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Research paper

Valorization of tomato pomace by sequential lycopene extraction and anaerobic digestion

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ABSTRACT

Tomato pomace, a major byproduct of tomato paste production, is an abundant solid waste stream from food processing in California. Tomato pomace is a rich source of lycopene, a red carotenoid and antioxidant, and lignocellulose, the recalcitrant but energy-rich polysaccharide matrix that comprises plant cell walls. Harvesting both of these co-products could add substantial value to the pomace and potentially reduce waste. In this study, lycopene was extracted from tomato pomace using a mixed organic solvent approach. Yields of lycopene from the tomato pomace tended to be higher than most literature values reported for raw tomatoes, and consistent with many reported values for lycopene in tomato pomace and other products. However, review of the current literature indicates that reported lycopene content of tomatoes products varies by roughly two orders of magnitude, which suggests a need for investigation of the factors responsible for this unusually wide range. After lycopene extraction, direct bioconversion to methane via anaerobic digestion and pretreatment with the ionic liquid 1-ethyl-3-methylimidazolium acetate ahead of anaerobic digestion were explored. Under certain conditions, especially 100 °C for 1 h, pretreatment was beneficial to enzymatic digestion of cellulose. Extraction resulted in a statistically significant reduction in methane yield compared to raw pomace after 90 days of anaerobic digestion. However, supplementation of extracted pomace with the non-lycopene-containing aqueous fraction from the extraction is expected to restore the methane yield to that of raw pomace based on measured values for chemical and biochemical oxygen demand. Ionic liquid pretreatment decreased methane production of extracted pomace.

1. Introduction

Tomato pomace is the principal solid waste stream from tomato paste processing, comprised of skins, pulp, and seeds that are separated from the juice prior to evaporation. California grows and processes most of the United States' tomatoes, and accounts for just over a third of global production [1], which results in at least 60 kt of tomato pomace per season [2], much of which is routed to landfill or animal feed [3]. Since this value was published in 2007, annual production of tomato paste in California has increased by an average about 15% [4], indicating that greater annual quantities of tomato pomace are being produced currently. Value-added coproduct isolation and production from tomato pomace, therefore, represents an opportunity to manage these residues more sustainably, and creates an incentive for industries to facilitate the transition towards renewable bioproducts.

Tomatoes are a rich source of the lipophilic carotenoid lycopene [3], which accounts for up to 98% of carotenoids in tomato [5]. Since it was discovered to be a carotenoid with strong singlet oxygen quenching capability [6,7], lycopene has been characterized as an important dietary antioxidant that may play a protective role against cardiovascular disease and some cancers, and these biological activities have been reviewed previously [8–12]. Additionally, its bright red color allows it to be used as a natural food colorant to replace artificial food dyes that are decreasing in consumer popularity [13].

Many studies over decades have evaluated extraction of lycopene from tomato fruit [3,14], tomato skins [15] and/or tomato products [16,17]. It has been shown previously that lycopene tends to concentrate more in the skins and pulp of tomatoes compared to the water-soluble portions of the fruit [18], and that the quantity therein is often dependent on the cultivar of tomatoes used and the growing conditions [19]. Indeed, several studies have already been

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conducted for extracting lycopene from tomato pomace using both traditional solvents [3,20,21] and supercritical carbon dioxide [3,20,21], and values for lycopene yield tend to be higher than those for whole tomatoes. Currently, lycopene extract for use in food, as outlined by the Food and Agriculture Organization and World Health Organization, is made using crushed whole tomatoes of a variety that tends to be highest in lycopene [22]. The FDA has approved lycopene from tomato as a food additive [23], but not lycopene derived from other sources or made synthetically. Established protocols for lycopene extraction from tomato from the FDA, outlined in the United States Code of Federal Regulations (CFR) [23] and the FAO [22] utilizes traditional solvent extraction with ethyl acetate. Traditional solvent extraction is also used for many other color and nutrient additives obtained from fruit and vegetable extracts listed in the CFR. Solvents are also used in other extraction processes, including soybean oil, where solvent extraction is the most widely used method of oil extraction [24]. Updating the process to utilize tomato pomace, an existing low-value waste stream, as a source of lycopene instead of whole tomatoes expressly grown for lycopene extraction could help to reduce food waste.

In addition to lycopene, tomato pomace is also a source of both simple sugars (roughly 26%) [25] and the more complex carbohydrates that comprise the plant cell wall, also known as lignocellulose (approximately 65% on a dry mass basis) [25,26]. Together, these carbohydrates can serve as a feedstock for biofuel production technologies such as anaerobic digestion. Anaerobic digestion utilizes a diverse community of microorganisms to degrade and convert larger biological molecules into methane through a sequential process consisting of four stages— hydrolysis, acidogenesis, acetogenesis, and methanogenesis [27]. Biomass that is rich in lignocellulose, particularly graminaceous biomass such as wheatgrass or corn stover, is often difficult to ferment because of the recalcitrance of the lignocellulose network. Pretreatment is often used to increase the accessibility or digestibility of this matrix to enzymes and/or microorganisms prior to fermentation. Many types of pretreatment exist for these types of feedstocks, and among the most effective is the use of ionic liquids – salts that are molten at room temperature [28–36]. These solvents have particular appeal because most of them are non-toxic and have the ability to be recycled and reused [37]. Most pretreatment research has focused on these graminaceous residues, and investigations of the pretreatment of fruit and vegetable wastes have been scarce. In a recent study, it was demonstrated that ionic liquid pretreatment can significantly increase the efficacy of enzymatic digestion of tomato pomace with cellulases. However, this effect did not translate to anaerobic digestion process, where ionic liquid pretreatment was shown to have a detrimental effect on methane yield compared to untreated pomace [26]. There is some evidence that a small amount of residual ionic liquid can remain in the pretreated biomass even after thorough rinsing [38], and this could have played a role in reactor performance, as ILs have been demonstrated to be toxic to both yeasts [39] and bacteria [40,41], and it has been demonstrated that adding ILs directly to anaerobic reactors inhibits performance [42]. However, several studies of lignocellulosic biomass have found a beneficial effect of ionic liquid pretreatment on methane production during anaerobic digestion [29,30,42], so it was concluded unlikely that residual IL was the main culprit of the reduction in methane potential. It was hypothesized that antimicrobial compounds may be generated under the high temperature of pretreatment due to reactions between compounds in the pomace that are not typically abundant in conventional lignocellulosic feedstocks, such as water soluble sugars, protein, and oil. Lycopene extraction prior to pretreatment for anaerobic digestion may help to remove other reactive components that contribute to inhibitor formation through pathways such as Maillard browning. Coupling these two processes can also incentivize industry rerouting of waste, and offset the use of fossil fuels for energy.

