ORIGINAL PAPER

Optimization of Lycopene Extraction from Tomato Processing Waste Using an Eco-Friendly Ethyl Lactate–Ethyl Acetate Solvent: A Green Valorization Approach

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Abstract

Lycopene is a highly-prized antioxidant with associated health benefits and is abundant in natural sources. A green valorization approach was used to extract lycopene from tomato processing waste. Ultrasound-assisted extraction was applied to the tomato waste using an eco-friendly solvent mixture containing ethyl lactate and ethyl acetate for the extraction of lycopene. Extraction parameters were: X_1 = extraction temperature (°C), X_2 = proportion of ethyl acetate in solvent mixture (% v/v), X_3 = solvent:sample ratio (mL/g), and X_4 = extraction time (min). A Box–Behnken design was used to define experimental conditions, and response surface methodology was then conducted to determine the optimized conditions: X_1 = 63.4 °C, X_2 = 30% (v/v), X_3 = 100 mL/g, and X_4 = 20 min. The experimental optimized extraction yield of lycopene was 1334.8 µg/g (d.w.), in agreement with the predicted yield. At the same conditions without ultrasound, a yield of 1209.5 µg/g (d.w.) was obtained (9.4% lower). Ultrasound increases extraction yield, and tomato processing by-products are a viable alternative source of extractable lycopene. This represents a greener strategy for the extraction of lycopene in comparison to conventional methods using organic solvents, and shows a promising alternative use for a food processing waste.

Keywords By-product · Green extraction · Carotenoids · Box–Behnken design · Response surface methodology · Biomass utilization · Extraction/separation

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Introduction

The use of agricultural by-products as source material for the extraction of high value-added compounds has been heavily investigated in recent years [1, 2]. Due to their organic composition, these wastes represent alternative raw materials that could be exploited for the recovery of bioactive compounds that have positive effects on human health and also widespread technological applications, as coloring agents and antioxidants. Lycopene is an example of a high-value biomolecule that could be potentially recovered from food processing wastes, as a valorization approach to minimize the environmental impact of waste disposal and increase the sustainability of the overall process. Lycopene is a carotenoid and a valuable dietary antioxidant, with activity against diseases such as cancer and cardiovascular diseases [3–5]. It is widely used by various industries, as a dietary supplement for human nutrition, and as a natural colorant and antioxidant in products with high lipid content [6].

Lycopene is usually extracted from natural sources, especially red-coloured vegetables such as tomatoes [6],

red pepper [7] and watermelon [8]. Synthetic routes for lycopene synthesis have been developed [9], but given the abundance of the compound in natural materials and the market preference for natural products, the extraction of the carotenoid from these sources is preferred. The major source of lycopene is tomato, due to its high lycopene content [3] and low cost compared to other lycopene-rich fruits and vegetables [6]. Many companies extract lycopene from tomatoes worldwide, and a high-lycopene tomato variety, containing around 150–250 mg/kg (fresh weight, f.w.), has been developed specifically to be used as source for lycopene extraction [10]. Commercial extraction of lycopene involves crushing the raw tomatoes to separate fibrous materials (such as skin and seeds) from the juice, then extracting the compound from the juice using an organic solvent. However, most of the lycopene in tomatoes (72–92%) is found in the skin fraction [11]. Therefore, tomato pomace, the by-product from industrial processing, can be considered a potential source of lycopene as it is mostly composed of skin and seeds.

The extraction of lycopene from vegetables requires the use of organic solvents, such as hexane, due to the hydrophobic nature of the compound. Hexane has advantages of low cost, good capacity for solubilizing lipophilic compounds, and low boiling point [12], and is considered as a food grade solvent by the FDA in residual concentrations of up to 25 ppm [13]. However, this solvent has a high environmental impact as it is obtained from petroleum [14], thus requiring safe handling and storage procedures. Alternative strategies can be developed using the principles of green chemistry to overcome the disadvantages associated with the use of organic solvents for extraction, where green chemistry promotes the reduction or elimination of hazardous materials and processes. Green solvents are alternatives to conventional organic solvents due to their low toxicity, high biodegradability, and sustainable production from non-petroleum sources. One example is ethyl acetate (ethyl ethanoate) (EA), an environmentally friendly organic solvent commonly used for extraction of lipophilic compounds [15]. Although overexposure to EA may cause health hazards such as skin irritation and unconsciousness, it is considered a low-toxicity solvent due to its rapid hydrolysis to ethanol and acetic acid during metabolism [16]. Its presence in food products is accepted in concentrations of up to 25 mg/kg, and it is even used as a flavouring agent due to its fruity aroma [17]. EA is considered a green alternative to hexane because it can be produced from bio-based ethanol, and is biodegradable in air and water [18]. The solvent has been used for extraction of lycopene from tomato, resulting in yields that are comparable to hexane extraction [19–21]. Another promising green solvent is ethyl lactate (ethyl 2-hydroxypropanoate) (EL), a non-corrosive, non-toxic (non-carcinogenic and non-teratogenic), non-ozone depleting, biodegradable, considered as generally recognized as safe (GRAS) solvent [22]. EL can

be commercially obtained from lactic acid and ethanol, both of which can be obtained from fermentation processes [23]. Recently, EL has shown good potential for the extraction of carotenoids from tomato and other plant materials [24–27]. Therefore, both EA and EL represent promising green alternatives to hexane for carotenoid extraction.

