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The Effect of Ionic Liquid Pretreatment on the Bioconversion of Tomato Processing Waste to Fermentable Sugars and Biogas

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Abstract Tomato pomace is an abundant lignocellulosic waste stream from industrial tomato processing and therefore a potential feedstock for production of renewable biofuels. However, little research has been conducted to determine if pretreatment can enhance release of fermentable sugars from tomato pomace. Ionic liquids (ILs) are an emerging pretreatment technology for lignocellulosic biomass to increase enzymatic digestibility and biofuel yield while utilizing recyclable chemicals with low toxicity. In this study, pretreatment of tomato pomace with the ionic liquid 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]) was investigated. Changes in pomace enzymatic digestibility were affected by pretreatment time and temperature. Certain pretreatment conditions significantly improved reducing sugar yield and hydrolysis time compared to untreated pomace. Compositional analyses suggested that pretreatment primarily removed water-soluble compounds and enriched for lignocellulose in pomace, with only subtle changes to the composition of the lignocellulose. While tomato pomace was effectively pretreated with [C2mim][OAc] to improve enzymatic digestibility, as of yet, unknown factors in the pomace caused ionic liquid pretreatment to negatively affect anaerobic digestion of pretreated material. This result, which is unique compared to similar studies on IL pretreatment of grasses and woody biomass, highlights the need for additional research to determine how the unique chemical composition of tomato pomace and other lignocellulosic fruit residues may interact with ionic liquids to generate inhibitors for downstream fermentation to biofuels.

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Introduction

Lignocellulosic plant biomass is a potential feedstock for production of renewable biofuels to offset the use of fossil fuels. However, lignocellulose, the structurally complex network of cellulose, hemicellulose, and lignin that comprises the plant cell wall, is highly resistant to enzymatic digestion. This recalcitrance limits the rate and amount of fermentable sugars released by deconstructive enzymes and is detrimental to the economics of biofuel production. As a result, pretreatment is often required to disrupt the highly ordered structure of lignocellulose and make the cell wall polysaccharides more accessible to deconstructive enzymes. A variety of pretreatment methods exist that strip away lignin and hemicellulose, disrupt the crystalline structure of cellulose, or otherwise alter the interaction of enzymes with cellulose.

Ionic liquid (IL) pretreatment is a promising emerging pretreatment technology. Pretreatment involves dissolution of plant biomass in ionic liquids, which are salts that are molten at or near room temperature. IL preatreatment can lead to disruption of the hydrogen bonds responsible for cellulose crystallinity, along with partial removal of lignin and hemicellulose, resulting in increased digestibility of the pretreated biomass [1–5]. In several types of biomass, such as grasses and woody biomass, IL pretreatment outperforms more established techniques such as dilute acid or alkaline pretreatment [6, 7]. Additionally, IL pretreatment is appealing due to the non-toxic nature of many ILs and their potential to be recycled.

A wide variety of lignocellulosic feedstocks will be required to meet the global need for renewable energy. Most IL pretreatment research has focused on dedicated bioenergy crops and woody residues from forests and agriculture. However, lignocellulosic residues from food processing are also an abundant and readily available feedstock. IL pretreatment has been performed on select food processing residues including corn cobs [8], wheat straw [9], and empty palm fruit bunches [10]. However, no research has reported IL pretreatment of waste biomass derived directly from fruits, such as the residual skins and seeds that result from processing of many fruits into pastes, purees, and juices. These residues can have unique properties relative to woody and graminaceous biomass, such as differing hemicellulose and lignin composition, increased pectin and protein content, greater moisture content, and elevated levels of fermentable non-structural carbohydrates, which may alter optimal pretreatment conditions. To date, pretreatment research for biofuel production from fruit pomaces has been limited to dilute acid or alkaline pretreatment of pomaces from apples [11, 12], olives [13], and grapes [14].

In this study, IL pretreatment of tomato pomace was investigated. Tomato pomace is the primary solid waste from tomato processing and is comprised of tomato skins and seeds that are separated from the juice prior to evaporation to form tomato paste. In California, where over 90 % of the USA's processing tomatoes are grown and processed (totaling roughly 35 % of global production) [15], approximately 60,000 dry Mg/year of tomato pomace are produced each season [16], most of which ends up as landfill or animal feed [17]. Utilization of tomato pomace to produce biofuels could reduce waste, offset a fraction of the processing costs, and decrease reliance on fossil fuels for energy.

To this end, ionic liquid pretreatment could potentially enhance sugar release during enzymatic digestion of pomace cell wall polysaccharides and subsequently improve the yield of biofuels following fermentation.

A response surface study was conducted to determine optimal pretreatment conditions with 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]), an IL that has garnered attention for its superior efficacy in dissolving lignocellulosic biomass [18–21]. [C2mim][OAc] is of particular interest because, unlike other effective ILs, it is not formed from a halide anion and therefore presents less toxicity risk to human and environmental health. [C2mim][OAc] also has a lower melting point than many chloride-based ILs, including [C2mim][Cl], such that preparation can be performed at room temperature and room temperature cooling will not result in IL solidification. There is also evidence that [C2mim][OAc] can better disrupt larger particle sizes compared to other ILs, which could reduce the energy and infrastructure needed to pulverize pomace ahead of pretreatment [22].

Tomato pomace was pretreated with [C2mim][OAc] under varying conditions and pretreatment efficacy was assessed using an enzymatic digestibility assay. Furthermore, the pretreatment performance of recycled [C2mim][OAc] was examined. Compositional analyses were performed on untreated and pretreated tomato pomace to determine if certain cell wall components were selectively removed during pretreatment. A variety of waste biomass bioconversion methods exist to produce different biofuels. In this study, anaerobic digestion to produce biomethane was considered as one potential biofuel production method. Anaerobic digestion was conducted using both pretreated and untreated pomace samples to investigate the potential of IL pretreatment to enhance biomethane production.

Materials and Methods

Tomato Pomace

Tomato pomace from paste production was collected from an industrial processing facility in Dixon, CA in 2013. The pomace consisted of the residual skins and seeds following juice separation in a finisher. The moisture content of the fresh pomace was 57.6 % (fresh weight basis). To stabilize the pomace, the material was solar dried for 1 week to achieve a moisture content of 5.1 % (fresh weight basis). Dried pomace was stored in sealed tubs at room temperature until use. To reduce the particle size to <1 mm and improve sample uniformity, 700 mL batches of pomace were homogenized in a Waring laboratory blender for 30 s on the high setting prior to pretreatment.

Ionic Liquid Pretreatment

For each pretreatment, 0.5 g of pomace was added to 9.5 mL of 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]) (BASF, Ludwigshafen, Germany) in a glass test tube. Tubes were heated in an oil bath at either 100, 130, or 160 °C for 1, 2, or 3 h. Following pretreatment, tubes were cooled to <100 °C and 20 mL of DI water was added to the mixture of biomass and ionic liquid to halt the pretreatment and regenerate cellulose. Pretreated solids were isolated and washed five times in DI water via vacuum filtration through grade 389 filter paper (Sartorius, Bohemia, NY). Washed solids were dried in a vacuum oven overnight at 45 °C and then weighed to determine dry weight yield. Dried solids were stored at 4 °C until further use.