Previously, extraction of lycopene with traditional solvents has been conducted using moderate temperatures, such as room temperature [3,14,16,17,21,43] to 40 °C [15] and up to 60 °C [20]. Higher temperatures have been investigated for supercritical carbon dioxide extraction, where it has been shown that higher temperatures generally lead to higher lycopene yields up to 70 °C [44], 80 °C [45], 90 °C [46], 86 °C [47], 100 °C [48], and even 110 °C [49]. In addition, enzymatic digestion prior to supercritical CO_2 extraction has been demonstrated to enhance lycopene yield [50]. However, higher temperatures have not been well investigated for traditional solvent extraction, as is evidenced by the literature review summarized in Table 3. It is, however, well established that higher temperatures play an important role in pretreatment of the lignocellulosic material. Often, very high temperatures above 150 °C are used with steam, liquid hot water, or organic solvents and/or caustics, but some studies have investigated lower temperatures to enhance biomass digestibility. Supercritical carbon dioxide extraction at high pressures has been shown to increase the enzymatic digestibility of corn stover and switchgrass at 120 °C [51], and even as low as 60 °C in sugar cane bagasse and crystalline cellulose preparation [52]. Lycopene extraction at higher temperatures and pressures has the potential to affect the digestibility of the tomato pomace and act as a pretreatment to improve lignocellulose bioconversion. However, the benefits of using an extraction procedure as a de facto pretreatment for lignocellulose must be weighed against the possibility of stripping nutrients that could benefit downstream anaerobic digestion.

In this study, lycopene was extracted from tomato pomace using a mixed-solvent approach, using a central composite design to optimize the temperature and extraction duration for maximal lycopene yield. This mixed-solvent approach yielded two phases of extract: a nonpolar phase containing lycopene and other nonpolar compounds, and a polar phase containing soluble sugars, proteins, and other polar compounds. Lycopene in the nonpolar extracts was quantified using a spectrophotometric assay and standard solutions. Reducing sugar content, soluble protein content, chemical oxygen demand (COD), and biochemical oxygen demand (BOD) were determined for the polar extracts. To determine any effect of the extraction process on the enzymatic digestibility of cellulose, extracted pomace was tested for reducing sugar yield during cellulase digestion. Moreover, methane yield of extracted pomace during anaerobic digestion was determined and compared to raw (non-extracted) pomace.

It was previously hypothesized that inhibitor generation during ionic liquid pretreatment stifled methane production during anaerobic digestion [26]. As a follow-up investigation to this phenomenon, some extracted pomace was pretreated with ionic liquid, using pretreatment parameters chosen based on earlier digestibility studies. Reactants for creation of inhibitory compounds were likely to be compounds not found in other graminaceous biomass such as soluble sugars, oils, and unique proteins; therefore, extraction was hypothesized to mitigate the negative effect of pretreatment on the anaerobic digestion process. To test this hypothesis, the extracted and pretreated pomace was also tested for both enzymatic digestibility and methane yield during anaerobic digestion alongside the extracted and raw pomace.

2. Materials and methods

2.1. Tomato pomace

Tomato pomace, consisting of residual skins and seeds from

paste production, was collected from an industrial processing facility in Dixon, California in 2015. Tomatoes were of a proprietary processing variety of *Solanum lycopersicum*. The wet basis moisture of the fresh pomace was 56.12%, as determined by drying in a vacuum oven for 24 h. Pomace was divided into three batches: one that was solar dried for 1 week, one that was convection air dried at 55 °C to constant mass (24 h), and one that was frozen fresh in sealed bags at −20 °C in the dark. Both forms of dried pomace were stored in sealed plastic bags in the dark under ambient conditions. Immediately prior to use, frozen pomace was dried in a vacuum oven at 45 °C in the dark to constant mass (18 h). Frozen pomace was dried within 8 weeks of collection. After drying, all samples regardless of drying method were extracted within one week. To reduce the particle size to <1 mm and improve sample uniformity, pomace was homogenized in a Waring laboratory blender for 30 s on the high setting prior to utilization.

2.2. Lycopene extraction

Dried, homogenized pomace was extracted in batches using a protocol adapted from Periago [16] and Sadler [17], with the batch size adjusted to 0.5 g. Pomace was combined with 25 cm^3 hexane (HPLC grade, Sigma Aldrich, St. Louis, MO), 12.5 cm^3 acetone (HPLC grade, Fisher Scientific, Hampton, NH), 12.5 cm^3 ethanol (200 proof Koptec, Decon Labs Inc., King of Prussia, PA) in a pressure tube (Ace Glass, Vineland, NJ), covered with foil and placed in a heated oil bath. Extraction conditions varied with respect to temperature and time. Following completion of the extraction, samples were cooled to room temperature and filtered through a vacuum filtration apparatus using grade 389 filter paper (Sartorius, Bohemia, NY). To the filtered extract, 10 cm^3 of water were added, resulting in separation into two distinct phases: a polar phase consisting of water, acetone, ethanol, and polar extractives; and a nonpolar phase consisting of mostly hexane, lycopene, and other nonpolar extractives. This mixture was transferred to a separatory funnel and dispensed into two corresponding centrifuge tubes. Extracts were dried in a centrifugal evaporator (SpeedVac SPD2010, Thermo Scientific, Waltham, MA) under vacuum at 45 °C until dried, approx. 18 h, weighed, covered with foil, and stored at −20 °C until analysis. Filtered solids were washed with water, dried in a vacuum oven for 18 h at 45 °C, and weighed.

2.3. Lycopene quantification

Lycopene standards (Sigma Aldrich, St. Louis, MO) were suspended in pure HPLC grade hexane and multiple concentrations were prepared by serial dilution. Hexane extracts were also suspended in pure hexane and transferred to a microplate (Costar #3370, Corning Inc., Kennebunk, ME) along with the standard solutions. The plate was covered with an optically transparent seal (VWR, Radnor, PA) to prevent evaporation and protect plate reading equipment. The plate was read immediately at 472 nm [14,16], and concentrations of lycopene were determined with a standard curve, and yield of lycopene per unit dry basis was calculated using the starting mass of pomace.

