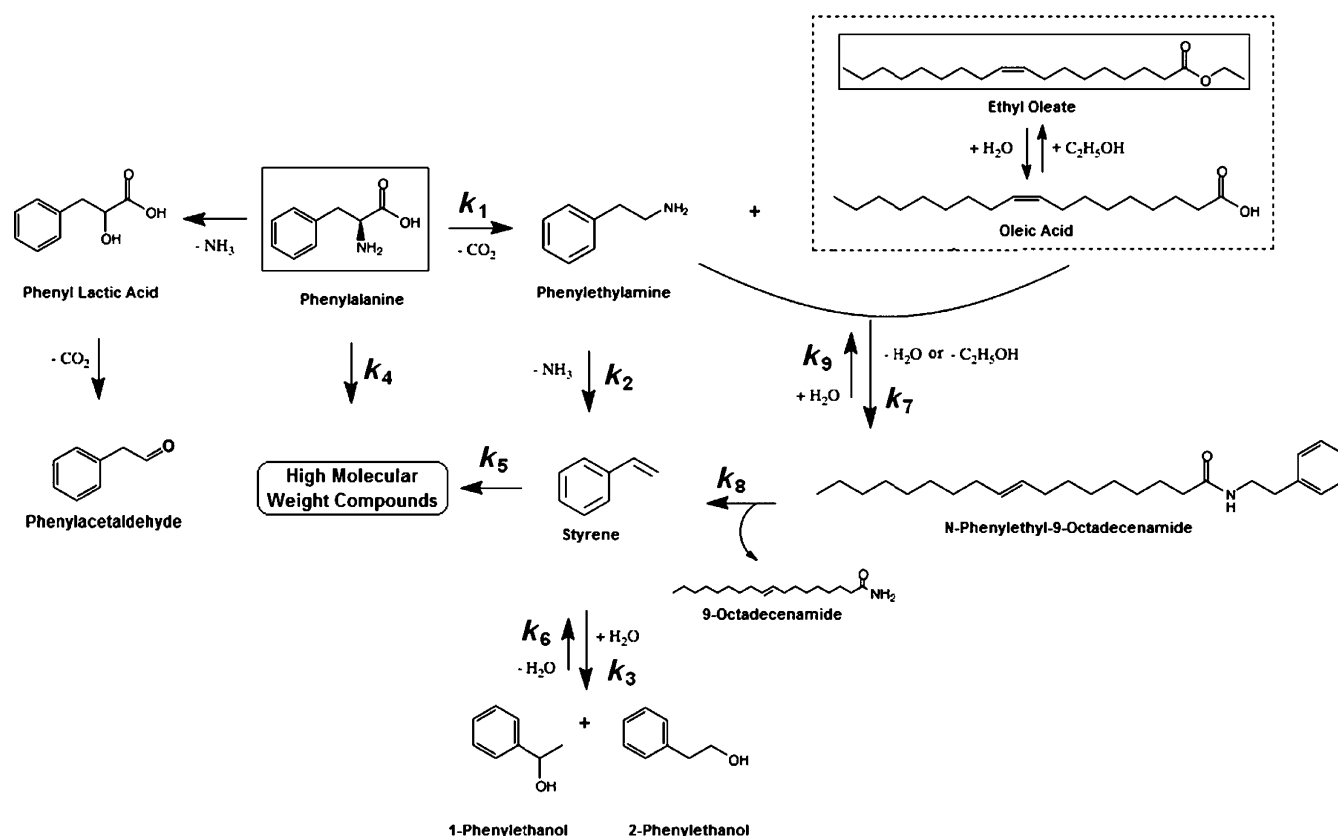
$$\frac{dC_{PEA}}{dt} = k_1 C_{PA} - k_2 C_{PEA} - k_7 C_{PEA} + k_9 C_{PEODM} \quad (10)$$

$$\frac{dC_{STY}}{dt} = k_2 C_{PEA} - k_3 C_{STY} - k_5 C_{STY} + k_6 C_{PEOH} + k_8 C_{PEODM} \quad (11)$$

$$\frac{dC_{PEOH}}{dt} = k_3 C_{STY} - k_6 C_{PEOH} \quad (12)$$

$$\frac{dC_{PEODM}}{dt} = k_7 C_{PEA} - k_8 C_{PEODM} - k_9 C_{PEODM} \quad (13)$$

Table 5 compares the values of the rate constants obtained for the different pathways in the reaction network for different ratios of ester to phenylalanine. Note that the rate for formation of PEODM (k_7) is taken to be first-order in phenylethylamine, but zero order with respect to oleic acid. This approximation simplified the modeling and allowed us to maintain focus on the pathways for phenylalanine. The model fits the temporal variation of the experimental product concentrations very well at all of the molar ratios explored for the binary mix-



Scheme 3. Reaction network for a binary mixture of ethyl oleate and phenylalanine in HTW.

