Sustainable approach for lycopene extraction from tomato processing by-product using hydrophobic eutectic solvents

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Abstract Lycopene, a non-polar antioxidant compound with important effects on human health and wide commercial applications, was extracted from tomato processing wastes using innovative hydrophobic eutectic mixtures (HEMs) replacing traditional organic solvents. HEMs were prepared using DL-menthol as hydrogen-bond acceptor (HBA) and lactic acid as hydrogen-bond donor (HBD), and the ultrasound-assisted extraction (UAE) was optimized using a Box–Behnken design to evaluate extraction conditions: extraction temperature (°C), molar ratio of eutectic mixture (moles HBA: mol HBD), solvent to sample ratio (volume to mass, mL/g), and extraction time (min), with lycopene extraction yield (µg/g d.w.) as the response variable. Optimization of parameters was performed using response surface methodology, and the optimized extraction conditions were determined to be 70 °C, 8:1 mol HBA/mol HBD, 120 mL/g solvent: sample, and 10 min. The experimental optimal yield was 1446.6 µg/g, in agreement with the predicted optimal yield, indicating the

validity of the model. This new technique for lycopene extraction, using a HEM as extraction solvent in replacement of hazardous organic solvents, and tomato pomace as source material, represents a viable and more sustainable approach for obtaining a high value-added bioactive compound, and can contribute towards the development of greener extraction processes.

Keywords Green extraction · Deep eutectic solvents · Tomato pomace · Response surface methodology · Box–Behnken design

Introduction

Lycopene, a carotenoid found in tomatoes, is one of the most important dietary antioxidants for human health (Rao and Agarwal 1999). Tomato pomace is an industrial processing by-product comprising of peel and seeds. The tomato processing industry produces 600 thousand to 2 million tonnes of discarded pomace that represents between 1.5 and 5% of the 40 million tonnes of tomato processed yearly (WPTC 2016). As such, the valorization of tomato pomace for extracting lycopene is an attractive alternative to for this under-utilized resource in place of waste disposal.

Hexane is the organic solvent of choice for carotenoid extraction, although less polluting alternatives are constantly being explored. Deep eutectic solvents (DES) are a new class of green solvents for extraction processes. These systems are formed by mixing two or more Lewis/Brønsted–Lowry acids and bases, with one of the compounds acting as a hydrogen bonding acceptor (HBA) and the other as a hydrogen bonding donor (HBD). The DES system has a lower freezing point than its initial constituents (Abbott

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et al. 2004). Some of the benefits of DES are that they are sustainable (not derived from petroleum), cheap, easily prepared, biodegradable and have high purity (Dai et al. 2013; Espino et al. 2016; Zhang et al. 2012).

DES have been extensively applied as green solvents for the extraction of many bioactive compounds (Dai et al. 2013). However, most of these solvents are hydrophilic, and have been used to extract polar compounds, such as phenolics, anthocyanins and polysaccharides (Li and Row 2016; Ruesgas-Ramón et al. 2017; Zainal-Abidin et al. 2017; Zhang et al. 2016). Research into hydrophobic eutectic mixtures (HEMs) for the extraction of non-polar bioactive compounds is much less explored.

Amongst several reported DES systems, choline-chloride-based systems have shown good potential for extraction of non-polar compounds (Cao et al. 2017a, b; Chen et al. 2016; Zhang et al. 2014). The extraction of astaxanthin, a hydrophobic carotenoid, from shrimp by-products using choline-chloride-based low hydrophobicity DES has been investigated by Zhang et al. (2014). Recently, DL-menthol-based solvent systems have been reported to be cheap and efficient for the extraction of low polarity compounds (Ribeiro et al. 2015; Van Osch et al. 2015). Until now, there has been no report available on the use of eutectic solvents for extraction of lycopene.

In this paper we demonstrate a sustainable approach for lycopene extraction, using tomato pomace as raw material and HEM as a green extraction solvent. After preliminary experiments, a DL-menthol and lactic acid HEM was selected for optimization of ultrasound-assisted extraction (UAE) of lycopene using Box-Behnken design and response surface methodology (RSM).