2.4. Enzymatic digestion and reducing sugar assay

Extracted pomace was tested for enzyme digestibility using a cellulase mixture followed by a reducing sugar assay as described previously [26]. Two enzymatic digestion studies were conducted: (A) contained the same CCD space as the lycopene extraction study, and (B) compared (1) raw pomace, (2) extracted pomace, (3) pomace that was pretreated at 100 °C for 1 h, (4) pomace that was pretreated at 160 °C for 3 h, (5) extracted pomace that was pretreated at 100 °C for 1 h, (6) extracted pomace that was pretreated at 160 °C for 3 h. In brief, samples of pomace were enzymatically digested at 45 °C with a cellulase mixture from *Trichoderma reesei* (Sigma Aldrich, St. Louis, MO), with time points taken at 0, 1, 2, 3, 5, 7, and 24 h. Raw (frozen, vacuum-dried, but not extracted) pomace was used as a control. Samples were assayed for reducing sugar content in a dinitrosalicylic acid (DNS) assay [26] using glucose standard solutions; therefore, results were expressed as the equivalent mass of glucose.

2.5. Nutrient analysis of polar extractives

The polar fraction of the tomato pomace extract was analyzed for reducing sugars, protein, and total BOD. Reducing sugar content was also determined using the DNS assay. The protein content in extracts was determined using the Bradford technique [53]. Bovine serum albumin (BSA) from a Pierce kit (Thermo Fisher Scientific, Waltham, MA) and glucose were used as standards for the Bradford and reducing sugar assays, respectively. Four samples of polar extractives were analyzed, and triplicate measurements were made for both reducing sugar and protein content. BOD measurements were made using a HACH BOD protocol. Dried polar extractives were re-suspended in DI water and added to a BOD bottle along with one nutrient pillow (#14160–66, Hach, Loveland, CO). Bottles were seeded for bacteria with anaerobic digester sludge, and both seeded bottles and unseeded bottles with no sample (containing only water and nutrient pillows) were used as controls. Initial dissolved oxygen was measured in each bottle, then they were incubated at 20 °C for 5 days, and final dissolved oxygen content was measured using a HACH HQ40d instrument (Hach, Loveland, CO). BOD was calculated using initial and final dissolved oxygen content and the volume and mass of sample added and the volume of the BOD bottle, and adjusting by subtracting the BOD values from controls. COD measurements were made using HACH high-range COD vials (#2565115, Hach, Loveland, CO). Dried polar extractives were re-suspended in DI water and added to a COD vial, along with a blank of only DI water and a COD standard solution (#2253929, Hach, Loveland, CO). Vials were heated at 150 °C for 2 h, cooled to room temperature and the absorbance was read at 620 nm using a HACH DR-870 colorimeter (Hach, Loveland, CO) to determine COD. COD in extractives was calculated using the volume and mass of extract added to each vial.

2.6. Ionic liquid pretreatment

For pretreatment, 0.5 g of raw or extracted pomace was added to 9.5 cm^3 of 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]) (Sigma Aldrich, St. Louis, MO) in a glass test tube. The extraction conditions chosen for subsequent ionic liquid pretreatment – 100 °C for 90min – were selected using the results of the response surface study for lycopene quantification. Samples were pretreated at either 100 °C for 1 h or 160 °C for 3 h based on results of an earlier study that showed effects on enzymatic and microbial digestion between these pretreatment conditions [26]. Pretreated solids were collected in the same manner as the previous study using a vacuum filtration apparatus, and washed five times with water to remove residual ionic liquid. For three replicates, a sixth wash was conducted, and the rinse water dried to confirm it was free of residual IL. Solids were dried in a vacuum oven and stored in a desiccator at room temperature until further use.

2.7. Anaerobic digestion

Anaerobic digestion was conducted in batch digesters with periodic monitoring of methane production as described previously

[26]. Digester sludge was derived from the stabilization tank of a nearby larger-scale digester, where residual labile organic matter is exhausted ahead of disposal. Prior work has established that there is minimal background methanogenesis in this sludge [26]. However, in this study, amounts were increased to 1.00 g of pomace combined with 100 cm^3 of sludge to improve sensitivity. Batch digesters were initially purged with nitrogen and incubated at 55 °C for 90 days, with measurements of methane made every 198 min for the duration of the study. The MicroOxymax system, which uses infrared absorbance technology to measure methane and carbon dioxide, was operated in anaerobic mode according to manufacturer's instructions. To determine the effect of combined extraction/ionic liquid pretreatment on biogas production during anaerobic digestion, four categories of pomace were tested: (1) raw pomace, (2) extracted pomace, (3) extracted pomace that was pretreated at 100 °C for 1 h, (4) extracted pomace that was pretreated at 160 °C for 3 h. Groups (3) and (4) were tested in a ratio of 1:1 treated pomace:raw pomace to restore nutrients that may have been lost during pretreatment and avoid the confounding effects of nutrient limitation when assessing ionic liquid pretreatment efficacy. Biogas quality was calculated as the percentage of methane out of the combined volume of methane and carbon dioxide produced. Quality calculations are based on initial production (intervals 1–50), as CO_2 production tended to drop below the detection threshold in most samples throughout the remainder of the experiment duration. Volatile solids content of pomace samples was determined by combusting dry material in a furnace set to 550 °C until a constant mass of ash was achieved (about 5 h).

2.8. Experimental design and analysis

The effects of extraction temperature and duration on lycopene yield and enzymatic digestibility were examined using a face-centered, 3 × 3 central composite design (CCD) experiment, with extraction temperatures of 80, 100, and 120 °C, and extraction durations of 30, 60, and 90 min. The center point (100 °C, 60min) was repeated 5 times to gauge variability. For extraction, lycopene yield was used as the response variable. For enzymatic digestion experiments, reducing sugar yield after 24 h was used as the response variable. First- and second-order effects of each variable as well as any interaction effects between the two variables were tested, and parameters were fitted to a response surface as previously described [26]:

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

where $Y(t,T)$ is the response, lycopene or reducing sugar yield, t represents extraction time, T represents extraction temperature, β_0 is a constant that describes the intercept, β_t is the main effect of extraction time on the response, β_T is the main effect of extraction temperature on the response, β_{tT} is the interaction effect between extraction time and temperature on the response, β_{tt} is the second-order effect of extraction time on the response, and β_{TT} is the second-order effect of extraction temperature on the response. These parameters were fitted using the standard least squares model fitting function in JMP Pro (SAS, ver. 12.0.1).

Results of the enzymatic digestion and anaerobic digestion experiments that tested raw vs. extracted pomace were analyzed using a two-tailed t-test in JMP Pro. Results that tested multiple categories of pomace were analyzed using one-way ANOVA and Tukey's post-hoc analysis in JMP Pro at $\alpha = 0.05$.