Rate constant (<i>k</i>) in Scheme 3	Units	Initial ratio of ethyl oleate to phenylalanine			
		0	0.2	1.0	5.0
1	min ⁻¹	1.65	1.91	2.02	2.22
2	min ⁻¹	0.018	0.029	0.035	0.043
3	min ⁻¹	0.004	0.013	0.018	0.023
4	L mol ⁻¹ min ⁻¹	11.62	12.07	11.25	12.75
5	min ⁻¹	0.0002	0.0016	0.0131	0.0217
6	min ⁻¹	0.0006	0.0920	0.1024	0.1316
7	min ⁻¹	–	0.0084	0.0379	0.0501
8	min ⁻¹	–	0.0375	0.0340	0.0361
9	min ⁻¹	–	0.0431	0.0413	0.0391

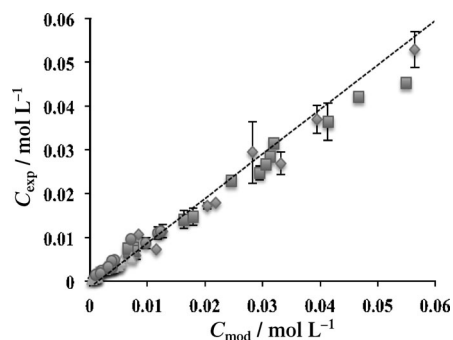


Figure 8. Parity plot of experimental and model concentrations for \blacklozenge phenylethylamine, \blacksquare styrene, \blacktriangle phenylethanol, and \bullet *N*-phenylethyl-9-octadecenamide for binary mixture at 350 °C and different batch holding times.

ture of phenylalanine and ethyl oleate as is seen in the parity plot in Figure 8 and in the Supporting Information, Scheme 1.

Table 5 shows that the decarboxylation rate constant k_1 increases by 35% at the highest molar ratio. Li and Brill^[29] reported that the rate constant for decarboxylation of phenylalanine nearly doubled as the pH decreased from 5.3 to 2.5 (at 330 °C). A similar effect of pH could be at work in this binary system, as more oleic acid would be generated at the higher ester concentrations, thereby lowering the pH of the solution.

The addition of ester has an even more marked effect on the rate constants k_2 and k_3 (increasing by 140 and 530%, respectively, at the highest molar ratio of 5.0), as deamination of phenylethylamine to styrene and hydration of styrene to phenylethanol is enhanced upon adding ester. The rate constant for oligomerization of phenylalanine (k_4) is independent of added ester, suggesting that the rate of this path is not pH dependent. However, the rate constant for styrene conversion to high molecular weight compounds (k_5) increases by two orders of magnitude as more ester is added, probably because of enhanced acid catalysis at the higher ethyl oleate concentrations. Dimers of styrene (1,3-diphenylbutane) were identified in the GC analysis but not quantified (see the Supporting Information, Figure 1). Lastly, the dehydration of phenylethanol is increased nearly 230-times in the presence of ethyl oleate at a molar ratio of phenylalanine to ethyl oleate of 5.0. Once again, the acidic environment

(in the presence of fatty acid from ester hydrolysis) would be favorable for dehydration of alcohols as has been previously reported.^[52–54] The rate constant for formation of PEODM (k_7) increases by an order of magnitude as the ethyl-oleate-to-phenylalanine molar ratio increases from 0.2 to 5.0, but this increase can probably be attributed to the model not explicitly accounting for the higher oleic acid concentrations present at the higher ratios. The rate constant for formation of styrene from PEODM (k_8) and that for hydrolysis of PEODM (k_9) does not change significantly with change in ester concentration, suggesting that they are not affected by a change in the pH. For instance, Duan et al.^[63] have shown that the rate of hydrolysis of *N*-methylacetamide is increased at a low pH (< 2), but remains constant at near-neutral pH.