Enzymatic Digestion and Reducing Sugar Assay

Pretreated pomace was evaluated for enzyme digestibility using a cellulase mixture followed by a reducing sugar assay. Samples of pretreated tomato pomace were enzymatically digested in a 1-mL reaction volume containing 30 mg pomace (dry weight), 50 mM sodium acetate (pH 5.0), 0.3 % (*m*/*v*) sodium azide, and 9.7 EGU/mL cellulase from *Trichoderma reesei* (Sigma Aldrich, St. Louis, MO), which contained multiple cellulases and some hemicellulase activity. Untreated (raw) pomace and pomace that had been washed five times in DI water to remove soluble sugars were used as controls. The washed pomace was used to reduce the confounding effect of signal from non-cell wall soluble sugars during reducing sugar assays. Digestions were conducted at 45 °C and 200 rpm agitation. Ten-microliter samples were taken from reaction tubes at 0, 1, 2, 3, 5, 7, and 24 h (with an additional 12 h time point for the recycling study), diluted in 50 μL DI water, boiled for 5 min, and then stored at −20 °C. Samples were assayed for reducing sugar content using the dinitrosalicylic acid (DNS) assay. For each reaction, 23.3 μL of digestion sample was combined with 46.7 μL of DNS reagent (14 g/L dinitrosalicylic acid, 14 g/L sodium hydroxide, and 280 g/L potassium sodium tartrate), heated for 5 min at 95 °C, diluted to 30 % concentration in DI water, and the absorbance at 540 nm was measured. Standard solutions of 0, 1, 2, 3, 5, and 10 mg/mL glucose were processed identically to digestion samples. As a result, reducing sugar mass values were calculated as the equivalent mass of glucose.

Ionic Liquid Recycling

A pooled mixture of spent ionic liquid and water (approximate volumetric ratio 1:2) from pretreatment experiments was stored at 4 °C. To isolate IL for recycling, 100 mL samples of aqueous spent IL were combined with 25 g of potassium phosphate (K_3PO_4) in a separatory funnel. Addition of the potassium phosphate induced separation of the IL from the aqueous phase that formed with the hygroscopic salt [23]. The IL-rich phase at the bottom of the separatory funnel was drained and dried of residual water at 105 °C for 1 h prior to reuse in pretreatment.

Recovered IL was used to pretreat tomato pomace using the methods described previously. Pretreatments were performed at 130 °C for 1 h. Pomace pretreated with either recycled IL or with fresh IL were tested for enzymatic digestibility. Non-pretreated (raw) pomace was used as a control. Three replicate samples were processed for each treatment and control.

Anaerobic Digestion

Methanogenic sludge was obtained from a thermophilic anaerobic digester used to process mixed organic waste at the University of California, Davis. To prepare batch anaerobic digesters, 0.5 g of pomace was added to 50 mL of sludge in a 250-mL glass media bottle and the headspace was purged with nitrogen gas. Bottle lids contained check valves (0.29 psi cracking pressure, #80103, Qocina, Ronkonkoma, NY) to allow biogas to vent and prevent oxygen contamination. Biogas was captured by tubing that connected check valves to gas collection vessels. Gas collectors were connected to a MicroOxymax respirometry system (Columbus Instruments, Columbus, OH) for periodic measurement of methane, carbon dioxide, and hydrogen gases. Batch digesters were incubated at 55 °C for 28 days. Concentration and cumulative production of target gases

were measured every 2 h for the duration of the study. Biogas quality was calculated as the volumetric percentage of methane in the cumulative volume of biogas (estimated as the combined cumulative volumes of methane and carbon dioxide) produced over the incubation period.

To determine if pretreatment was effective for enhancing biogas production during anaerobic digestion, pretreated pomaces were tested against non-pretreated pomace. Water-washed pomace was also examined to remove the confounding effect of soluble carbohydrate leaching and isolate the effect of sugar release due to cellulose digestion. In a subsequent anaerobic digestion study, pretreated pomace was combined with untreated pomace at a mass ratio of 2:1 to investigate if supplementation with untreated pomace may compensate for nutrients lost from the pomace during pretreatment.

Analytical Methods

The moisture content of fresh and dried pomace was determined by drying in a vacuum oven at 45 °C for 24 h until the dry weight stabilized. Pomace composition was determined via fractionation followed by gravimetric and HPLC analyses. The operations and mass flows for each of the methods are depicted in Fig. 1. Gravimetric analysis was performed on both raw pomace and pomace pretreated at 130 °C for 1 h based on the updated fiber analysis protocols of Van Soest [24, 25]. Neutral detergent solution and acid detergent solution were prepared according to established methods with the modifications suggested by Van Soest: decahydronaphthalene was omitted and 2-ethoxyethanol was replaced with the safer

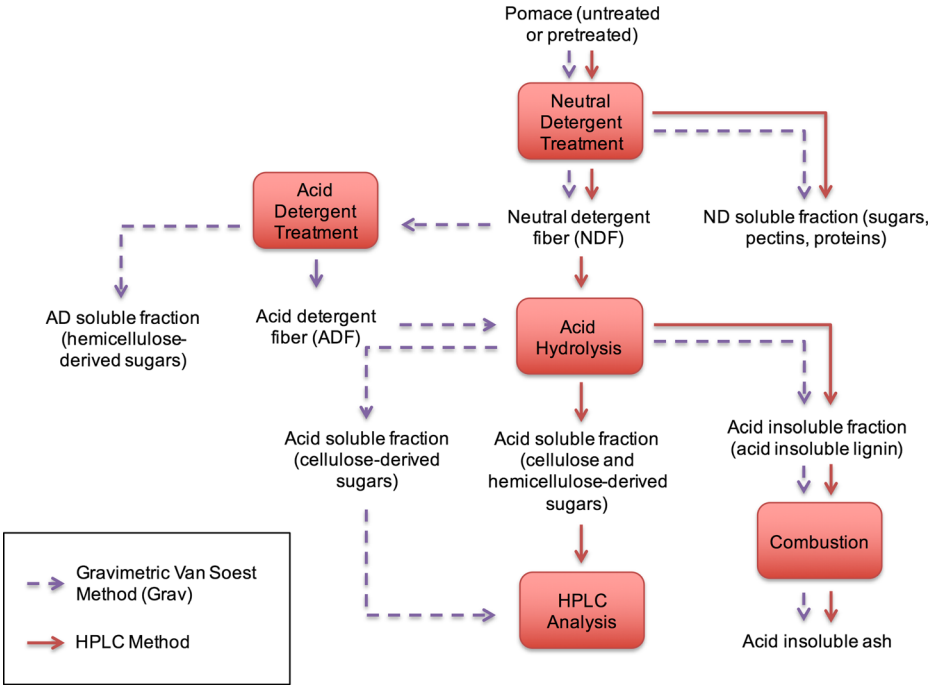

Fig. 1 Flow diagram illustrating operations and material flows for the two analysis methods used to characterize pomace composition

triethylene glycol (99 %, Alfa Aesar, Haverhill, MA), and 50 μL of α-amylase (heat-stable, #A3306, Sigma Aldrich) was added to the neutral detergent solution.