3. Results

3.1. Lycopene extraction

The lycopene content of solar dried pomace was below the 5 μg g^{-1} detection threshold for all replicates in the design space. For convection oven-dried pomace, the maximum lycopene obtained was 26.9 μg g^{-1}, and some samples contained undetectable levels. For the frozen, vacuum-dried pomace, lycopene levels obtained were between 293 and 476μg g^{-1} dry pomace. The results of the CCD experiment are shown in Fig. 1 and Table 1. Significant first- and second-order effects were detected for extraction temperature, while significant first-order effects were detected for extraction time. A significant interaction between temperature and time was also detected, with time having less of an effect with increasing temperature. The generated response surface confirmed that the maximum yield of lycopene within the design space was 100 °C for 90 min.

3.2. Solids recovery

At the optimal extraction conditions, the average recovery of solids was 80.08% (SD 1.512%), the average yield of dry polar extractives was 5.724% of initial mass of pomace (SD 0.5031%), and the average yield of dry hexane extractives including oils and lycopene was 13.55% of initial dry mass of pomace (SD 1.200%). This accounts for a mean total recovery of 99.35%; however, 100% is in the 90, 95, and 99% confidence intervals for the mean.

Pomace that underwent lycopene extraction at the selected conditions, as well as raw pomace, were subjected to ionic liquid pretreatment. Solids recovery following these different treatments is depicted in Table 2. Recovery declined substantially with increasing pretreatment temperature, and the cumulative recovery from sequential extraction and pretreatment was predictably low.

3.3. Enzymatic digestion

The enzymatic digestion of extracted pomace yielded no significant differences between extraction parameters within the CCD design space based on 24 h reducing sugar yields (data not shown). Therefore, the parameters that yielded maximum lycopene yield – 100 °C for 90 min – were chosen for further investigation.

Results of the enzymatic digestion of different treatment combinations – extraction and/or ionic liquid pretreatment – are shown in Fig. 2. Most notably, no differences were found between raw and extracted pomace, regardless of whether recovery was taken into account. In addition, significant differences were found between several pretreatments and the raw and extracted pomaces, but these differences were dependent on whether solids recovery was taken into account. With or without recovery, both extracted and non-extracted 100 °C pretreatments performed significantly better than both raw and extracted pomace. Without recovery, the extracted and non-extracted 160 °C pretreated pomace performed significantly better than both raw and extracted pomace. However, with recovery, the non-extracted 160 °C pretreated pomace did not perform better than raw or extracted pomace, and the extracted 160 °C pretreated pomace performed significantly better than only the extracted pomace, not raw pomace.

3.4. Nutrient analysis of polar extractives

Nutrient analysis of the polar extractives yielded a protein content of 223.3 μg g^{-1} dry extract (SD 36.27μg) as determined by the Bradford assay, a reducing sugar content of 8.793 mg g^{-1} (SD

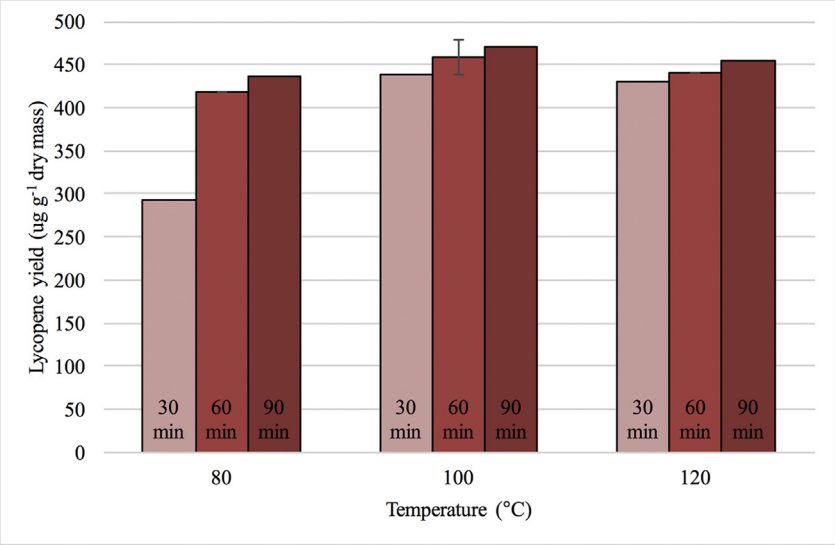

Fig. 1. Lycopene yield of various extraction conditions. Lycopene yield in ug g^{-1} dry mass of pomace for different extraction conditions.

Table 1
Parameter estimates for lycopene yield.

Parameter	Estimate[a]	Standard error	P-value[b]
β_0	462.4	10.90	**<0.0001**
β_t	29.48	9.750	**0.0076**
β_T	33.45	9.750	**0.0137**
β_{tT}	−29.8	11.94	**0.0308**
β_{tt}	−40.04	14.62	0.298
β_{TT}	−14.94	14.62	**0.0196**

[a] Parameter estimates are based on the response surface model using units of ug lycopene recovered per g dry pomace and coded values for the independent variables.
[b] Bold values indicate P-values that are below the 0.05 threshold for statistical significance.

Table 2
Solids recovery following different treatments.

Treatment	Recovery (%)	SD (%)
Extraction	80.08	1.512
100 °C 1 h Pretreatment	66.33	1.539
160 °C 3 h Pretreatment	40.65	2.562
Extraction & 100 °C 1 h Pretreatment	54.94[a]	2.004
Extraction & 160 °C 3 h Pretreatment	32.32[a]	2.200

[a] Indicates the cumulative recovery after both extraction and subsequent pretreatment.

0.6926 mg) as determined by the DNS assay, a COD of 1783 mg g^{-1}, and a BOD of 1544 mg g^{-1}.