Implications for hydrothermal liquefaction of algae

The results from this study of phenylalanine reactions in HTW have several implications for hydrothermal liquefaction of algae. Several products identified in bio-oil from algae could have originated from amino acids within the proteins in the cells. Crude bio-oil obtained during algae liquefaction contains amines, which can be formed via decarboxylation of amino acids. Bio-oil also contains high molecular weight compounds, which can arise due to oligomerization during the reaction of amino acids in HTW.

Secondly, the phenylalanine pathway to oligomerized products can be important at the temperatures at which hydrothermal liquefaction of algae is carried out, for example, 350 °C. This path following second-order kinetics implies that oligomerization will become more significant at higher concentrations (higher biomass loadings). Oligomerization products can also arise from styrene, which contains a vinyl group permitting it to undergo addition reactions to form dimers and trimers. One strategy to reduce the rate of oligomerization is to hydrogenate the double bond in styrene when it is formed. Thus, one might anticipate that algae liquefaction in the presence of a known hydrogenation catalyst and H₂ might produce a bio-oil with less of a high molecular weight component, as demonstrated by Duan and Savage.^[12] Oligomerization occurring with phenylalanine or other amino acids is difficult to control. Some strategies that can be used to reduce oligomerization are to use a chain-terminating molecule that can reduce the possibility of this type of step polymerization or perform liquefaction at lower temperatures or lower biomass loadings.

We have discovered that salts and boric acid accelerate phenylalanine conversion but decrease the yield of phenylethylamine and increase the yield of oligomers. Salts are present in 5–15 wt% in dry algae. It seems that their presence in algae can lead to undesirable oligomer formation from proteins and reduce the quality of bio-oil obtained from algae.

Lastly, the binary mixture of ethyl oleate and phenylalanine gave valuable insights for the reactions of complex systems such as triglycerides and proteins. The rate of deamination of phenylethylamine to styrene increases in the presence of ester. Deamination liberates ammonia, which can react with fatty acids/esters to form amides. Similarly, phenylethylamine can

react with fatty acids/esters to form the corresponding amides, which could potentially produce additional styrene at higher temperatures or longer batch holding times. The amide formation is undesirable as it adds to the nitrogen content in the bio-oil and decreases the fatty acid yields.

Conclusions

Under the conditions studied, phenylalanine readily underwent decarboxylation to form phenylethylamine. Subsequently, phenylethylamine underwent deamination to styrene (favored at high temperatures), and styrene underwent either hydration to form phenylethanol or oligomerization. Phenylethanol can dehydrate to a small extent in high-temperature water (HTW) to styrene. Phenylalanine can also oligomerize at high temperatures and longer batch holding times.

We propose a reaction network based on the above observations. A phenomenological kinetics model based on the proposed network accurately describes the trends in the data and typically estimates the concentrations of the reactant and products within their experimental errors. The rate constant for phenylethylamine formation is largest amongst all the first-order rate constants. Thus, we elucidate broadly through this study of phenylalanine that many different types of reactions (e.g., decarboxylation, hydration, oligomerization, and dehydration) occur during hydrothermal liquefaction of algae.

Experiments that explored the effects of salts and boric acid on the rate of decarboxylation of phenylalanine revealed that NaNO₃ and K₂HPO₄ have the most influence on phenylalanine conversion. However, the phenylethylamine yield and the carbon balance both decrease, indicating that oligomerization is favored in the presence of salts.

Mixtures of ethyl oleate and phenylalanine showed several interesting results. As the concentration of ethyl oleate increases, deamination of phenylethylamine and hydration of styrene are increasingly favored. Thus, the yield of phenylethylamine decreases and those of styrene and phenylethanol increase with increasing ester amount. As the amount of ester increases, phenylalanine also undergoes deamination, as a parallel reaction, to form phenylacetaldehyde, possibly favored due to lowering of the pH. Conversely, as the relative amount of phenylalanine is increased, the conversion of ethyl oleate and yield of oleic acid increased. New reaction products (9-octadecenamide, *N*-ethyl-9-octadecenamide, and *N*-phenylethyl-9-octadecenamide) are also formed in the binary mixture due to the interaction of phenylethylamine/ammonia with ethyl oleate/oleic acid. The total yield of the amides remains similar with changing molar ratios of the binary components. Due to the formation of these amides, the yields of oleic acid and phenylethylamine decreases at longer times.