The use of assisting technologies, such as the application of ultrasound, to improve the efficiency of solvent extraction from biological materials is another strategy for reducing the environmental impact of extraction, and has been widely studied in recent years [28, 29]. The increase of extraction yield observed in ultrasound-assisted processes has been associated with the acoustic cavitation phenomenon, in which ultrasound waves cause a violent collapse of gas bubbles present in the solvent [30]. Ultrasonication has been applied as assisting technology for the extraction of several bioactive compounds from food processing wastes, such as phenolic compounds from sunflower seed cake [31] and from apple peels [32], flavonoids from citrus peels [33], among many others. Also, it has been previously studied for the extraction of lycopene from tomato [21, 34] and tomato processing by-products [35–37]. However, to the best of our knowledge, no study has yet been published evaluating the ultrasound-assisted extraction of lycopene from tomato pomace using EL, nor its use combined with EA.

In this study, a three-fold strategy was applied to develop a greener alternative for lycopene extraction using (1) food by-products (i.e. tomato processing waste) as source material, (2) green solvents to replace conventional organic solvents, and (3) ultrasound to improve extraction yield. Although EL has shown good potential for the extraction of carotenoids from plant materials, this is the first report that combines these three approaches for extraction of lycopene from tomato-based material. Thus, the objective of this study was to establish optimal conditions for the ultrasound-assisted extraction (UAE) of lycopene from tomato pomace with a green solvent mixture of ethyl lactate and ethyl acetate. Extraction parameters investigated were temperature, proportion of ethyl acetate in solvent mixture, solvent:sample ratio and time. A Box–Behnken experimental design and response surface methodology (RSM) were used to optimize extraction conditions, which were experimentally verified, and compared to extraction without ultrasound.

Materials and Methods

Chemicals

Ethyl lactate (CAS 687-47-8) and ethyl acetate (CAS 141-78-6) were purchased from Sigma-Aldrich (Oakville, ON, Canada), and were of analytical grade. The lycopene

standard (CAS 502-65-8) was purchased from Fisher Scientific (Ottawa, ON, Canada).

Plant Material

Tomato pomace was collected from a tomato processing plant located in the state of Goiás (Brazil), immediately after being produced. The moisture content of the fresh pomace was ~62.8%, and it was composed of 61.5% skin and 38.5% seeds. The sample was freeze-dried in a bench-top freeze dryer (Liotop L108, Liotop, São Carlos, Brazil) for ~16 h to a final moisture content of ~5.6%. The moisture content of samples was determined in vacuum oven (Lindberg Blue M, Thermo Scientific, Asheville, NC, USA) at 72 °C and 14 psi until constant weight (AOAC method 934.06) [38]. Freeze-dried samples were vacuum packed, transported to Halifax (NS, Canada), and kept at −20 °C until further analysis. Immediately before extraction, samples were ground using a manual grinder (Smartgrind, Black and Decker, Mississauga, ON, Canada) and sieved through a 0.5-mm (32 mesh) sieve.

Box–Behnken Experimental Design

Optimization of UAE was performed using a Box–Behnken design (BBD) to determine the experimental points. The extraction parameters used in the experimental design were chosen based on results from other lycopene extraction studies [15, 21, 34, 39]. The parameters were X_1 = extraction temperature (°C), X_2 = proportion of ethyl acetate in the ethyl lactate–ethyl acetate solvent mixture (%, v/v), X_3 = solvent:sample ratio (mL/g) and X_4 = extraction time (min). The experimental design was established according to a BBD for four factors [40]. In this design, each variable (X_i) assumes three levels (k_i), coded as −1, 0, and +1. Extreme high (k_i = +1) and low (k_i = −1) natural values of each parameter were defined based on preliminary studies, and the centre level (k_i = 0) was then calculated as the median of the extremes. The natural (X_i) and coded (k_i) levels used in the design for each variable are shown in Table 1.

The BBD matrix is presented in Table 2, with six centre points. To reduce possible effects of external variations, the experiments were conducted in randomized order as shown in Table 2. The six centre point runs (runs 25–30) were not randomized, but evenly interspersed among the other experimental points, since they provide a measure of process stability and allow estimating inherent variability of the process [41] and therefore must be regularly verified throughout the experiment.