Materials and methods

Chemicals

Analytical grade choline-chloride ((2-Hydroxyethyl)trimethylammonium chloride), DL-menthol (2-Iso-propyl-5-methylcyclohexanol), 85–90% lactic acid solution (2-Hydroxypropionic acid), levulinic acid (4-Oxopentanoic acid) and ethyl acetate were from Sigma-Aldrich (Oakville, ON, Canada). The lycopene standard was from Fisher Scientific (Ottawa, ON, Canada).

Plant material

Tomato pomace was obtained from an industrial processing plant at 62.8% moisture content (fresh weight—f.w.) and immediately freeze-dried in benchtop freeze dryer (Liotop L108, Liotop, São Carlos, Brazil) to a moisture content of 4.6% (f.w.), vacuum packed and stored at

– 20 °C until further analysis. Prior to extraction, samples were ground using a manual grinder (Smartgrind, Black & Decker, Mississauga, ON, Canada) and sieved through a 0.5-mm (32 mesh) sieve.

Screening of hydrophobic eutectic mixtures (HEMs) for lycopene extraction

Preliminary experiments were conducted to select the HEM solvent for optimizing the UAE of lycopene. The HEMs evaluated were choline-chloride combined with levulinic acid (Cho-Lev) and DL-menthol combined with lactic acid (Men-Lac) (Cao et al. 2017b; Ribeiro et al. 2015). For the preparation of each HEM, the solid HBA was weighed in a glass vial wrapped in aluminium foil, and the liquid HBD was added at the required ratio (2:1, 1:1 or 1:2 mol/mol). The mixtures were heated using a magnetic stirring-heating plate at 1200 rpm until no solid particles remained. Deionized (Milli-Q) water was then added to the liquid HEM in ratios varying from 0 to 40% (v/v), and then vortexed for 30 s. These green solvents were prepared before extraction and used within the same day. Extraction of lycopene from the pomace was then performed with these HEMs in a shaking water bath at 60 °C for 30 min. This showed that Men-Lac resulted in better lycopene extraction, corresponding to an intense red colour, while the Cho-Lev mixtures remained colourless. Water could be easily incorporated into the Cho-Lev solvent, but not in Men-Lac, where two separate layers remained distinct after vortexing, indicating the strong hydrophobicity of this solvent. Therefore, Men-Lac, without any water added, was selected as the solvent for UAE experiments.

Box–Behnken experimental design

The variables selected for optimization were X_1 = extraction temperature (°C), X_2 = molar ratio of menthol/lactic acid (moles HBA/mol HBD), X_3 = solvent:sample ratio (mL/g) and X_4 = extraction time (min). These parameters and levels were chosen based on preliminary experiments and prior DES extraction studies (Cao et al. 2017a; Duan et al. 2016). A Box–Behnken design (BBD) was used for the UAE optimization experiments (Box et al. 2005), with the natural (X_i) and coded (k_i) levels shown in Table 1.

Ultrasound-assisted extraction (UAE)

The UAE of lycopene from tomato pomace using HEMs was conducted in an ultrasound water bath (Branson 2510R-DTH, Branson Ultrasonics Corp., Danbury, CT, USA) with fixed frequency (40 kHz) and power (100 W). The solvent was prepared immediately before use as in “Screening of hydrophobic eutectic mixtures (HEMs) for

Table 1 Box–Behnken design matrix for optimization of lycopene yield using hydrophobic eutectic solvent (DL-menthol combined with lactic acid)