In brief, pomaces were first subjected to a neutral detergent extraction to remove extractives and pectin, followed by an acid detergent extraction to selectively remove hemicelluloses, and a final 1-h digestion in 72 % sulfuric acid at room temperature to hydrolyze cellulose. Between each extraction step, the solids fractions were isolated in porcelain filtering crucibles (25 mL, medium porosity, Fisher Scientific, Hampton, NH) using a vacuum filtration apparatus with crucible adapters. Isolated solids were dried in a drying oven at 105 °C and then weighed to determine the change in mass between each extraction. These data were used to calculate the mass fractions of the compounds targeted by each digestion or extraction step. Residual solids obtained following the final digestion step was considered to be acid-insoluble lignin. The acid-insoluble ash content of the sample was determined by measuring the residual mass following combustion of the sample in a muffle furnace at 550 °C for 4 h. Together, these mass fractions defined the compositional mass balance for the pomace.

To further resolve structural carbohydrate composition in pomace samples, the solids fractions recovered after neutral detergent and acid detergent extractions were analyzed by HPLC. Solids fractions underwent hydrolysis in 72 % sulfuric acid at room temperature for 1-h followed by dilute acid hydrolysis in 4 % sulfuric acid in pressure tubes (Ace Glass, Vineland, NJ)) at 121 °C for 1 h in an autoclave [26]. The hydrolysates were neutralized using calcium carbonate to a pH of approximately 5.5, filtered through 0.2 μm syringe filters and stored at 4 °C until analysis. Soluble sugar compositions were analyzed by HPLC (Shimadzu Scientific Instruments, Columbia, MD) using Aminex HPX-87P with de-ashing and Carbo-P guard cartridges (BioRad, Hercules, CA). 10 μL of each sample was injected. The flow rate was 0.6 mL/min at 80 °C using HPLC-grade water as mobile phase. Sugar concentration was determined by a refractive index detector (RID-10A, Shimadzu Scientific Instruments, Columbia, MD). Standard solutions of D-glucose, D-xylose, D-mannose, D-galactose, D-cellobiose, and L-arabinose were processed in parallel with pomace samples as calibration standards.

Carbohydrate degradation products were measured using Aminex HPX-87H with H-guard cartridge (BioRad, Hercules, CA). 10 μL of each sample was injected. The flow rate was 0.6 mL/min at 45 °C using 0.005 M sulfuric acid as mobile phase. Degradation products were determined using photodiode array (PDA) and refractive index detectors with running time 60 min. The standard solutions of galacturonic acid, L-lactic acid, formic acid, acetic acid, levulinic acid, 5-hydroxy-2- furaldehyde (HMF), and furfural were used as calibration standards to determine the level (if any) of sugar degradation.

Sugar recovery standards were run in parallel to the pomace samples during the acid hydrolysis, autoclave step, and neutralization to quantify any loss of sugars resulting from the hydrolysis procedure. Triplicate analyses were conducted for all samples.

Experimental Design and Analysis

The effects of pretreatment temperature and duration on biomass solubilization, enzymatic digestibility, and anaerobic digestion were examined using a face-centered, 3×3 central composite design (CCD) experiment. The three pretreatment temperatures and durations described in the pretreatment section defined the design space. The low, medial, and high values for each variable were coded as -1, 0, and +1, respectively. The center point (130 °C, 2 h) was repeated five times to gauge variability. Three to five replicates of untreated and washed pomace controls were used depending on the experiment. Release of reducing sugars

during pomace digestion was calculated by first subtracting initial absorbance at 540 nm values from subsequent absorbance readings to remove background signal and then comparing the absorbance to the glucose standard curve. The sugar release versus time data was used to fit parameters in a saturation model of the form:

$$S(t) = \frac{S_{\max} t}{K_S + t} \tag{1}$$

where $S(t)$ is the glucose equivalent mass of reducing sugar released at time t (g reducing sugar/g dry solids), $S_{\max}$ is the maximum glucose equivalent mass of reducing sugar that can be released (g reducing sugar/g dry solids), t is the time (h), and K_S is the time required to achieve sugar releasing totaling half of $S_{\max}$ (h). Parameter fitting was accomplished using the non-linear least squares curve fit (lsqcurvefit) function in Matlab R2013b (version 8.2.0.701, Mathworks, Natick, MA) with parameter estimates constrained to positive values.

Response data from CCD experiments were used to fit parameters in a response surface model of the form:

$$Y(t,T) = \beta_0 + \beta_t t + \beta_T T + \beta_{tT} tT + \beta_{tt} t^2 + \beta_{TT} T^2 \tag{2}$$

where $Y(t,T)$ is the response of interest, t is the coded value for pretreatment time, T is the coded value for pretreatment temperature, β_0 is a constant describing the intercept, β_t is the main effect of pretreatment time on the response, β_T is the main effect of pretreatment temperature on the response, β_{tT} is the interaction effect between pretreatment time and temperature on the response, β_{tt} is the second-order effect of pretreatment time on the response, and β_{TT} is the second-order effect of pretreatment temperature on the response. Parameters in the response surface model were fitted with JMP Pro statistical analysis software (version 12.0.1, SAS, Cary, NC) using the *fit model* command and a standard least squares fit.

Results

Solids Recovery

Recoverable solids, or the yield of dry solids (as a percentage of raw pomace dry mass), from each pretreatment as well as from washed pomace are shown in Table 1 and Fig. 2. Significant main and second-order effects were observed for pretreatment temperature. Increased temperature resulted in decreased recoverable solids with a precipitous decrease in recoverable solids at a pretreatment temperature of 160 °C. The amount of recoverable solids from pomace washed with water was approximately 80 %, representing the loss of solids due to dissolution of water-soluble compounds and removal of small particulates during the washing process. Pretreated pomace exhibited additional loss of solids over water-washed pomace, indicating additional solubilization of material during pretreatment.

Enzymatic Digestion

The enzymatic digestibility of pretreated tomato pomace was tested against non-pretreated raw and washed pomace controls using cellulase from *T. reesei* and a reducing sugar assay. Results for the enzymatic digestion analyses are shown in Fig. 3, Tables 2 and 3.