Table 1 Input variables used in Box–Behnken design (BBD), their coded (k_i) and natural (X_i) values, and the equation for conversion from k_i to X_i

Input variable	Natural unit	Levels			Equation
		−1	0	+1	
X_1 = Extraction temperature	°C	40	55	70	$k_1 = \frac{X_1 - 55}{15}$
X_2 = Proportion of EA in solvent mixture	%, v/v	30	65	100	$k_2 = \frac{X_2 - 65}{35}$
X_3 = Solvent:sample ratio	mL/g	50	75	100	$k_3 = \frac{X_3 - 75}{25}$
X_4 = Extraction time	min	20	35	50	$k_4 = \frac{X_4 - 35}{15}$

Ultrasound-Assisted Extraction (UAE) of Lycopene

Preliminary analyses indicated that the proposed solvent mixture EL–EA could obtain 73% higher lycopene yield than a mixture of hexane and acetone, both at 1:1 (v/v) [42]; therefore, its use as solvent for the UAE was further evaluated. The UAE of lycopene from tomato pomace 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), with internal dimensions of 24 × 14 × 10 cm (L × W × D) and 2.8 L capacity, where a constant water level was used as well as the placement of samples in the bath. The solvent mixture was prepared immediately before use, and placed in water bath to achieve extraction temperature before being added to the sample. According to the experimental condition (Table 2), the appropriate amount of sample was added to 10 mL screw-capped glass tubes. Then, 5 mL of the solvent mixture were added, the tubes were vortexed for 10 s, and placed in the ultrasound bath. The extraction temperature was controlled within ± 1 °C of the set temperature with a calibrated thermometer and adjusted using hot and cold water as needed. At the end of the extraction time, the tubes were removed from the bath and centrifuged (Sorvall RT1, Thermo Scientific, Madison, WI, USA) at 2916×g for 10 min at 20 °C for separation of the pellet from the lycopene extract. The supernatant (extract) was then transferred to another tube for spectrophotometric analysis. Extraction was performed under reduced light to reduce carotenoid degradation.

Determination of the UAE Lycopene Yield

Lycopene Calibration Curves

A lycopene standard curve was determined for each solvent mixture with different EL:EA ratio, where spectrophotometric analyses were performed under dimmed light to reduce carotenoid degradation. A 500 μg/mL lycopene stock solution was prepared by dissolving pure lycopene standard in

Table 2 Box–Behnken design matrix with experimental factors in coded (k_i) and natural (X_i) values, experimental and predicted results for lycopene extraction yield (µg/g d.w.)

Assay	Run order	Factor[a]				Lycopene yield (µg/g d.w.)		Prediction error (%)
		Extraction temperature	Proportion of EA in solvent mixture	Solvent:sample ratio	Sonication time	Experimental	Predicted	
		k_1 (X_1) (°C)	k_2 (X_2) (%, v/v)	k_3 (X_3) (mL/g)	k_4 (X_4) (min)			
1	20	− 1 (40)	− 1 (30)	0 (75)	0 (35)	1147.1	1125.3	− 1.9
2	22	+ 1 (70)	− 1 (30)	0 (75)	0 (35)	1004.1	1047.1	4
3	10	− 1 (40)	+ 1 (100)	0 (75)	0 (35)	849.5	878.7	3.4
4	11	+ 1 (70)	+ 1 (100)	0 (75)	0 (35)	857.5	800.5	− 6.6
5	17	0 (55)	0 (65)	− 1 (50)	− 1 (20)	1070.3	1016.7	− 5.0
6	5	0 (55)	0 (65)	+ 1 (100)	− 1 (20)	1249.4	1256.2	0.5
7	14	0 (55)	0 (65)	− 1 (50)	+ 1 (50)	1108.7	1086.7	− 2.0
8	6	0 (55)	0 (65)	+ 1 (100)	+ 1 (50)	931.3	969.6	4.1
9	29	− 1 (40)	0 (65)	0 (75)	− 1 (20)	1065.9	1000.7	− 6.1
10	3	+ 1 (70)	0 (65)	0 (75)	− 1 (20)	1187.8	1151.6	− 3.0
11	9	− 1 (40)	0 (65)	0 (75)	+ 1 (50)	1145.0	1121.5	− 2.1
12	16	+ 1 (70)	0 (65)	0 (75)	+ 1 (50)	809.0	814.3	0.6
13	4	0 (55)	− 1 (30)	− 1 (50)	0 (35)	1067.3	1115.9	4.6
14	21	0 (55)	+ 1 (100)	− 1 (50)	0 (35)	760.9	869.4	14.3
15	23	0 (55)	− 1 (30)	+ 1 (100)	0 (35)	1264.5	1177.1	− 6.9
16	15	0 (55)	+ 1 (100)	+ 1 (100)	0 (35)	1022.4	930.5	− 9.0
17	18	− 1 (40)	0 (65)	− 1 (50)	0 (35)	1009.8	1030.5	2.0
18	27	+ 1 (70)	0 (65)	− 1 (50)	0 (35)	1017.8	952.3	− 6.4
19	12	− 1 (40)	0 (65)	+ 1 (100)	0 (35)	1031.0	1091.7	5.9
20	2	+ 1 (70)	0 (65)	+ 1 (100)	0 (35)	903.1	1013.5	12.2
21	26	0 (55)	− 1 (30)	0 (75)	− 1 (20)	1193.5	1200.6	0.6
22	28	0 (55)	+ 1 (100)	0 (75)	− 1 (20)	873.7	954.1	9.2
23	24	0 (55)	− 1 (30)	0 (75)	+ 1 (50)	1081.9	1092.4	1.0
24	8	0 (55)	+ 1 (100)	0 (75)	+ 1 (50)	915.0	845.8	− 7.6
25	1	0 (55)	0 (65)	0 (75)	0 (35)	1137.7	1082.3	− 4.9
26	7	0 (55)	0 (65)	0 (75)	0 (35)	991.5	1082.3	9.2
27	13	0 (55)	0 (65)	0 (75)	0 (35)	1034.9	1082.3	4.6
28	19	0 (55)	0 (65)	0 (75)	0 (35)	1189.0	1082.3	− 9.0
29	25	0 (55)	0 (65)	0 (75)	0 (35)	1104.4	1082.3	− 2.0
30	30	0 (55)	0 (65)	0 (75)	0 (35)	1012.4	1082.3	6.9