Assay	Run order	Factor ⁽¹⁾				Lycopene yield (µg/g)		Error (%)
		Extraction temperature k_1 (X_1 , °C)	Molar ratio k_2 (X_2 , moles HBA/mol HBD)	Solvent: sample ratio k_3 (X_3 , mL/g)	Time k_4 (X_4 , min)	Experiment	Predicted	
1	9	− 1 (30)	− 1 (2)	0 (90)	0 (30)	898.1	821.7	− 8.51
2	17	+ 1 (70)	− 1 (2)	0 (90)	0 (30)	1125.5	1137.6	1.08
3	10	− 1 (30)	+ 1 (8)	0 (90)	0 (30)	1029.2	1064.7	3.45
4	18	+ 1 (70)	+ 1 (8)	0 (90)	0 (30)	1462.8	1380.6	− 5.62
5	2	0 (50)	0 (5)	− 1 (60)	− 1 (10)	883.6	863.2	− 2.31
6	28	0 (50)	0 (5)	+ 1 (120)	− 1 (10)	1172.6	1126.5	− 3.93
7	29	0 (50)	0 (5)	− 1 (60)	+ 1 (50)	1064.4	1067.5	0.29
8	7	0 (50)	0 (5)	+ 1 (120)	+ 1 (50)	966.2	943.6	− 2.34
9	11	− 1 (30)	0 (5)	0 (90)	− 1 (10)	846.4	937.8	10.80
10	19	+ 1 (70)	0 (5)	0 (90)	− 1 (10)	1167.9	1253.7	7.35
11	14	− 1 (30)	0 (5)	0 (90)	+ 1 (50)	918.6	948.5	3.25
12	22	+ 1 (70)	0 (5)	0 (90)	+ 1 (50)	1223.8	1264.4	3.32
13	4	0 (50)	− 1 (2)	− 1 (60)	0 (30)	811.1	843.8	4.03
14	5	0 (50)	+ 1 (8)	− 1 (60)	0 (30)	1052.9	1086.8	3.22
15	26	0 (50)	− 1 (2)	+ 1 (120)	0 (30)	1025.1	913.5	− 10.89
16	25	0 (50)	+ 1 (8)	+ 1 (120)	0 (30)	1153.8	1156.5	0.23
17	12	− 1 (30)	0 (5)	− 1 (60)	0 (30)	988.9	908.3	− 8.15
18	20	+ 1 (70)	0 (5)	− 1 (60)	0 (30)	1325.4	1224.2	− 7.64
19	13	− 1 (30)	0 (5)	+ 1 (120)	0 (30)	977.8	978.0	0.02
20	21	+ 1 (70)	0 (5)	+ 1 (120)	0 (30)	1248.9	1293.9	3.60
21	3	0 (50)	− 1 (2)	0 (90)	− 1 (10)	870.6	873.3	0.31
22	24	0 (50)	+ 1 (8)	0 (90)	− 1 (10)	1102.8	1116.3	1.22
23	27	0 (50)	− 1 (2)	0 (90)	+ 1 (50)	774.1	884.0	14.20
24	6	0 (50)	+ 1 (8)	0 (90)	+ 1 (50)	1161.0	1127.0	− 2.93
25	1	0 (50)	0 (5)	0 (90)	0 (30)	930.2	1000.2	7.53
26	8	0 (50)	0 (5)	0 (90)	0 (30)	1014.2	1000.2	− 1.38
27	15	0 (50)	0 (5)	0 (90)	0 (30)	1038.2	1000.2	− 3.66
28	16	0 (50)	0 (5)	0 (90)	0 (30)	1008.0	1000.2	− 0.77
29	23	0 (50)	0 (5)	0 (90)	0 (30)	977.6	1000.2	2.31
30	30	0 (50)	0 (5)	0 (90)	0 (30)	997.0	1000.2	0.32

⁽¹⁾ Coded level (Natural value)

lycopene extraction” section, but without adding any water. The fresh solvent was placed in the water bath to achieve extraction temperature before contacting the sample. UAE was performed as previously reported (Silva et al. 2018), following Table 1. The supernatant (extract) was then analyzed for lycopene content.

Lycopene analysis

The lycopene concentration of each extract was determined according to Strati and Oreopoulou (2011), with adaptations. Firstly, calibration curves of pure lycopene standards

were constructed by plotting absorbance $\times$ concentration ($\mu\text{g/mL}$), using concentrations between 5 and 30 $\mu\text{g/mL}$ of lycopene in each Men-Lac solvent (2:1, 5:1 and 8:1 mol/mol) diluted with ethyl acetate (1:1, v/v). Wavelength scans from 350 to 550 nm using a UV–Vis spectrophotometer (Genesys 10S UV–Vis, Thermo Scientific, Madison, WI, USA) were used to confirm the characteristic lycopene three-peak absorbance spectrum (Britton 1995), and the wavelength of peak absorbance (477 nm) for all solvents. Linear regression equations ($R^2 > 0.99$) were obtained for each calibration curve and used to determine the lycopene content ($\mu\text{g/mL}$) in each extract of the

Table 2 Analysis of variance (ANOVA) results for the final reduced quadratic model for ultrasound-assisted lycopene extraction with hydrophobic eutectic solvent (DL-menthol combined with lactic acid)