Table 1 Parameter estimates for the response surface model describing recoverable solids as a function of pretreatment time and temperature

Parameter	Estimate[a]	Standard error	*P* value[b]
β_0	0.629	0.008	*<0.0001*
β_t	−0.003	0.008	0.6906
β_T	−0.063	0.008	*0.0001*
β_{tT}	−0.008	0.010	0.4708
β_{tt}	−0.005	0.012	0.6754
β_{TT}	−0.035	0.012	*0.0208*

[a] Parameter estimates are based on the response surface model using units of g solids recovered/g dry pomace for the dependent variable and coded values for the independent variables

[b] Values in italics indicate *P* values that are below the 0.05 threshold for statistical significance

Data from enzymatic digestion reactions describing reducing sugar release versus time were sufficient for fitting the non-linear model given in Eq. 1 (Supplementary Figure 1). The first- and second-order effects of temperature were statistically significant for both the maximum projected yield of reducing sugars (S_{max}) (Fig. 3a, Table 2) and the enzyme kinetics, indicated by the time required for the release of reducing sugars to reach half of S_{max} (K_s) (Fig. 3b, Table 3) ($P<0.05$). The 100 and 130 °C pretreatment temperatures performed better than the untreated and washed controls with respect to yield of reducing sugars. The center points in particular performed significantly better than washed and untreated controls as determined by one-way ANOVA analysis ($P<0.01$ for both).

However, the significant second-order effect was evidenced by the lower S_{max} values observed at 160 °C, which were lower than the control samples. Conversely, pretreatment at 160 °C led to more rapid digestion, as indicated by greater K_s values compared to control samples. For subsequent experiments, 1 h was determined to be a suitable pretreatment time since the effect of time was insignificant for both digestibility response metrics ($P>0.05$).

S_{max} values denote the maximum projected yield of reducing sugars per unit mass of pretreated material. Reducing sugar yields were also normalized per unit mass of pomace prior to pretreatment (or washing in the case of washed pomace controls). The total yields of

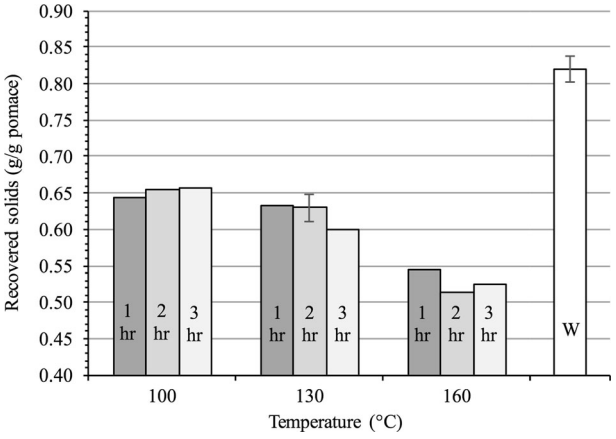

Fig. 2 Recoverable solids from tomato pomace after IL pretreatment at various temperatures and times. Pretreatment times are indicated within each *bar*. Tomato pomace that was not pretreated but was washed similarly to pretreated pomace (*W*), is presented as a control. *Error bars* represent one standard deviation for yields observed at the center-point treatment ($n=5$) and in non-pretreated, washed tomato pomace ($n=3$)

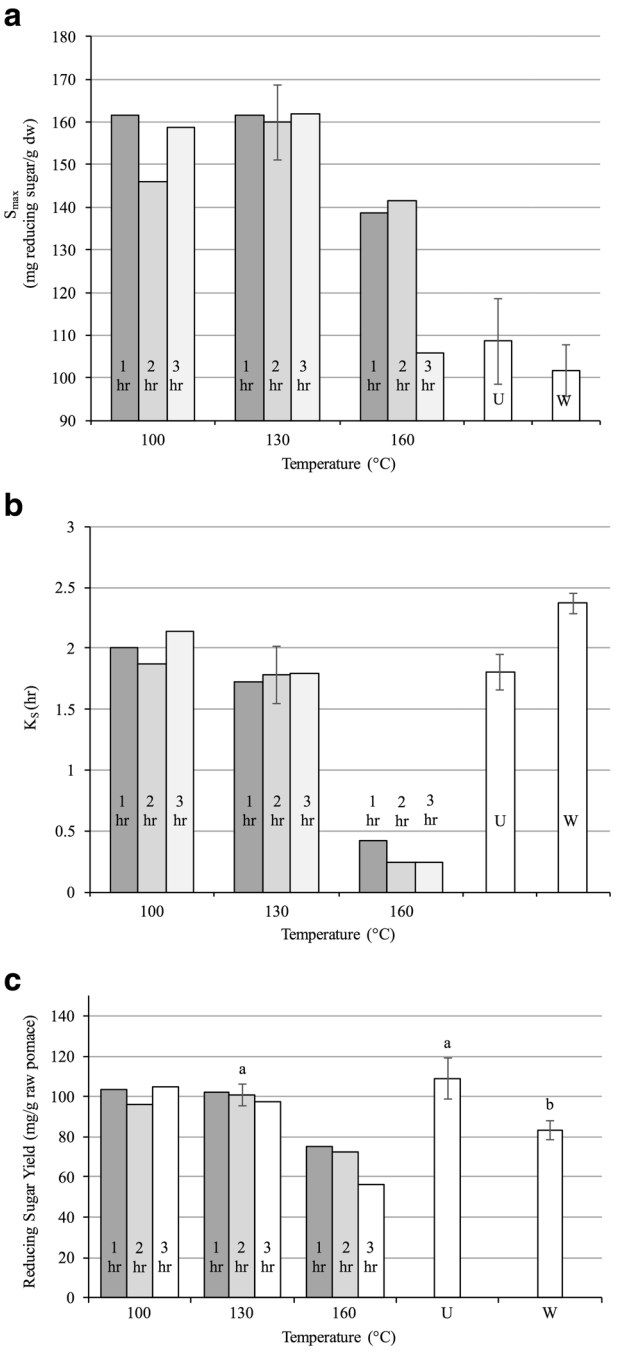

Fig. 3 Fitted parameters from non-linear regression models describing enzymatic digestion of pretreated pomace. Parameters indicate **a** the projected maximum release of reducing sugars per unit mass of pretreated pomace and the **b** time to achieve half of the projected maximum sugar release during enzymatic digestion. S_{max} values were normalized to determine the **c** projected reducing sugar yield per unit mass of original raw pomace during enzymatic digestion of pretreated tomato pomace. Pretreatment durations are indicated within bars. Tomato pomace that was untreated (*U*) and pomace that was not pretreated but was washed similarly to pretreated pomace (*W*), are presented as controls. *Error bars* represent one standard deviation. Where presented, *values that do not share a letter* are significantly different ($P < 0.05$). $n = 5$ for the centerpoint, untreated, and washed treatments

reducing sugars per gram of starting pomace for pretreated, untreated, and washed pomaces are shown in Fig. 3c. Fitted parameters for the corresponding response surface model are shown in Table 4. Within the design space, the greatest pretreatment temperature resulted in significantly lower reducing sugar yield, as indicated by significant first- and second-order effects for

Table 2 Parameter estimates for the response surface model describing S_{max} as a function of pretreatment time and temperature