[a]Coded level (natural value)

EA, which was kept at − 20 °C for up to 1 week for determination of the standard curve. The stock solution was diluted with the appropriate solvent mixture as needed to give concentrations varying from 5 to 30 µg/mL. Absorbance readings were performed immediately after dilution using a UV–Vis spectrophotometer (Genesys 10S UV–Vis, Thermo Scientific, Madison, WI, USA) in duplicate for each dilution. Wavelength scans from 350 to 550 nm were used to confirm the lycopene three-peak absorbance spectrum, which consisted of peaks at approximately 450, 470 (maximum), and 505 nm, with the specific wavelength varying according to the solvent used [4, 43]. The maximum absorbances (at

Table 3 Wavelength for peak absorbance (λ_{max}) and lycopene standard equation for each solvent (L is lycopene concentration in µg/mL and A_{max} is the absorbance at the λ_{max})

Solvent (% EA, v/v)	λ_{max} (nm)	Regression equation	R^2
30	477	$L = 9.65 \times A_{max}$	0.9999
65	474	$L = 7.51 \times A_{max}$	0.9985
100	473	$L = 6.98 \times A_{max}$	0.9995

approximately 470 nm, Table 3, Online Resource 1) for the lycopene solutions were used to construct standard curves for each solvent mixture, and linear regression equations were subsequently obtained by plotting absorbance against lycopene concentration (µg/mL), going through the origin. The regression equations obtained for each calibration curve are shown in Table 3, where L is lycopene concentration in µg/mL and A_{peak} is the absorbance value at wavelength of the absorption peak (λ_{peak}). The standard equations ($R^2 > 0.99$) were used to calculate lycopene concentration for each extract obtained from the BBD experimental conditions.

UAE Lycopene Yield

The lycopene extraction yield obtained from each treatment was determined according to Strati and Oreopoulou [25]. Since lycopene is the major carotenoid in tomatoes and tomato products [44, 45], lycopene standard curves have been used by other researchers for estimating the total carotenoid content in tomato products [39, 46]. The concentration of lycopene (µg/mL) in each extract was therefore determined using the peak absorbance in the UV–Vis spectra in relation to the appropriate standard curve (Table 3). Then, the lycopene extraction yield was calculated according to Eq. 1, where L is the concentration of lycopene in the extract calculated using the regression equations (µg/mL), V is the final volume of extract obtained (mL), w is the sample weight used for extraction (g), and m is the moisture of the sample. Results are expressed as µg of lycopene per g dry weight (d.w.).

$$Lycopene\ yield\ (\mu g/g) \; = \; \frac{L \times V}{w \times (1 - m)} \tag{1}$$

Response Surface Analysis

RSM was used to optimize lycopene yield under the studied conditions, using Minitab® version 17.3.1 software (Minitab Inc., PA, USA). Responses were fitted to a second-degree polynomial equation, as shown in Eq. 2, where Y is the response variable (lycopene yield, µg/g), β_0 is the constant (or model intercept); β_i is the linear coefficient of each variable X_i; β_{ii} is the quadratic coefficient; β_{ij} is the cross-product coefficient of interaction between variables X_i and X_j; and X_i and X_j are the independent variables. Models were obtained for coded and natural factors, and analysis of variance (ANOVA) was performed on the full model to determine the significant variables affecting extraction yield ($p < 0.05$). The initial full quadratic model was refined by stepwise backward elimination of non-significant terms [47], using a significance level of 5%. ANOVA was used to evaluate the significance and goodness-of-fit of the final reduced model, which was used to predict lycopene yield

(Y_{pred}). Three-dimensional surface plots of lycopene yield *versus* each combination of 2 factors (with remaining two factors held at centre level, $k_i = 0$) were used to illustrate the influence of the variables in the response.

$$Y = \; \beta_0 \; + \; \sum_{i=1}^{4} \beta_i X_i \; + \; \sum_{i=1}^{4} \beta_{ii} X_i^2 \; + \; \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j \tag{2}$$

Optimization, Model Validation and Comparison Without Ultrasound

The optimal extraction conditions for maximizing lycopene yield with UAE were determined from the final model using the response optimizer function in Minitab® (v. 17.3.1). Experiments were then performed in triplicate at the optimal extraction conditions to validate the model. In addition, results were compared with solvent extraction without ultrasound (SE) at the same optimal extraction conditions, to evaluate the effect of ultrasound on extraction yield.