Source	DF	Adj SS	Adj MS	F value	P value
<i>Model</i>	6	602,251	100,375	25.85	< 0.0001
Linear	4	491,438	122,860	31.65	< 0.0001
X_1 = Temperature	1	299,376	299,376	77.11	< 0.0001
X_2 = HBD:HBA molar ratio	1	177,147	177,147	45.63	< 0.0001
X_3 = Solvent:sample ratio	1	14,571	14,571	3.75	0.065
X_4 = Time	1	344	344	0.09	0.769
Square	1	73,337	73,337	18.89	< 0.0001
X_1^2	1	73,337	73,337	18.89	< 0.0001
2-Way interaction	1	37,476	37,476	9.650	0.005
$X_3 * X_4$	1	37,476	37,476	9.65	0.005
<i>Error</i>	23	89,294	3882		
Lack-of-fit	18	82,387	4577	3.31	0.095
Pure error	5	6907	1381		
<i>Total</i>	29	691,545			

R^2 : 87.09%, $R^2(\text{adj})$: 83.72%, $R^2(\text{pred})$: 77.42%

experimental procedure. These extracts were diluted with pure ethyl acetate (1:1, v/v) prior to spectrophotometric analysis. Then, lycopene yield in each extract was obtained as previously described (Silva et al. 2018). Results were expressed as μg of lycopene per g dry weight (d.w.).

Response surface methodology (RSM) analysis and modeling

Process optimization was performed using RSM (Silva et al. 2018), where a second-order polynomial model was fit to the experimental results ($p < 0.05$). The optimal extraction conditions for maximizing lycopene yield were determined using the response optimizer function in Minitab® (v. 17.3.1).

UAE optimization and effect of ultrasound

UAE was experimentally performed, in triplicate, under the predicted optimal extraction conditions, and the results compared to the theoretical yield. In addition, to evaluate the effect of ultrasonication, solvent extraction (SE) was performed in triplicate under the same optimal extraction conditions but without the use of ultrasound, and SE yield was compared to the optimized UAE yield.

Results and discussion

The lycopene yield ($\mu\text{g/g}$ d.w.) (Table 1) varied from 774.1 to 1462.8 $\mu\text{g/g}$, and the six centre point replicates averaged 994.2 ± 37.2 $\mu\text{g/g}$, which represents a coefficient of variation of 3.7%, indicating good stability. The final reduced models ($p < 0.0001$), in terms of coded and natural

variables, are shown in Eqs. 1 and 2, respectively, and the ANOVA results are shown in Table 2. Although the linear terms X_3 ($p = 0.065$) and X_4 ($p = 0.769$) did not have a significant effect, both were included in the model for hierarchical purposes, as the 2-way interaction ($X_3 * X_4$) was significant. The predicted yield for each treatment, calculated using the final model, showed less than 5% difference for most experimental conditions. Also, the lack-of-fit was not significant ($p = 0.095$), further suggesting that model goodness-of-fit is satisfactory (Berger et al. 2018).

$$\begin{aligned} \text{Lycopene}(\mu\text{g/g}) = & 1000.2 + 157.9 \times k_1 + 121.5 \times k_2 \\ & + 34.8 \times k_3 + 5.4 \times k_4 + 100.9 \times k_1^2 \\ & - 96.8 \times k_3 \times k_4 \end{aligned} \quad (1)$$

$$\begin{aligned} \text{Lycopene}(\mu\text{g/g}) = & 485 - 17.33 \times X_1 + 40.50 \times X_2 + 6.00 \\ & \times X_3 + 14.79 \times X_4 + 0.2523 \times X_1^2 \\ & - 0.1613 \times X_3 \times X_4 \end{aligned} \quad (2)$$

The three-dimensional plot of the 2-way interaction between factors X_3 (solvent:sample ratio) and X_4 (time) is shown in Fig. 1, and indicates that a saddle point is present. This indicates that similarly high lycopene yields can be achieved at either high solvent:sample ratio and shorter times, or at low solvent:sample ratio at longer times. At lower solvent:sample ratios, the solvent is saturated with the extracted compound more quickly, and mass transfer rates are reduced, thus requiring more time to achieve high yields. At higher solvent:sample ratios, there is a greater initial concentration difference, therefore the mass transfer of the lycopene into the solvent occurs at a greater rate, and high yields are obtained in a shorter time.