3.5. Anaerobic digestion

An anaerobic digestion study indicated that extraction of tomato pomace resulted in decreased production of methane. Furthermore, extraction did not mitigate the previously observed detrimental effect of ionic liquid pretreatment on pomace anaerobic digestion. A time course of methane production in raw and extracted pomace over the course of the experiment (90 days) is shown in Fig. 3. At several time points, this difference was statistically significant. Significance reached a peak near 60 days with $P < 0.01$, whereas no significant differences in methane production were detected at or before 15 days. At 90 days, the average yields of methane were 108.0 cm^3 for raw pomace and 78.56 cm^3 for extracted pomace, and these data remained under the threshold of significance with $P = 0.0427$. Average final yields at the termination of data collection for pretreated pomaces were 40.35 cm^3 for extracted pomace pretreated at 100 °C for 1 h, and 46.83 cm^3 for extracted pomace pretreated at 160 °C for 3 h. These values, as well as results from one-way ANOVA analysis and subsequent Tukey's post-hoc analysis, are visualized in Fig. 4. The 100 °C, 1-h pretreatment produced significantly less methane than both raw and extracted pomace, and the 160 °C, 3-h pretreatment produced significantly less methane than raw pomace. The 160 °C, 3-h pretreatment also produced less methane than the extracted pomace, but this difference was not statistically significant. The volatile solids content of the various pomace samples were measured to be: 97.1% ± 0.13%; 97.0% ± 0.36%; 98.5% ± 0.03%; 98.5% ± 0.11% for raw; extracted; extracted and 100 °C pretreated; and extracted and 160 °C pretreated pomace, respectively. Biogas quality was similar for raw (67.9% ± 6.18%) and extracted (71.3% ± 3.70%) pomace, and a *t*-test revealed no significant difference in biogas quality between the two treatments ($P = 0.37$). CO_2 production for both pretreated pomace samples was consistently below the detection threshold, and therefore biogas quality could not be reliably calculated for these samples.

4. Discussion

Lycopene yield was found to be significantly affected by drying method of the pomace. Solar drying and hot air drying at 55 °C were both found to be detrimental to lycopene content of the tomato pomace. The range of lycopene yields obtained for frozen, vacuum-dried pomace of 293–476μg g^{-1} dry pomace, aligns well with many other successful extractions of tomato products. However, reported values for lycopene content of even raw tomato vary by up to two orders of magnitude in the literature. A sampling of previous research on the lycopene content of tomatoes and tomato pomace is presented in Table 3. Factoring in the high moisture content of about 92% in raw tomatoes, and the fact that lycopene concentrates in the skin, many of the values reported for whole tomato fruits on a wet basis become much closer to the same range as values reported for those on a dry basis. It should also be highlighted that profound differences between different tomato

Table 3
Sampling of previous results for lycopene yield from tomatoes and tomato pomace.

Product	Extraction Method	Maximum Lycopene Yield (ug g^{-1})[a]	Handling Notes	Reference
Raw tomato	Hexane, acetone, ethanol, room temp (RT), 30 min	47.2 (wet)	Homogenized, stored at −80 °C	[16]
Raw tomato	Acetone	33.5 (wet)	Frozen at unspecified temp	[3]
Raw tomato	Hexane, acetone, ethanol, RT	119 (wet)	Seeds removed, homogenized	[83]
Raw tomato	Hexane, methanol, acetone	125.4 (wet)	Homogenized, not stored	[68]
Raw tomato	Hexane, acetone, ethanol, RT	630 (dry)	Air dried at 42 °C for 18 h	[63]
Raw tomato	Hexane, acetone, ethanol, RT	47.6–55.9 (wet)	Ground	[69]
Raw tomato	Tetrahydrofuran (THF), 0 °C	173-236 (wet)	Homogenized	[55]
Raw tomato	Chloroform, acetone, hexane	2010 (?)	Homogenized, sealed in cans, frozen at −40 °C, wet/dry basis not specified	[64]
Raw tomato	Hexane, acetone, ethanol, RT	3310 (?)	Homogenized, wet/dry basis not specified	[67]
Raw tomato	THF	880-940 (dry)	Homogenized	[66]
Raw tomato pulp	Hexane, acetone, ethanol, RT	1300 (dry)	Frozen at −40 °C, skins, seeds removed	[61]
Fresh tomato juice	Hexane, ethanol	55-181 (wet)	Microwaved, pulp removed, frozen at −32 °C	[54]
Raw tomato (high pigment cultivar)	Hexane, acetone, ethanol, RT	213 (wet)	Seeds removed, homogenized	[83]
Raw tomato skins, separated	Acetone: hexane Soxhlet	770.8 (dry)	Dried in air oven at 35 °C for 24 h, stored at −5 °C	[45]
Raw tomato skins, separated	Supercritical CO_2, 27.58 MPa, 80 °C	644.1 (dry)	Dried in air oven at 35 °C for 24 h, stored at −5 °C	[45]
Tomato pomace	Acetone	119.8 (wet)	Reported as 78% skins, frozen at unspecified temp	[3]
Tomato pomace	Chloroform Soxhlet	820 (dry)	Ground, drying method not specified, reported as 37% skins	[46]
Tomato pomace	Supercritical CO_2, 40 MPa, 90 °C	459 (dry)	Ground, drying method not specified, reported as 37% skins	[46]
Tomato pomace	Supercritical CO_2, 46.0 MPa, 80 °C	314 (dry)	Frozen at unspecified temp, drying method not specified	[84]
Tomato pomace	THF, methanol	734 (dry)	Dried in air oven at 65°C-50 °C for 48 h, stored at −30 °C	[21]
Tomato pomace	Chloroform	24.5 (dry)	Stored at −20 °C, reported as 30.5% skins	[47]
Tomato pomace	Supercritical CO_2, 34.5 MPa, 86 °C	14.86 (dry)	Stored at −20 °C, reported as 30.5% skins	[47]
Tomato pomace	Supercritical CO_2, 40 MPa, 100 °C	31.25 (wet)	Air dried (unspecified), ground	[48]
Tomato pomace	Hexane, acetone, ethanol, RT	19.8 (dry)	Skins separated, air dried at unspecified temp, ground	[20]
Tomato pomace	THF	739 (wet)	Heat treated at 100 °C, freeze dried, ground	[70]

[a] Wet or dry basis, if given, is indicated in parentheses. A question mark (?) is listed for references that do not clearly state which basis the yields were given.