Results and Discussion

Lycopene Standard Curves in Different Solvent Mixtures

The absorbance spectrum of a carotenoid is highly dependent on the solvent in which the compound is dissolved [43]. To allow the correct estimation of the carotenoid content of the extracts, standard curves of pure lycopene were obtained for each solvent mixture used in the extraction (Fig. 1). The increase in the amount of EA in the solvent mixture from 30 to 100% caused a hyperchromic effect in all concentrations used [48].
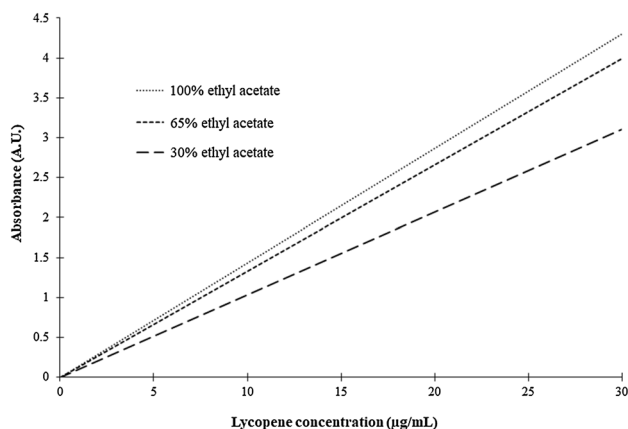
Fig. 1 Lycopene standard curves in different solvent mixtures: (·········) 100% ethyl acetate, (— — —) 65% ethyl acetate–35% ethyl lactate, and (—— ——) 30% ethyl acetate–70% ethyl lactate

The position of the absorption peak (λ_{max}) of carotenoids depends on several factors, such as the molecular environment in which the carotenoid molecule is inserted [4, 48]. For this reason, the same compound shows different absorption peaks for different solvents. Considering the three solvent mixtures evaluated, the increase in the amount of ethyl acetate in the solvent mixture caused a hypsochromic shift, that is, a displacement in the λ_{max} to a shorter wavelength [48]. The λ_{max} observed for lycopene in 100% EA (473 nm, as shown in Table 3) was the same [25] or within a ± 1 nm variation [49] from the values reported in other studies.

Lycopene Extraction Yield and Modeling

The lycopene yield at each experimental treatment is shown in Table 2. Experimental lycopene yield varied between 760.9 and 1264.5 µg/g. The centre point replicates ranged from 991.5 to 1189.0 µg/g. Besides providing a measure of process stability and variability, the centre points also provide an estimation of quadratic terms [41]. In this study, the centre points average was 1078.3 ± 77.7 µg/g, representing a coefficient of variation of 7.2%.

ANOVA testing was performed on the full quadratic polynomial model to determine the variables with significant effect on extraction yield ($p < 0.05$). The ANOVA results for the final reduced quadratic model are shown in Table 4. Although the linear terms X_1 ($p = 0.075$) and X_3 ($p = 0.158$) did not have a significant effect on extraction, both were included in the model for hierarchical purposes. The final reduced model was significant ($p < 0.0001$), indicating that

the quadratic model fitted the observed experimental results well. Also, the lack-of-fit was not significant ($p = 0.652$), further suggesting that model goodness-of-fit is satisfactory [50].

The reduced models containing only significant terms are shown in Eqs. 3 and 4, using coded (k_i, Eq. 3) and natural factors (X_i, Eq. 4). The regression model was also used to calculate the predicted yields for each treatment, thus allowing the comparison of the experimental yield with the corresponding predicted values (Table 2), which shows that the prediction error was below 10% for most of the treatments, indicating good adequacy of the predicted model. Equation 3 shows that the strongest effect on lycopene yield (largest coefficient) is due to the negative first-order effect of factor X_2. Here, the proportion of EA in the solvent mixture is the factor influencing the extraction yield the most, and an increase in the proportion of EA causes a decrease in lycopene yield.

$$Lycopene\ (\mu g/g) = 1082.3 - 39.1k_1 - 123.3k_2 + 30.6k_3 - 54.1k_4 - 60.3k_1^2 - 59.1k_2^2 - 114.5k_1k_4 - 89.1k_3k_4 \tag{3}$$

$$Lycopene\ (\mu g/g) = -1129 + 44.7X_1 + 2.75X_2 + 9.54X_3 + 42.2X_4 - 0.27X_1^2 - 0.05X_2^2 - 0.51X_1X_4 - 0.24X_3X_4 \tag{4}$$