Parameter	Estimate[a]	Standard error	*P* value[b]
β_0	160.5	4.5	*<0.0001*
β_t	−5.9	4.4	0.220
β_T	−13.4	4.4	*0.019*
β_{tT}	−7.8	5.4	0.201
β_{tt}	−0.2	6.5	0.980
β_{TT}	−18.3	7.8	*0.025*

[a] Parameter estimates are based on the response surface model using units of mg reducing sugar/g dry solids for the dependent variable, S_{max}, and coded values for the independent variables

[b] Values in italics indicate *P* values that are below the 0.05 threshold for statistical significance

temperature in the response surface model. The significant second-order effect was reflected in the data as a notable decrease in yield for pomace pretreated at 160 °C in contrast to the enhanced yield observed for pretreatment at 100 or 130 °C. Moreover, there was a significant negative main effect for pretreatment time. Results of a one-way ANOVA comparing untreated, washed, and the center point pretreatment of 130 °C for 1 h indicated that both the pretreated ($P<0.01$) and untreated pomace ($P<0.01$) resulted in higher theoretical reducing sugar yields than the washed pomace.

Ionic Liquid Recycling

S_{max} and K_S values were determined for the enzymatic digestion of pomace pretreated with fresh IL and recycled IL. Non-pretreated pomace was analyzed as a control. There were no statistically significant differences in K_S values between the three groups ($P=0.192$) (Fig. 4a). However, there was a significant difference between treatments for S_{max} ($P=0.000231$). Tukey's post-hoc analysis determined that pomace pretreated with recycled IL yielded a significantly lower S_{max} value than that obtained from pretreatment using fresh IL ($P<0.05$) (Fig. 4b). Furthermore, pretreatment with either fresh or recycled IL resulted in significantly greater S_{max} values than that obtained from non-pretreated raw pomace ($P<0.01$ for both).

Table 3 Parameter estimates for the response surface model describing K_s as a function of pretreatment time and temperature

Parameter	Estimate[a]	Standard error	*P* value[b]
β_0	1.76	0.08	*<0.0001*
β_t	<0.01	0.08	0.952
β_T	−0.85	0.08	*<0.0001*
β_{tT}	−0.08	0.10	0.439
β_{tt}	0.06	0.11	0.639
β_{TT}	−0.64	0.11	*0.001*

[a] Parameter estimates are based on the response surface model using units of h for the response variable, K_s, and coded values for the independent variables

[b] Values in italics indicate *P* values that are below the 0.05 threshold for statistical significance

Table 4 Parameter estimates for the response surface model describing reducing sugar yield (S_{max}) normalized per gram of raw pomace as a function of pretreatment time and temperature

Parameter	Estimate[a]	Standard error	*P* value[b]
β_0	97.72	1.835	<0.0001
β_T	−14.11	1.804	*0.0001*
β_t	−4.575	1.804	*0.0389*
β_{tT}	−4.7425	2.209	0.0689
β_{TT}	−12.33	2.658	*0.0024*
β_{tt}	0.01534	2.658	0.9956

[a] Parameter estimates are based on the response surface model using mg reducing sugar/g pomace for the response variable and coded values for the independent variables

[b] Values in italics indicate *P* values that are below the 0.05 threshold for statistical significance

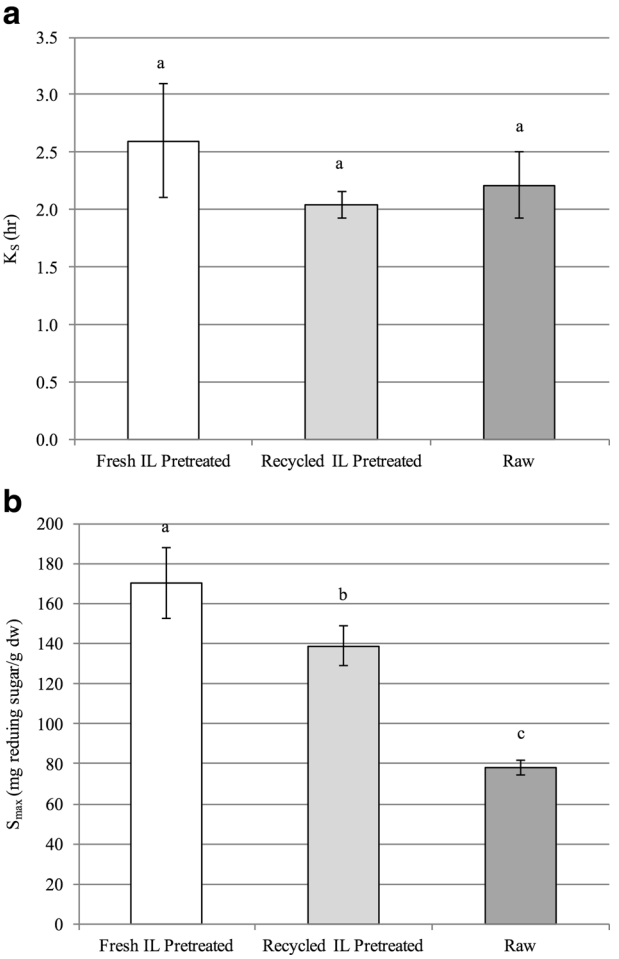

Fig. 4 Enzymatic digestibility of tomato pomace pretreated using fresh IL and recycled IL. Non-pretreated (*raw*) pomace served as a control. Empirical data were used to estimate parameter values for **a** K_S and **b** S_{max} from Eq. 1. *Values that do not share a letter* are significantly different ($P < 0.05$)

Biomass Composition

Compositional analyses were performed on raw and pretreated pomaces to gauge the effect of IL pretreatment on retention of cell wall components. The results from the compositional analyses are shown in Fig. 5a. Since negligible levels of oxidized compounds were detected, it was concluded that minimal degradation occurred (data not shown). The sequential extraction and gravimetric analysis generally led to higher estimates of cellulose and hemicellulose content compared to levels measured via HPLC. As HPLC provides a more targeted measurement of cell wall polysaccharides, hemicellulose, and cellulose levels measured in this way are likely more accurate. Both analyses agreed that pretreated pomace contained lower levels of extractives and were enriched for lignin. Moreover, both analyses suggested an enrichment