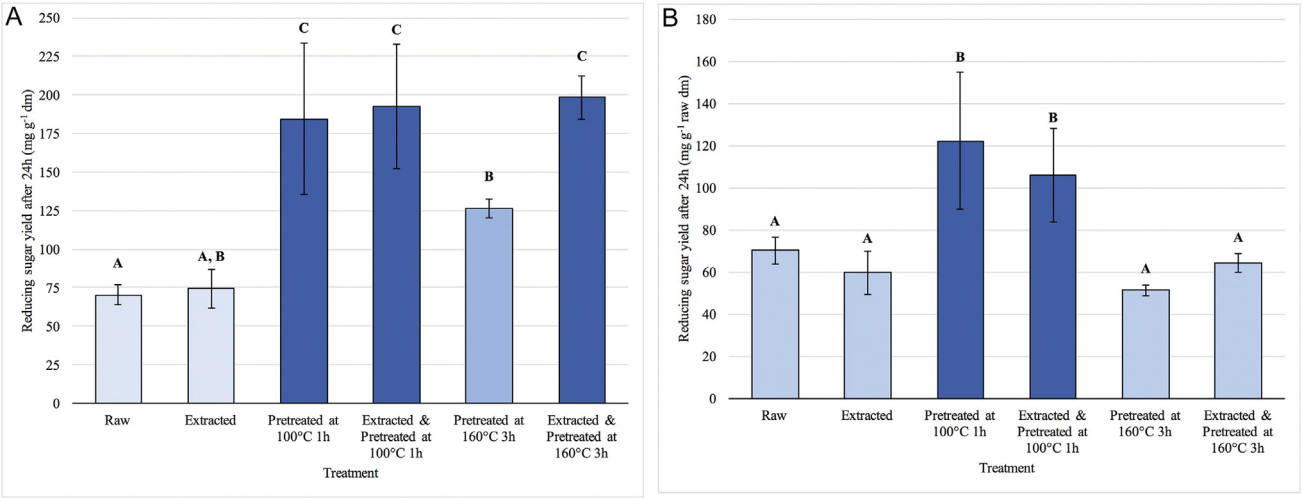

Fig. 2. Reducing sugar yields following enzymatic digestion for different treatments. (A) Gross reducing sugar yield for raw pomace and various treatments after 24 h of digestion with cellulase enzyme cocktail from *T. reesei* in mg g^{-1} dry mass. Values in (B) are adjusted to mg g^{-1} dry mass of raw pomace to account for recovery of solids following the different treatments. Columns that do not share a letter are significantly different.

varieties and cultivars across different years have been reported, even in California processing tomatoes alone [54]. For tomato byproducts such as pomace, differing processing methods may also play a role. A study found that statistically significant losses of lycopene between 9% and 28% were found during tomato processing into paste [55]. However, cultivar and processing method of tomatoes are not consistently reported. Of the twenty references included in Table 3, nine did not specify tomato variety. And while several papers conducted their own processing analyses for raw tomato, which will be discussed subsequently, of the eight that examined commercial tomato pomace, only three included any information regarding processing conditions. For this reason, drawing definitive conclusions regarding the factors leading to the wide range of reported lycopene contents is difficult.

Some of these differences may be attributed to extraction method, but even among very similar extraction methods for tomato pomace, for example, a wide range of reported values can be observed. Drying method (if applicable) may also be a contributing factor, but the effect of drying on lycopene content is somewhat inconsistent; some studies involving drying report values in a normal or even high end of the range, while others report abnormally low values. Previous studies on degradation of lycopene

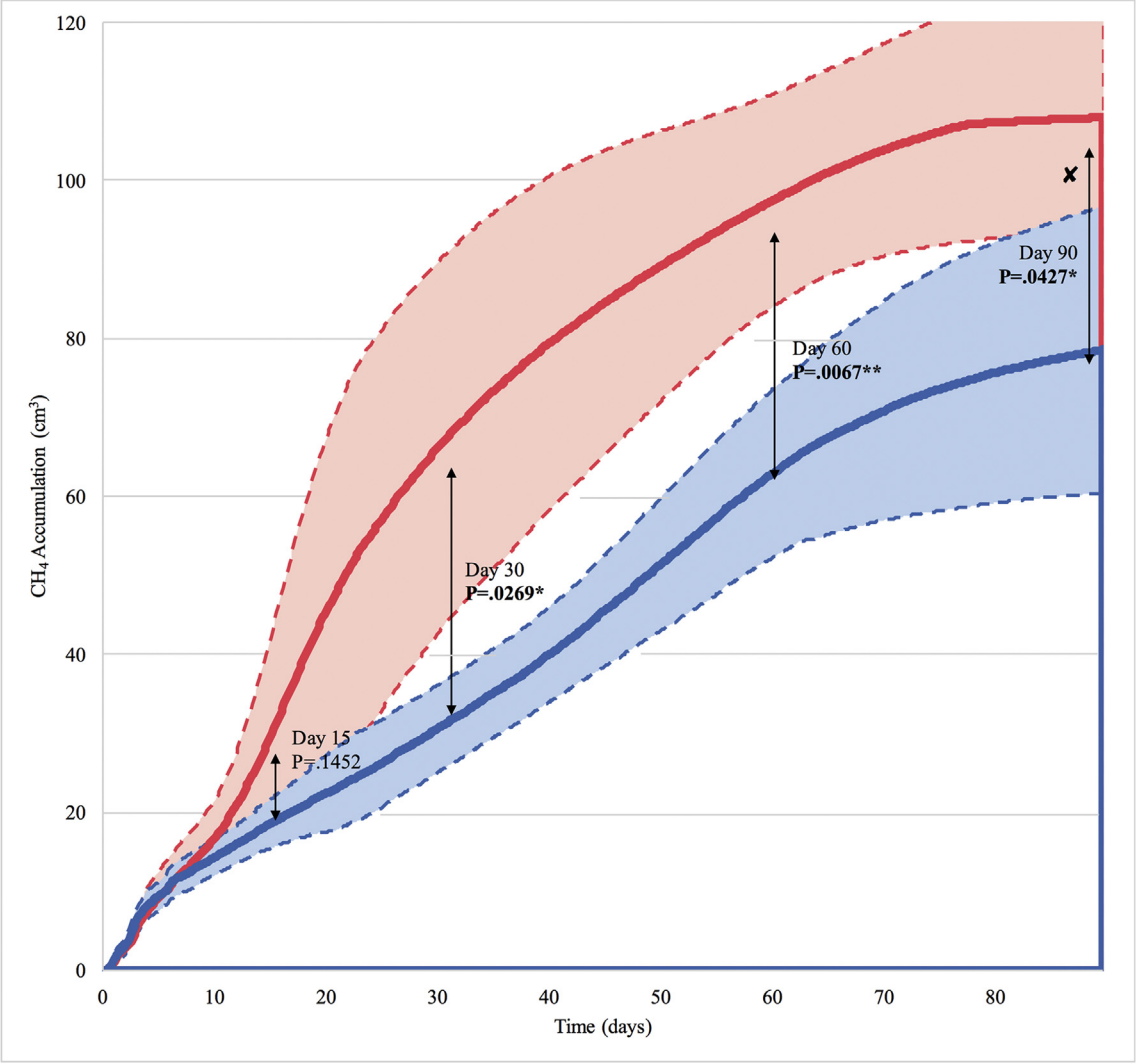

Fig. 3. Methane production of raw and extracted pomace. Cumulative methane production of anaerobic digestion reactors containing raw and extracted pomace in cm^3, adjusted per gram of VS content. Dark solid lines represent average methane production for all replicates of each treatment (n = 4) at each time interval (n = 645). Pale color fill capped by dashed lines represents one standard deviation above and below the average. The marked "X" represents the theoretical methane yield for extracted pomace supplemented with the polar fraction of the extract based on calculations from its BOD. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