Factors X_1 (temperature) and X_2 (proportion of EA in solvent mixture) showed negative quadratic effects on lycopene yield. For temperature, while an initial increase will increase mass transfer rates and extraction yield, a further increase in temperature decreases lycopene yield. This is likely due to thermal degradation of lycopene at high temperatures [51, 52]. High extraction yields can be observed at higher temperatures due to several effects, including the decrease in surface tension and solvent viscosity, leading to improved sample wetting and matrix penetration; the increase in molecular motion of the solvent, leading to higher solubility; and the destruction of the plant matrix, increasing the availability of the target compound to the solvent [53, 54]. However, an upper temperature limit must be defined to avoid thermal degradation. In the present study, the higher temperature likely caused carotenoid degradation, since the optimal temperature was below the maximum of 70 °C.

The negative quadratic effect of EA proportion in the solvent mixture (X_2) indicates that increasing the amount of EA beyond an optimal value causes a decrease in the lycopene yield, suggesting that EL is more efficient for extraction than EA. The extraction of carotenoids from tomatoes has been previously investigated using EA [19–21] or EL [24–27] individually, with yields comparable to or higher than hexane. Our results show that the mixture of EL with EA was effective for obtaining high lycopene extraction yield (between 760.9 and 1264.5 µg/g), and the highest yield was achieved with the lowest proportion of EA, indicating that

Table 4 ANOVA results of significant factors in quadratic model for UAE of lycopene

Source	DF	SS	MS	F-value	p value
Model	8	376,968	47,121	9.00	< 0.0001
Linear	4	247,118	61,780	11.80	< 0.0001
X_1 = Temperature	1	18,342	18,342	3.50	0.075
X_2 = %Acetate	1	182,380	182,380	34.83	< 0.0001
X_3 = Solvent:sample	1	11,221	11,221	2.14	0.158
X_4 = Time	1	35,175	35,175	6.72	0.017
Square	2	45,623	22,812	4.36	0.026
X_1^2	1	25,860	25,860	4.94	0.037
X_2^2	1	24,831	24,831	4.74	0.041
2-Way interaction	2	84,226	42,113	8.04	0.003
$X_1 \times X_4$	1	52,441	52,441	10.02	0.005
$X_3 \times X_4$	1	31,786	31,786	6.07	0.022
Error	21	109,947	5236		
Lack-of-fit	16	79,740	4984	0.82	0.652
Pure error	5	30,207	6041		
Total	29	486,914			

*DF* degrees of freedom, *SS* sum of squares, *MS* mean square

EL is the major component responsible for the extraction efficiency.

The positive linear effect of solvent:sample ratio (X_3) shows that an increase of this ratio (more solvent for a fixed amount of sample) could further increase the extraction yield. However, the maximum ratio would be limited by cost and environmental considerations for the amount of solvent used. Thus, the maximum amount of solvent recommended was fixed at 100 mL/g of sample, which is a reasonable level close to the maximum amount tested in other studies [34, 39].

Extraction time (X_4) showed a negative linear effect, where higher lycopene yields were observed at lower extraction times. The decreased yield observed at higher extraction time could have been caused by the longer exposure of the bioactive compound to agents that cause carotenoid degradation, such as light and oxygen [49, 52]. Although the exposure to these agents was minimized during the experimental procedure, their presence could not have been completely excluded, similarly to the conditions that would be observed in a large-scale process. Therefore, it is possible that some level of thermal- and photo-degradation could have taken place when the extraction procedure was carried out for a longer period of time.