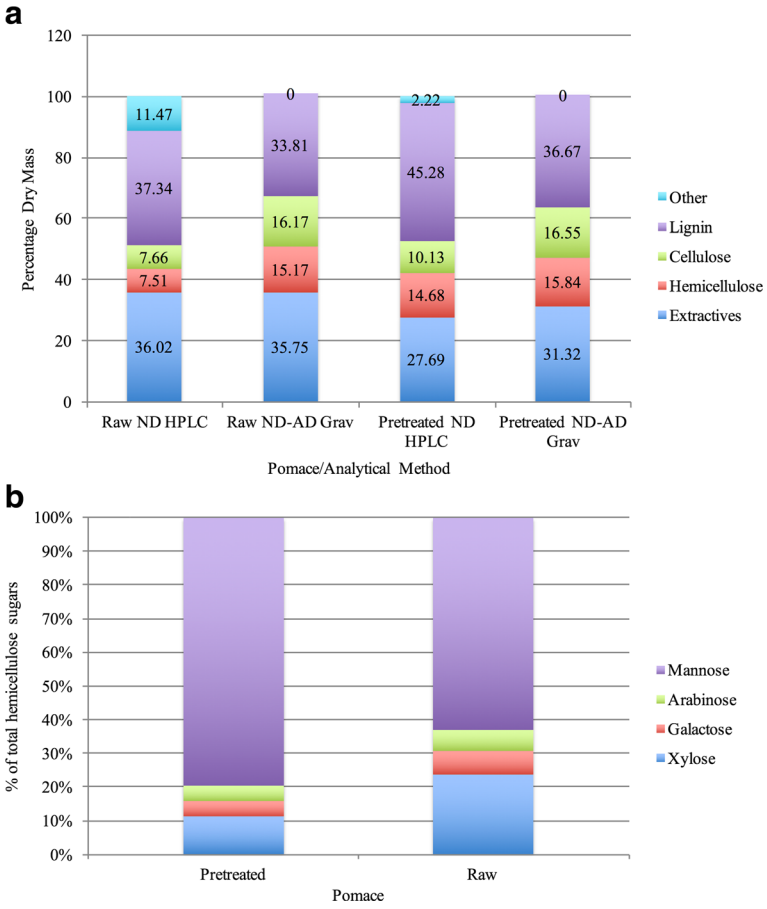

Fig. 5 Compositional analyses of pretreated and untreated raw tomato pomace. **a** Mass percentages of cell wall polysaccharides, lignin, extractives, and other (material present in cell wall hydrolysates that could not be classified as cellulose or hemicellulose via HPLC) in non-pretreated tomato pomace and tomato pomace pretreated at 130 °C for 1 h. Data are presented for pomace samples processed using two different analytical methods: *ND HPLC* HPLC analysis of structural carbohydrates in the hydrolysate of the neutral detergent fiber; *ND-AD grav* gravimetric determination of structural carbohydrates via sequential neutral detergent extraction, acid detergent extraction, and acid hydrolysis. **b** Relative abundance of hemicellulose-specific sugars within the hemicellulose fraction of untreated raw pomace and pretreated pomace. Values are given as the mean of three replicates

of cellulose and hemicellulose in the pretreated material, although the effect was more apparent when using HPLC to directly measure cellulose- and hemicellulose-derived sugars. Additionally, HPLC was used to measure the composition of sugars uniquely derived from hemicellulose to gauge whether IL pretreatment selectively affected particular hemicellulose polysaccharides (Fig. 5b). The data showed that pretreatment led to a significant enrichment of mannose ($P = 0.0229$) with a corresponding significant decrease in xylose ($P = 0.0253$). While there was also enrichment of arabinose and galactose in the pretreated material, the effect was not statistically significant.

Pomace composition and overall solids recovery data were used to calculate the recovery of individual biomass components following pretreatment. Retention of each component was calculated as a percentage of the original mass of that component in the pomace prior to pretreatment (Table 5). The data for both compositional analysis methods showed that approximately half the total extractives were removed during pretreatment. Additionally, the data for both analytical techniques indicated that there was loss of some cellulose and lignin during pretreatment. However, the more targeted HPLC-based quantification method suggested greater recovery of cellulose, hemicellulose, and lignin following pretreatment compared to the purely gravimetric sequential extraction analytical method. For both methods, measurement of hemicellulose retention was most variable. Although the mean hemicellulose retention for the HPLC-based method was greater than 100 %, the 100 % retention value falls within the 95 % confidence interval for the mean. Consequently, this result can likely be attributed to the considerable variability in the hemicellulose retention data.

Anaerobic Digestion and Biogas Production

Methane and carbon dioxide production were measured during anaerobic digestion of pretreated, raw, and washed tomato pomace samples. There was no significant difference in the total quantity of methane produced per unit mass of pomace between pretreated samples and the control untreated and washed samples. Furthermore, there were no significant differences in methane yield among the pretreatment conditions examined in the response surface design space (data not shown). However, there were statistically significant differences in biogas quality, measured as the fraction of methane in the total combined volume of methane and carbon dioxide produced, within the design space (Fig. 6, Table 6). Within the design

Table 5 Recovery of components in pretreated pomace according to different analytical methods, expressed as percentages of quantity in raw untreated pomace

Component	ND HPLC recovery[a,b]	ND-AD grav recovery[a,c]
Extractives	48.81 ± 8.69	54.78 ± 1.25
Cellulose	83.38 ± 6.75	64.48 ± 3.63
Hemicellulose	123.1 ± 31.36	65.75 ± 16.82
Lignin	84.38 ± 14.49	61.87 ± 7.27

[a] Values are given as mean ± standard deviation

[b] Component mass fractions measured using gravimetric determination of neutral detergent extractives, HPLC measurement of structural carbohydrates in the hydrolysate of the neutral detergent fiber, and gravimetric measurement of lignin via combustion of residue following hydrolysis

[c] Component mass fractions measured gravimetrically via sequential neutral detergent extraction, acid detergent extraction, acid hydrolysis, and combustion

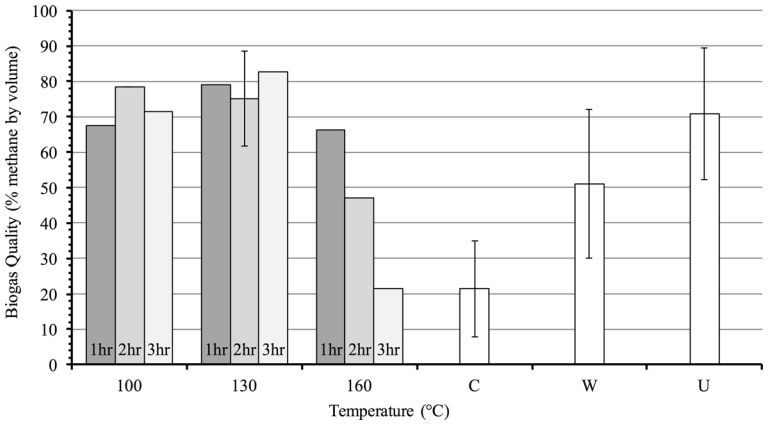

Fig. 6 Mean biogas quality from anaerobic digestion of tomato pomace pretreated using various pretreatment times and temperatures. Pretreatment times are listed within bars. Biogas quality data is also provided for control samples containing no pomace (C), washed, non-pretreated pomace (W), and raw, untreated pomace (U). *Error bars* indicate one standard deviation. $n = 5$ for the center-point and $n = 3$ for the control treatments

space, there was a significant negative main and second-order effect of pretreatment temperature on biogas quality. This is evidenced by the fact that pomaces pretreated at either 100 or 130 °C showed high biogas quality comparable to untreated control pomace while pomace pretreated at 160 °C exhibited markedly lower biogas quality compared to the controls. While the interaction effect between pretreatment time and temperature was not found to be statistically significant, the P value for the interaction effect ($P = 0.087$) approached the $P = 0.05$ threshold for significance. The interaction effect was apparent in the data as pretreatment time appeared to have a greater negative impact on biogas quality for pretreatment at 160 °C compared to lower pretreatment temperatures.