during different drying and storage conditions have been conducted. Light exposure appears to be one of the most destructive environmental condition for lycopene degradation. A study found 94% loss of pure lycopene after 144 h (6 days) of light exposure at room temperature [56]. Another found almost 100% loss of lycopene in tomato peel after 42 h of light exposure at room temperature [57]. However, another found maximum lycopene losses of only about 20% after exposure of tomato pulp to light for 12 days [58], and another found a loss of only 25% of lycopene in vegetable juice after 8 days of light exposure at 4 °C [59]. Therefore, despite some consensus that light is destructive to lycopene, there does not seem to be a definitive answer for just how sensitive it is, and how the tomato matrix may affect that sensitivity.

Other commonly cited factors of lycopene degradation are oxygen exposure and high temperature, especially their combination. Elevated temperatures in the presence of oxygen have been found to reduce lycopene levels in tomato peel substantially over 10 h (by 21% at 50 °C, 47% at 100 °C) [60]. Another study found losses of 10% of lycopene after only 30 min of heat treatment at 60 °C [61]. However, other studies have seen a minimal effect of drying or other high temperature treatment with oxygen. One study found only a 4% loss of lycopene after air drying tomato at 95 °C for 10 h [62]. Another found that drying at 42 °C for 18 h resulted in losses of lycopene of approximately 10–20%, depending on the variety [63]. And yet another study found that heat treatments at 88 °C for 30 min actually increased extractable lycopene by more than twofold [64]. Other studies have presented mixed results depending on drying parameters. One group found that drying tomato at 50 °C (for unspecified time) to 4% moisture content had negligible effect on lycopene content, similar to freeze drying, while drying at 80 °C resulted in almost 100% loss [57]. Another found that at 90 °C temperatures, lycopene remains relatively stable for 1–2 h, but losses of roughly 50% lycopene can occur over a period of 6 h, and high temperatures above 100 °C over a few hours speeds degradation significantly [65]. Conversely, another study found that drying at 80 °C for 7 h resulted in undetectable lycopene losses, while at 110 °C losses were roughly 20% [66]. A pure lycopene standard was shown to be very unstable at 150 °C, with losses close

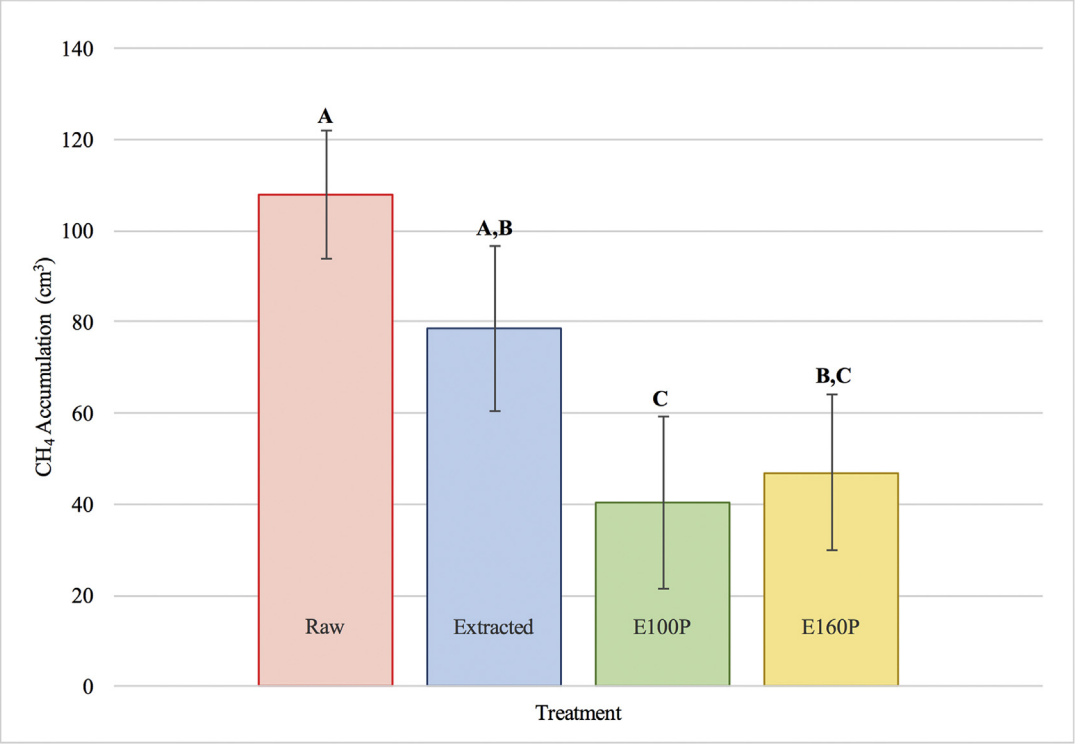

Fig. 4. 90-Day methane yields for all treatments. Average yields of methane for raw pomace; extracted pomace; extracted pomace pretreated at 100 °C for 1 h (E100P); and extracted pomace pretreated at 160 °C for 3 h (E160P) after 90 days of digestion, adjusted per gram of VS content. Columns that do not share a letter are significantly different.

to 100% after 30 min, but at 100 °C losses of only 10% after 30 min and 40% after 60 min. In this same study, lycopene was better retained in the tomato matrix, regardless of cooking method and temperature, where even the most extreme heat treatment of pan-frying at 165 °C, roughly 30% of lycopene was retained [67]. Although the literature seems unanimous that very high temperatures above 100 °C cause faster degradation of lycopene, there does not seem to be a consensus on an appropriate time frame of exposure, or to what extent or how rapidly moderately high temperatures below 100 °C contribute to degradation.