Fig. 1 Response surface plot of lycopene yield showing the effect solvent:sample ratio and time when fixed at 50 °C and 5 mol HBA/mol HBA

RSM analysis was performed and the optimal extraction conditions obtained were $X_1 = 70$ °C, $X_2 = 8:1$ mol HBA/mol HBD, $X_3 = 120$ mL/g solvent:sample, and $X_4 = 10$ min, with a predicted lycopene yield of 1506.9 µg/g. The average experimental yield obtained under these conditions was 1446.6 µg/g indicating good agreement with the model.

The optimum extraction occurred at the highest temperature investigated. DESs often have high viscosities which would limit the ability of the solvent to penetrate the plant matrix during an extraction process, therefore, the use of high temperatures is usually required to reduce the viscosity of the solvent system (Cao et al. 2017a; Zhang et al. 2012). DL-menthol-based DESs have lower viscosity when compared to other eutectic mixtures (Ribeiro et al. 2015), but still higher than conventional organic solvents such as hexane. Although higher yields were predicted at the highest extraction temperature, it is important to establish an upper limit of temperature for lycopene extraction, since the carotenoid is susceptible to thermal degradation (Henry et al. 1998). The short extraction time required to achieve optimal yield indicates that once the solvent has penetrated the plant matrix, the target compound is easily solubilized. In addition, it is a positive aspect to consider for industrial purposes, since could reduce the energy requirement of the extraction process, and likely reduce costs and increase the overall sustainability of the process.

Although tomato processing by-products have been previously investigated for lycopene extraction, the yields vary extensively, depending on the characteristics of the raw material and the extraction conditions applied. A recent review has shown that yields between 6.39 and 6772 mg/kg (d.w.) have been previously reported (Strati

and Oreopoulou 2014). The optimized lycopene extraction yield achieved in this study (predicted: 1506.9 µg/g, experimental: 1446.6 µg/g d.w.) was higher than an optimized extraction from the same tomato pomace, using a solvent mixture of ethyl-acetate and ethyl-lactate (predicted: 1343.9 µg/g, experimental: 1334.8 µg/g d.w.) (Silva et al. 2018). Similar yields were reported by Calvo, Dado and Santa-Maria (2007) (approximately 1200 mg/kg d.w.) using the organic solvent ethyl acetate. Strati and Oreopoulou (2011) used the environment-friendly solvent ethyl lactate, but obtained a much lower yield, of 243.00 mg/kg d.w.

The SE yield was 896.6 µg/g, 38% lower than with UAE. Ultrasound increases extraction yields by promoting the collapse of gas bubbles in the solvent (Suslick 1990), which degrades the vegetal matrix thus increasing mass transfer rates. The effectiveness of UAE depends on several factors, such as characteristics of the solvent system (viscosity), plant matrix (moisture, particle size distribution, etc.), and ultrasound equipment (power, frequency, shape, and size) (Chemat et al. 2017). The result from this study indicates that ultrasound improved solvent penetration in the plant matrix, possibly due to a reduction in the resistance to mass transfer usually exhibited by eutectic solvents. This is in agreement with Cao et al. (2017b) who reported that UAE was the most efficient method for DES extraction in comparison to other extraction methods such as magnetic stirring, heating, water-bath shaking, and air-bath shaking.

This HEM solvent system represents an important development in the field of green chemistry. Indeed, one of the greatest challenges in improving the sustainability of the chemical industry is to establish more environment-friendly processes for obtaining important value-added compounds such as lycopene (Chemat et al. 2012). The present study, using a by-product and a green solvent, provides an alternative for a more sustainable lycopene extraction process.

Conclusion

This is the first report on the use of a hydrophobic deep eutectic solvent for the extraction of lycopene from tomato pomace. The new Men-Lac HEM solvent system composed of DL-menthol and lactic acid showed a great capacity for extracting lycopene, and was comparable to conventional organic solvents. The optimum conditions for ultrasound-assisted extraction were 70 °C, 8:1 mol HBA/mol HBD, 120 mL/g solvent:sample, and 10 min, yielding 1446.6 µg/g (d.w.) of lycopene. These results represent a more sustainable approach for lycopene extraction.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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