A modified reaction network is proposed for the binary mixture, and the rate constants for this network were compared at different ratios of ethyl oleate to phenylalanine. Increasing the relative amount of ester led to higher rate constants for decarboxylation of phenylalanine, deamination of phenylethylamine, and hydration of styrene. The rate constant for dehydration of phenylethanol also increases by two orders of magnitude as

compared to its value with no ester added. The proposed network for the binary mixture accurately describes the trends in the data and typically estimates the concentrations of different products within their experimental errors.

The study of phenylalanine in HTW has implications to algae liquefaction. For instance, at 350 °C, when the bio-oil yield from algae is maximum, we obtain a low carbon balance, indicating oligomer formation. Ideally, one would want to avoid the formation of these oligomers to improve the quality of bio-oil. Strategies that involve hydrogenation of olefins in bio-crude or their cracking to smaller compounds might enhance bio-oil yields. Salts also enhance oligomer formation, whereas esters can form undesirable amides, which can increase the nitrogen content in the crude bio-oil.

Experimental Section

We carried out all experiments in batch reactors assembled from 3/8 inch 316 stainless steel Swagelok tube fittings (one port connector and two caps). Each reactor had an internal volume of approximately 1.5 cm³. Prior to using these reactors, we filled them with water and heated them in a fluidized sand bath at 300 °C for 60 min to remove any residual materials and expose the reactor walls to the HTW environment.

All chemicals were purchased from either Sigma Aldrich or Fischer Scientific in high purity ($\geq 99\%$) and used as received. The water was distilled and deionized prior to use.

Prior to loading the reactors, we prepared a stock solution by dissolving phenylalanine (1.5 g) in water (100 mL; giving a concentration of 15 000 ppm). Fresh stock solutions were prepared every two weeks. The reactors were then loaded at room temperature with suitable amounts of the stock solution. The amount of stock solution loaded for each experiment was such that the liquid phase would occupy about 95% of the reactor volume at the reaction conditions, based on the density of pure water at the same conditions, (i.e., 1.20, 1.14, 1.08, and 0.92 mL was loaded at 220, 250, 280 or 350 °C, respectively). Note that concentrations were calculated based on this volume throughout this report. Pressure at reaction conditions could be determined from the saturated steam tables as 2.3, 4.0, 6.4, and 17.0 MPa at 220, 250, 280, or 350 °C, respectively. The loaded reactors were sealed and placed in a pre-heated, fluidized sand bath (Techne SBL-2D) and maintained at the desired temperature using a temperature controller (Techne TC-8D). The sand bath was isothermal within ± 1 °C. The reactor heat-up time was 2–3 min as measured previously,^[8] which is short relative to the typical batch holding times used in this study. Upon reaching the desired batch holding time, the reactors were removed from the sand bath and quenched by immersing them in a cold water bath. The reactors were opened and the contents were recovered using methanol, which was able to dissolve both the reactants and the products formed during the reaction, thereby giving a clear homogeneous phase for further analysis.

We also performed experiments with the addition of various salts (NaCl, NaNO₃, Na₂SO₄, KCl, K₂HPO₄) and boric acid (H₃BO₃), at 250 °C and 30 min. Stock solutions were prepared by dissolving a salt (or boric acid; 40 mg,) in the phenylalanine stock solution (10 mL). The concentration of each additive in the phenylalanine solution was 4000 mg L⁻¹. The concentration of salt was chosen such that the ratio of salt to phenylalanine was between 20–25% (w/w), which is a typical ratio of ash/proteins in algae.^[2]

Reactions of a binary mixture were performed at 350 °C (10, 20, 30, 40, and 60 min) at three different molar ratios of phenylalanine to ethyl oleate ($\approx 0.2:1$; $\approx 1:1$; and $\approx 5:1$). Depending on the desired molar ratio of phenylalanine to ethyl oleate, we added stock solution (0.92 mL; either 4000, 15 000, or 22 000 ppm) along with the corresponding amount of ethyl oleate (either 10 or 40 μ L) to the reactor. Phenylalanine stock solution was prepared fresh for each mixture.