RSM Analysis and Optimization of UAE

RSM is a powerful tool for achieving high performance from processes at reduced costs. In this study, RSM was used to optimize the ultrasound-assisted extraction (UAE) of lycopene from tomato pomace. From the quadratic models obtained (Eqs. 3, 4), three-dimensional surface plots were constructed to illustrate the influence of the interaction of two factors at a time over the lycopene extraction yield. Figures 2 and 3 show the effect of the two significant
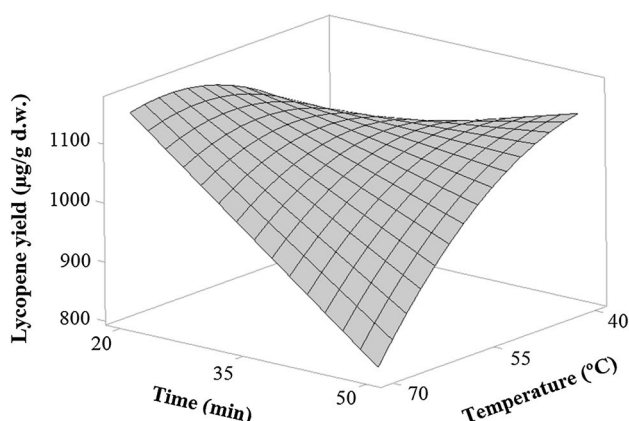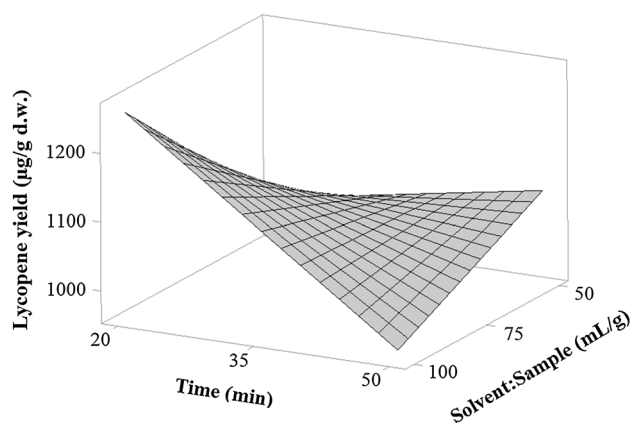
Fig. 3 Response surface plot for effect of interaction $X_3 \times X_4$ (Solvent:sample ratio × Time) over lycopene yield ($X_1 = 55$ °C, $X_2 = 65\%$ acetate in solvent mixture)

interactions (interaction $X_1 \times X_4$ and $X_3 \times X_4$, respectively) over lycopene yield. For each set of two parameters shown in the plots, the other two were held at the centre value ($k_i = 0$).

The interaction between time and temperature ($X_1 \times X_4$) (Fig. 2) shows that high lycopene yields can be achieved with a combination of either high temperature for short time or low temperature for longer time. This could be related to the ability of the solvent to penetrate the plant cell to extract the target compound, as discussed previously. At higher temperatures, mass transfer processes are enhanced, thus less time is required to achieve a high yield, while at lower temperatures more time is required for the solvent to reach the cell interior. The sharp decrease in lycopene yield at low temperature and short extraction time observed in Fig. 2 indicates this insufficient access of the solvent to the plant matrix. Interestingly, a sharp decrease was also observed at the combination of high temperature and high extraction time levels. This could indicate degradation of lycopene due to prolonged exposure of the sample to the high temperatures, since exposure to heat is one of the main factors that can cause lycopene degradation [52].

Figure 3 illustrates the influence of the interaction between solvent:sample ratio and time ($X_3 \times X_4$) on lycopene yield, with the highest yield obtained at high solvent:sample ratio and short extraction time. The relationship between solvent:sample ratio and time can be explained by the saturation of the solvent with the extracted compound. At higher ratios, more solvent is available to dilute the target compound, thus the mass transfer rates are maintained at high levels for longer [53]. As the highest yield is achieved with short extraction times, this suggests that the solvent penetrates the sample easily, quickly reaching the cell matrix and solubilizing the target compound.

The optimal extraction conditions obtained from RSM analysis were $X_1 = 63.4$ °C, $X_2 = 30\%$ (v/v) EA,

Fig. 2 Response surface plot for effect of interaction $X_1 \times X_4$ (Temperature × Time) over lycopene yield ($X_2 = 65\%$ acetate in solvent mixture, $X_3 = 75$:1 mL/g solvent:sample ratio)

$X_3 = 100$ mL/g solvent:sample, and $X_4 = 20$ min (Table 5), with a predicted lycopene yield of 1343.9 µg/g. Validation studies were performed in triplicate under the optimal conditions to verify the predicted yield. The average experimental yield at the optimal extraction conditions was then 1334.8 µg/g, showing good agreement with predicted values (only 0.7% error). These results further validate the model, indicating good prediction capacity.

RSM has been used to optimize the UAE of lycopene from tomatoes and its by-products using different extraction conditions (Table 6). Lycopene extraction yield can vary considerably among different studies, depending on the raw material and the extraction conditions applied, as shown by a recent review [54]. When using UAE, extraction yields also depends on the ultrasound equipment characteristics, such as power, frequency, shape, and size [28, 36]. For example, Kumcuoglu et al. [37] optimized UAE of dried tomato processing waste and obtained an extraction yield of 90.1 mg/kg (0.09 mg/g), much lower than the yield from the present work (1.3 mg/g), using a solvent mixture of hexane/acetone/ethanol (2:1:1) containing 0.05% (w/v) BHT, at a lower solvent:sample ratio. In comparison, Eh and Teoh [34] obtained a lycopene yield of 5.11 mg/g from dried tomato pulp by UAE using hexane/acetane/ethanol (2:1:1), at a lower temperature and amount of solvent, for a longer extraction time (Table 6).