Following this experiment, two additional pomace treatments were tested to explore whether loss of nutrients from the pomace during pretreatment impacted anaerobic digestion performance. Biogas production was monitored for digesters supplied with either untreated pomace or a 2:1 mixture (dry weight basis) of untreated pomace and pomace pretreated at 130 °C for 2 h. There was no significant difference in methane yield for anaerobic digestion of the supplemented pretreated pomace versus the untreated pomace control (data not shown),

Table 6 Parameter estimates for the response surface model describing biogas quality as a function of pretreatment time and temperature

Parameter	Estimate[1]	Standard error	P value[2]
β_0	77.535	5.87	*<0.0001*
β_t	−6.210	4.67	0.2413
β_T	−13.708	4.67	*0.0325*
β_{tT}	−12.165	5.72	0.087
β_{tt}	−0.367	7.19	0.961
β_{TT}	−18.562	7.19	*0.0494*

[a] Parameter estimates are based on the response surface model using the volumetric percentage of methane in the total biogas volume for the response variable and coded values for the independent variables

[b] Values in italics indicate P values that are below the 0.05 threshold for statistical significance

suggesting that loss of nutrients from the pomace during pretreatment was not the sole factor preventing enhanced anaerobic digestion of the pretreated pomace.

Discussion

Ionic liquid pretreatment increased the enzymatic digestibility of tomato pomace under certain pretreatment conditions. A significant increase in the S_{max} value and the reducing sugar yield value along with a significant decrease in the K_S value for pomace pretreated at 130 °C suggested that IL pretreatment can improve both the rate of release and the total quantity of fermentable sugars released from tomato pomace during enzymatic digestion. Differences in apparent reducing sugar release between pretreated pomace, untreated pomace, and pomace that was washed with water but otherwise not pretreated highlighted important considerations for interpreting enzymatic digestibility data. Unlike many conventional lignocellulosic feedstocks, which are typically woods or grasses, tomato pomace can contain appreciable quantities of water-soluble sugars due to the presence of residual pulp and juice. In particular, tomato juice contains glucose and fructose, which are both reducing sugars [27]. Leaching of these non-cell wall, soluble sugars during enzymatic digestion of the pomace can confound the measurement of reducing sugar release due to cell wall digestion. As a result, the water-washed pomace, which is stripped of confounding water-soluble reducing sugars, is a more appropriate control for gauging reducing sugar release during enzymatic digestion of cell wall polysaccharides. Likewise, water-washed pomace is a more suitable control for enzymatic digestion comparisons with IL-pretreated pomace, since the pretreatment and post-treatment washing processes likely also removed water-soluble sugars. Accordingly, comparisons between pretreated pomace and water-washed pomace were used to assess increases in digestibility due to pretreatment.

Enzymatic digestion data indicated a statistically significant 21 % increase in reducing sugar yield for pomace pretreated at 130 °C for 2 h compared to water-washed pomace. Solids recovery data suggested that there was a significant loss of solids as a result of IL pretreatment. Some of this loss may be attributed to removal of solutes by the IL during pretreatment. However, loss of precipitated cellulose and small biomass particulates during the post-pretreatment washing process may have also contributed to reduced solids recovery. Improving the post-pretreatment washing to retain more solids while removing residual IL may further enhance the reducing sugar yield from pretreated pomace relative to washed pomace.

While the increase in reducing sugar yield from the pretreated pomace can be reasonably assumed to be glucose from cellulose digestion, the cellulase mixture from *T. reesei* contained some hemicellulase activity according to the manufacturer's information. As a result, release of reducing sugars from hemicellulose degradation may also contribute to the observed increase in reducing sugar yield. While the increase in reducing sugar yield is notable, the magnitude of the yield improvement is not as great as that seen following IL pretreatment of many conventional lignocellulosic biofuel feedstocks. For instance, [C2mim][OAc]-pretreated cotton stalk showed 65 % digestibility (% reducing sugars/% solids) (more than 10-fold increase) [4]; 96 % cellulose conversion and 63 % xylan conversion were observed in switchgrass after pretreatment with imidazolium ILs, compared with just 2.7 and 8 %, respectively, for untreated switchgrass [5, 6]. A reducing sugar yield of 55 % (as % pretreated weight) was observed in wheat straw with [C2mim][DEP], as opposed to roughly 25 % observed with a similar

pretreatment with water [28]; 90 % glucan conversion was observed in pretreated *Pinus radiata* (compared to ~5 % untreated) [18]. The contrasting results for IL pretreatment of tomato pomace and conventional lignocellulosic feedstocks may stem from differences in cellulose digestibility between the materials. In prior studies, non-pretreated tomato pomace showed variable cellulose hydrolysis during digestion with *T. reesei* cellulases, with conversion values ranging 34 to 76 % [29, 30]. Despite the variability, these values are generally larger than those measured for the aforementioned non-pretreated lignocellulosic feedstocks. The greater digestibility of non-pretreated tomato pomace limits the maximum possible fold increase in digestibility such that it is less than that of more recalcitrant conventional lignocellulosic feedstocks.

Results from the IL recycling study suggested that recycled IL is effective at increasing the enzymatic digestibility of tomato pomace, though not as effective as fresh IL. Similar results have been observed in previous IL pretreatment studies involving other feedstocks [31, 32]. Carryover of moisture or dissolved biomass components in the recycled IL may contribute to decreased efficacy. Imidazolium IL has been shown to retain approximately 3 % of wood solids following pretreatment [31] and moisture contamination of ILs has been shown to decrease cellulose digestibility following pretreatment [33]. Given the current high cost of many ionic liquids, recovery and recycling of IL is crucial to improve the process economics of IL pretreatment at industrial scale [34]. However, additional research is required to determine what chemical modifications are made to IL during pretreatment of tomato pomace and to identify biomass-derived compounds that are retained in recovered IL that may affect pretreatment performance across multiple recycles.