We performed control experiments at room temperature to determine the recovery of phenylalanine in the absence and presence of salt (K₂HPO₄). We loaded different stock solutions (1 mL) into a reactor and then sealed the reactor. The reactors were placed in the fluidized sand bath at 22.5 ± 1 °C for 30 min. The post-reaction work-up procedure used for the experiments at the elevated temperatures was then performed.

To quantify phenylalanine, we used high pressure liquid chromatography (HPLC) in combination with a UV detector. A Zorbax ODS column (4.6 mm inner diameter \times 250 mm length) with 5 μ m particle size was used for the separation of phenylalanine. An isocratic method using a mobile phase consisting of water (90%) containing trifluoroacetic acid (0.1%) and acetonitrile (10%) containing trifluoroacetic acid (0.1%) was used at 1 mL min⁻¹ for a total runtime of 20 min. The phenylalanine peak eluted at about 7 min and had a maximum absorbance at 205 nm.

We used an Agilent Technologies model 6890N gas chromatograph (GC) equipped with an autosampler, autoinjector, and mass spectrometric detector to identify the main products from the HTW treatment of phenylalanine and binary mixtures. Phenylalanine was not amenable to GC analysis due to very poor peak resolution on the GC. A Wiley mass spectral library was used for compound identification by matching the mass fragments of observed peaks with those in the library. An Agilent Technologies model 6890 GC equipped with a flame ionization detector (FID) was used to quantify the products. The reaction products were separated on a HP-5 MS fused silica, non-polar capillary column (50 m length \times 0.20 mm inner diameter \times 0.33 μ m film thickness). We used an inlet temperature of 300 °C, a split ratio of 5:1, and an injection volume of 1.0 μ L. The temperature program involved an initial oven temperature of 40 °C followed by heating to 150 °C at a rate of 10 °C min⁻¹ (isothermal for 4 min), then ramping at 10 °C min⁻¹ to 225 °C (isothermal for 5 min), and a final ramp of 10 °C min⁻¹ to 300 °C (isothermal for 15 min), giving a total run time of 50 min. Helium served as the carrier gas (1 mL min⁻¹).

To detect high molecular weight compounds and oligomers of phenylalanine in the product mixture, a Agilent Q-TOF LC-MS system equipped with an Agilent 1290 binary pump was used. Agilent Zorbax Eclipse Plus C-18 column (2.1 \times 50 mm, 1.8 μ m particle size) was used as stationary phase. One Solvent (A) comprised water with formic acid (0.1%). Another solvent (B) comprised acetonitrile (95%), water (5%), and formic acid (0.1%). A gradient method starting with 5% solvent B (hold 1 min) transitioning to 100% B over 9 min (hold 2 min) was used. The flow rate to the column was maintained at 0.4 mL min⁻¹. Representative samples from reactions conducted at 250 °C and 30 min and 350 °C and 60 min were analyzed. These samples were recovered in a 1:1 (v/v) mixture of methanol and chloroform (to give a homogenous phase with water) and diluted to 10 mL to ensure that all the oligomers are soluble in the chloroform phase.

Lastly, ethyl oleate and oleic acid were quantified by using an Agilent Technologies model 7890N GC-FID. The separation was carried out on a DB-FFAP column (30 m length \times 0.32 mm inner diameter \times 0.25 μ m film thickness). The inlet temperature was set at 220 °C with a split ratio of 5:1 and an injection volume of 1.0 μ L. The tem-

perature program involved an initial oven temperature of 50 °C followed by heating to 120 °C at a rate of 5 °C min⁻¹ and ramping at 20 °C to 220 °C (isothermal for 6 min), giving a total run time of 25 min. Helium served as the carrier gas (1 mL min⁻¹).

We generated calibration curves using standards of pure phenylalanine, phenylethylamine, styrene, phenylethanol, ethyl oleate, and oleic acid to determine the corresponding concentrations of each of these compounds. Product molar yields were calculated by dividing the number of mols of the product by the number of mols of limiting reactant initially loaded into the reactor. Selected experiments at each temperature, all experiments with salts (or boric acid), and selected experiments with binary mixtures of phenylalanine and ethyl oleate were conducted in triplicate. Experimental uncertainty was calculated as the standard deviations in the product yields from these replicated experiments.

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