The optimized lycopene yield of 1.33 g/kg (d.w.) achieved in this study shows the effectiveness of a greener extraction process for obtaining a high value-added product from a widely available agro-industrial waste. The use of tomato pomace as raw material for lycopene extraction represents an efficient and environment-friendly use of a food waste, helping to reduce the environmental impact of food production [55]. In addition, the use of green solvents and ultrasound

Table 5 Optimized levels of experimental factors in coded (k_i) and natural (X_i) values

Input variable	Optimized level	
	Coded (k_i)	Natural (X_i)
$X_1 =$ Extraction temperature	0.6162	63.4 °C
$X_2 =$ Proportion of ethyl acetate in solvent mixture	−1	30%, v/v
$X_3 =$ Solvent:sample ratio	1	100 mL/g
$X_4 =$ Extraction time	−1	20 min

Table 6 Ultrasound-assisted extraction of lycopene from tomato or tomato by-products

Raw material	Extraction solvent	Optimized UAE conditions	Optimal lycopene yield	Reference
Tomato pomace, freeze-dried	EL/EA (7:3)	• 100 mL/g S:S • 63.4 °C • 20 min	• 1.33 mg/g (d.w.) • UAE increased yield by 10% compared to extraction under same conditions, without ultrasound	Present work
Tomato processing waste, dried	Hexane/ acetone/ ethanol (2:1:1) with BHT 0.05% (w/v)	• 35:1 mL/g S:S • 90 W • 30 min	• 90.1 mg/kg (0.09 mg/g) • UAE required less solvent, lower temperature, and less time to achieve similar yield to the optimized organic solvent extraction without ultrasound (93.9 mg/kg yield achieved at 50:1 mL/g S:S, 60 °C, 40 min)	Kumcuoglu et al. [37]
Tomato, fresh	EA	• 8.0:1 mL/g S:S • 86.4 °C • 29.1 min	• 89.4% of maximum lycopene yield achieved at optimized UAE • UAE required shorter period of time and lower solvent volume to achieve similar yield to solvent extraction with 2% dichloromethane in petroleum ether, without ultrasound	Lianfu and Zelong [21]
Tomato pulp, freeze-dried	Hexane/ acetane/ ethanol (2:1:1)	• 74.4:1 mL/g S:S • 47.6 °C • 45.6 min	• 5.11 mg/g (d.w.) • Same extraction conditions, without ultrasound: 1.23 mg/g (d.w.)	Eh and Teoh [34]

S:S solvent:sample ratio

to improve the extraction yield also reduces environmental impact of extraction processes, which generally involve hazardous organic solvents and large energy inputs to achieve desirable yields [28]. Furthermore, with the use of GRAS solvents there is potential for creating further value from the solids remaining after lycopene extraction, and further work should be conducted to investigate the extraction of macronutrients (dietary fiber, proteins, or oils), which would provide greater use of the waste.

Comparison of UAE with Solvent Extraction Without Ultrasound

To evaluate the effect of ultrasound on lycopene yield of the optimized process, solvent extraction (SE) was performed under the same optimal extraction conditions, without ultrasound. A yield of 1209.5 µg/g (d.w.) was obtained, which was 9.4% lower than with UAE, showing that the applied ultrasound treatment promoted an increase in extraction yield, when the other extraction parameters were kept constant, contributing towards a greener method for lycopene extraction. Ultrasonication has been reported to promote an increase in extraction yield due to acoustic cavitation occurring in the solvent [30]. The corresponding collapse of the gas bubbles in the liquid results in several physical effects on the plant matrix, such as fragmentation, erosion, capillarity, detexturation, and sonoporation [56], facilitating the release of the target compound from the plant matrix to the solvent media. This increase in the mass transfer rates contributes towards a greener process by the reduction of the amount of solvent and energy required to achieve a desired extraction yield. In comparison to this study, Eh and Teoh [34] reported a higher increase of 27% on lycopene extraction yield with the use of ultrasound compared to SE without ultrasound. The difference in the degree of improved extraction observed in the present study could be due to ultrasound equipment characteristics and could also indicate a higher efficiency of the green solvent mixture for penetrating plant cells and reaching the target lycopene compound.

Conclusions

A green valorization approach was used for lycopene extraction from tomato processing waste, and optimized using RSM. Tomato pomace was used as the raw material, a novel and efficient green solvent mixture (EL and EA) provided an alternative to conventional organic solvents, and ultrasound was applied to improve extraction yield. The optimized extraction conditions (63.4 °C, 30% (v/v) EA in solvent mixture, 100 mL/g solvent:sample ratio, and 20 min), achieved a high lycopene yield of 1334.8 ± 83.9 µg/g in a relatively short time with a mild temperature. The yield

without ultrasound at optimum conditions was 1209.5 µg/g which was 9.4% lower than with UAE, showing that the applied ultrasound treatment promoted an increase in extraction yield. This is the first study evaluating the ultrasound-assisted extraction of lycopene from tomato pomace using EL in combination with EA. These results indicate the effectiveness of the novel green solvent mixture proposed for lycopene extraction, and represent a more environmentally sustainable approach for lycopene extraction from tomato pomace and the valorization of industrial tomato processing waste.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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