As lycopene is an antioxidant molecule, the presence of oxygen as a key factor in its oxidation is supported by literature. However, there is not a well-defined consensus on how quickly this process occurs. Canned tomato juice with minimal oxygen exposure has been found to retain a majority of its lycopene content after 12 months at temperatures up to 37 °C in one study [68], which would indicate that oxygen plays a bigger role than temperature in lycopene degradation. However, another study found losses between 15% and 25% after only 10 weeks in sealed cans at 35 °C [69]. Again, a consensus on lycopene stability in tomato is hazy. It has been demonstrated that a water activity that is too low can contribute to more rapid degradation of lycopene during storage [70], which suggests involvement of the tomato matrix as well.

All of the aforementioned research suggests a need for lycopene extraction/utilization immediately after production or with minimal storage time after drying to minimize losses. It also highlights the need for more research to explain the discrepancies in lycopene content and degradation in the current literature, and how preservation of lycopene can be best achieved utilizing the tomato matrix itself as well as environmental factors.

Enzymatic digestion of different treatments confirmed that ionic liquid pretreatment under certain conditions can improve digestibility of tomato pomace, as has been demonstrated previously [26]. The enzymatic digestion also showed no significant differences in reducing sugar yield between raw and extracted pomace. This finding was promising, as a decrease in digestibility following extraction would be undesirable from a biofuel perspective, and the aim is to couple lycopene extraction with biogas production. The hydrolytic stage of anaerobic digestion has been demonstrated to become a rate-limiting step in the anaerobic digestion of lignocellulosic biomass, as microbial cellulases have reduced access to cellulose and hemicellulose due to the recalcitrant cell wall structure [71]. Additionally, other pretreatment studies have found a correlation between increased enzymatic saccharification and increased ethanol production from yeast fermentation [34,72,73] and methane production from anaerobic digestion [74–76]. Previous research indicated that while temperatures of 100 °C and 130 °C were beneficial to enzymatic digestion, the more extreme parameter of 160 °C was detrimental to enzymatic digestion, especially after 3 h [26]. In this study, prior extraction of the pomace mitigated this effect, as a significant difference was shown between pretreated pomace at 160 °C for 3 h versus pomace that was extracted prior to the same pretreatment. This finding provides support for the earlier hypothesis that inhibitor generation during pretreatment contributed the decreased digestibility; extraction could likely have removed precursors to inhibitor formation so fewer of these reactions occurred during pretreatment. However, similar to the previous study, this effect disappeared when recovery of solids was taken into account. The marked improvement in reducing sugar yield was not enough to overcome the combined recovery of less than 30%. The 100 °C 1 h pretreatment, with much higher solids yields and comparable reducing sugar yields, is a better candidate for potential pretreatment to combine with extraction.

Anaerobic digestion of extracted pomace compared to raw pomace indicated that extraction of tomato pomace results in

decreased production of methane, a difference of 29.44 cm^3, or roughly 27%. Throughout most of the time course of the experiment, the raw pomace produced significantly more methane. As the significance level between raw and extracted pomace decreased toward the end of the experiment, given more time, it is feasible the difference would diminish further as the extracted samples further approached their saturation points, to the point that significance could no longer be detected. However, these data nonetheless demonstrate that a trade-off to lycopene extraction is a small, but significant, reduction in methane potential of the extracted pomace. A logical explanation is that the lycopene extraction removes digestible nutrients that thereby depresses the methane potential of the pomace.

Results of an earlier study indicated that ionic liquid pretreatment of pomace was detrimental to methane production. In this anaerobic digestion study, it was determined that extraction of pomace prior to pretreatment does not mitigate this detrimental effect, as neither pretreatment produced more methane than its raw or extracted counterparts. As the goal of pretreatment is to improve digestibility of the material, based on these studies, ionic liquid pretreatment does not appear to be a viable option for improving methane yield of tomato pomace during anaerobic digestion.

Nutrient analysis of the polar fraction of the tomato pomace extract was conducted to determine if it would be a suitable substrate for supplementation to anaerobic digestion. A unique aspect of the extraction method used is the formation of two phases of extract. The nonpolar phase contains valuable lycopene, but the polar fraction need not go to waste. The protein and sugar content, as well as the appreciable COD and BOD, indicated that supplementing extracted pomace with this fraction could increase the methane yield. Stoichiometrically, a gram of COD removed translates to a methane production is about 350 cm^3 at STP [77]. Other studies on anaerobic digestion have supported the correlation between COD of substrates and biomethane potential [78–80]. However, actual biogas yields from anaerobic digestion can be lower than values estimated stoichiometrically by COD or VS assays, due to factors such as indigestible content like fiber, and the fact that some of the nutrients are used for biomass maintenance of the microbes themselves. The decrease in yields can vary, but for a mostly water-soluble, low-fiber fraction such as the polar extractives, yields are likely in the range of 90–95% per unit of COD, even assuming a biogas quality of 100% [81]. COD can tend to overestimate biodegradable compounds in a sample, whereas BOD has been shown to be closely correlated with biomethane potential [82]. Using the BOD of 1544 mg g^{-1} of the polar extractives, for anaerobic digestion of 1.00 g of extracted pomace, the addition of its corresponding polar extract would yield about 30.93 cm^3 of additional methane. Adjusted for the approximate 70% conversion rate observed in this study, this translates to roughly 21.65 cm^3 of methane. The average methane yield at 90 days of extracted pomace was 78.56 cm^3. Assuming this is close to the saturation point as the data indicate, it can be extrapolated that a conservative estimate for methane production of extracted pomace combined with polar extractives would be 100.2 cm^3. Since the average methane yield at 90 days of raw pomace was 108.0 cm^3 of methane, the yield of the extracted pomace could be quite comparable to that of raw pomace with enrichment of this otherwise low-value fraction.

5. Conclusions

A multi-co-product pipeline tomato pomace that targets both lycopene and biomethane has the potential to improve the value of this waste stream. Solvent extraction of tomato pomace from paste processing yielded a non-polar fraction with a lycopene content similar to that of many literature values for tomato products. Subsequent anaerobic digestion of extracted pomace indicated a slight trade-off between recovery of high-value lycopene and lower-value biogas, as extracted pomace produced less methane than raw, non-extracted pomace, and this difference was statistically significant. However, chemical and biochemical analyses of the polar, non-lycopene-containing fraction of tomato extract suggest that supplementation of this extract in anaerobic reactors has the potential to boost the biogas productivity of extracted pomace to rival that of the raw pomace. Under certain parameters, especially 100 °C for 1hr, ionic liquid pretreatment of extracted pomace increased digestibility during enzymatic digestion, but was largely ineffectual or detrimental to methane production during anaerobic digestion. Ionic liquid pretreatment, therefore, does not seem like an optimal pretreatment method for tomato pomace from an anaerobic digestion perspective.

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