Pomace composition data obtained prior to and following IL pretreatment provided insight into the nature of pomace structural changes that may result from pretreatment. Composition data for non-pretreated raw pomace aligned with previously published data, particularly for values of neutral detergent fiber [35, 36]. The measured cellulose:hemicellulose mass ratio of approximately 1:1 differed slightly from previous studies on tomato processing waste that found the ratio closer to 3:2 [37]. However, differences in tomato cultivar, season, and growing conditions may explain this subtle difference. Prior studies also indicated that nearly 40 % of the pomace cell wall matrix is composed of pectins. This agrees with the high quantity of neutral detergent-soluble material measured in the present work. The large amount of soluble material observed in the non-pretreated raw pomace was also consistent with the relatively high level of soluble carbohydrates previously measured in tomato biomass [37]. The results indicated that pretreatment served mainly to remove a portion of the extractives from the pomace, leading to hemicellulose, cellulose, and lignin constituting a greater mass fraction of the pretreated pomace. However, there were only subtle changes in the relative mass ratios of hemicellulose, cellulose, and lignin, indicating pretreatment did not selectively remove or enrich certain cell wall polysaccharides or lignin in the tomato pomace. Though some other feedstocks have shown the potential for strong selective enrichment or removal of particular structural components [6, 19, 38–41], this did not appear to be the case for tomato pomace pretreated with [C2mim][OAc]. In light of this, the mechanism for enhanced enzyme accessibility to substrates in IL-pretreated pomace may be due to disruption of cell wall component interactions rather than complete removal of any single component, as has been proposed for other IL-pretreated biomasses. Prior research has demonstrated such lignocellulose disruption. Studies showed that ionic liquid pretreatment decreased the crystallinity in regenerated cellulose for various feedstocks [6, 18, 20]. More research is needed to determine if compositional features that are unique to fruit pomaces compared

to conventional grass and woody feedstocks, such as greater soluble sugar, pectin, protein, and seed oil content, interact with either the lignocellulose or the ionic liquid to alter pretreatment mechanisms or performance.

In this study, HPLC analysis was employed as a more targeted method to verify the hemicellulose and cellulose levels measured by the gravimetric Van Soest method [24, 25], since tomato pomace is compositionally distinct from the graminaceous biomass typically analyzed via this method. The Van Soest method yielded greater measurements of hemicellulose and cellulose content than those provided by HPLC analysis the NDF hydrolysate. The overestimation of these components by the purely gravimetric method may be explained by several means. Firstly, the Van Soest method was originally designed for assessment of grasses, which have notable compositional differences from fruit residues such as tomato pomace. For instance, tomato biomass contains a relatively high amount of extractives compared to grasses, particularly pectin, which is known to affect the solubilization of tomato proteins in various solvents [42]. Moreover, insoluble seed proteins are known to exist in tomato pomace [43]. Insoluble cell wall proteins may also be embedded within the lignocellulose. As a result, the neutral detergent extraction step may not be sufficient to completely remove all non-cell wall material from the pomace. Subsequently, there may be carryover of non-fiber compounds to the downstream acid detergent, cellulose extraction, and acid-insoluble lignin determination steps. Extraction of non-target residual material in tandem with target compounds during these steps could affect the calculated values for hemicellulose, cellulose, or lignin. This phenomenon may have inflated the cellulose and hemicellulose content values for the gravimetric Van Soest method. It may have also contributed to the lower levels of acid-insoluble lignin measured by the Van Soest method relative to the HPLC method. The Van Soest method involved an extra acid detergent extraction step, which may have reduced the amount of non-target material carried over to the lignin measurement steps.

These findings suggest a need for caution when interpreting the results of compositional analyses for fruit residues, as the data may vary by analytical method. The ability to accurately characterize fruit residue composition will be important to advance pretreatment research for these materials. The field may benefit from additional research to adapt conventional analytical methods that were originally developed for very different types of biomass, such as the hybrid gravimetric and HPLC approach presented here. Moreover, additional analyses, such as measuring the composition of extractives removed during preatreatment, may further enable future work to improve fruit pomace pretreatment and bioconversion.

While IL pretreatment improved the enzymatic digestibility of tomato pomace in terms of both reducing sugar yield and hydrolysis rate, the enhanced digestibility did not translate to improved methane production during anaerobic digestion of pretreated pomace. Previous studies have successfully used pretreatment with imidazolium ILs to improve anaerobic digestion of water hyacinth, spruce, rice straw, and mango leaves [44].

The differing results between these studies and the IL-pretreated tomato pomace may be related to the unique properties of tomato pomace compared to wood or leaf biomass, such as increased soluble sugar, protein, and seed oil content. Several possibilities for microbial inhibitor generation exist during IL pretreatment based on the thermal degradation of compounds that are either unique to or present in greater quantities in tomato pomace relative to grass and wood biomass. Such inhibitory compounds could include Maillard reaction products, lipid oxidation products, and acetylation products, all of which have been described in previous research as being detrimental to microbial growth.

The relatively high content of sugars and protein in tomato pomace [35] may promote formation of Maillard products under high temperature conditions. Maillard products, such as melanoidins, are primary compounds formed from the reaction of reducing sugars and certain amino acids and have been found to differ in structure based on the temperature during formation, even within the range of 68-100 °C [45]. This could result in different melanoidin profiles between the three temperature treatments reported here. A mixture of Maillard products formed from arginine and xylose as well as histidine and glucose were found to inhibit the growth of *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*, with MICs ranging from 1–8 mg/mL [46]. A Maillard mixture formed from glucose and glycine at 90 °C inhibited growth of the bacteria *Aeromonas hydrophila*, *S. aureus*, and *Listeria monocytogenes* [47].

Reactions of compounds in tomato seed oil may also produce microbial inhibitors. Products of the chain reaction of lipid peroxidation under heat stress have been demonstrated to inhibit cell growth via disruption of membranes and damage to proteins and enzymes [48]. In addition, lipid interactions with proteins can form N-substituted amides, which are known to have antimicrobial activity [49]. Formation of such products has been observed at temperatures within the range of those used in this study [50]. Additional research is needed to confirm the formation of these inhibitory products.

Conclusions

Tomato pomace is an abundant lignocellulosic waste stream in the global tomato processing industry. In this study, ionic liquid pretreatment with [C2mim][OAc] was shown to increase the enzymatic digestibility of pomace lignocellulose and enhance release of reducing sugars that could be fermented into co-products. To the authors' knowledge, this represents the first study to apply ionic liquid pretreatment to a fruit pomace. Anaerobic digestion of ionic liquid-pretreated tomato pomace was investigated as one potential process to bioconvert pretreated material to biofuels. Interestingly, ionic liquid pretreatment of pomace did not significantly benefit biogas yield or quality during anaerobic digestion and, for some pretreatment conditions, actually proved detrimental to anaerobic digestion. These results, which differ from prior IL pretreatment studies involving more conventional wood and grass feedstocks, highlight the need to consider the unique properties of lignocellulosic fruit residues when developing pretreatment strategies. Existing pretreatment methods may need to be modified to account for the relatively high soluble sugar, protein, and lipid content of pomace compared to more conventional lignocellulosic feedstocks. These modifications might include extraction of pomace ahead of IL pretreatment to remove non-cell wall compounds, such as soluble sugars, proteins, and lipids, that do not require pretreatment and can be utilized directly in anaerobic digesters. Such extraction may also mitigate the observed inhibitory effect of ionic liquid pretreatment on anaerobic digestion by removing compounds that could degrade into microbial inhibitors under the high temperatures of pretreatment.

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