Title:

Microwave-assisted extraction of lycopene in tomato peels: effect of extraction conditions on all-*trans* and *cis*- isomer yields

Authors:

Ho, K.K.H.Y.[1], Ferruzzi, M.G.[1], Liceaga, A.M.[1], and San Martín-González, M.F.[1]

[1]Department of Food Science, Purdue University, 745 Agricultural Mall Dr, West Lafayette, IN, 47906, USA

Corresponding Author:

M. Fernanda San Martin-Gonzalez
Department of Food Science
Purdue University
745 Agriculture Mall Dr
West Lafayette, IN 47906
Tel. +1 (765) 496-1140
Fax +1 (765) 494-7953
fsanmartin@purdue.edu

Abstract

Lycopene is the primary carotenoid in tomato peels, a processing byproduct, and can be used as a natural color or bioactive ingredient. Unfortunately, extractions are inefficient as lycopene is extremely nonpolar and susceptible to degradation. As a rapid technique, microwave-assisted extraction (MAE) potentially offers efficient lycopene recovery. Thus, the objectives of this research were to: 1) optimize MAE of lycopene from tomato peels and 2) evaluate the effect of treatment on all-*trans* and isomer yields. Response surface methodology (RSM) was employed to optimize lycopene extraction with solvent ratio solid-liquid ratios, microwave power, and delivered energy equivalents as factors. High performance liquid chromatography with a diode array detector (HPLC-DAD) was used for isomer separation and quantification. Optimum MAE conditions were determined as: 0:10 solvent ratio at 400 W with a yield of 13.592 mg/100 g of extracted all-*trans*-lycopene. RSM suggested that ethyl acetate was a better MAE solvent for lycopene recovery as compared to hexane, which overall extracted less lycopene. HPLC-DAD indicated that MAE significantly improved all-*trans* and total lycopene yields, while conventional extraction demonstrated higher proportions of *cis*-isomer yields. Additionally,

electron micrographs showed that significant structural disruption occurred in MAE-treated samples, possibly allowing for the improved lycopene extraction.

KEYWORDS: all-*trans*-lycopene, *cis*-isomers, microwave-assisted extraction, response surface methodology

1. Introduction

The tomato industry is a multi-billion dollar market with the US being a top producer of tomatoes for processed foods (Thornsbury, 2012). In 2009, production exceeded 13 million tons (Economic Research Service, 2010), of which, 12% (the peel portion) was considered waste despite having more lycopene than the pulp by weight (Al-Wandawi, Abdul-Rahman, & Al-Shaikhly, 1985; George, Kaur, Khurdiya, & Kapoor, 2004). Lycopene, $C_{40}H_{56}$, is the primary pigment responsible for the red hue in tomatoes, watermelon, and blood oranges (Rodriguez-Amaya, 2001). As an acyclic, highly conjugated isoprenoid, lycopene is the most potent singlet oxygen quencher of all carotenoids (Di Mascio, Kaiser, & Sies, 1989). Consumption of lycopene from tomatoes has been associated with protection against oxidative DNA damage and anticancer properties (Agarwal & Rao, 2000), thus making it a compound of interest amongst medical and nutrition researchers.

Aside from potential health benefits, lycopene offers an alternative to synthetic food colorants. From a processing standpoint, extraction can be difficult as food grade solvent choices are limited. However, isolating lycopene from tomato peels can reduce the overall cost by adding value to an otherwise discarded waste product. Lycopene is insoluble in water and poorly soluble in organic solvents, which limits its removal from raw plant material. However, extraction efficiency of carotenoids can be improved by using solvent combinations to facilitate partitioning. Previous research indicated that solvent systems containing hexane and ethyl acetate are the most efficient for carotenoid extraction from tomato seeds and peels (Strati & Oreopoulou, 2011). Despite improvements, the extraction procedure itself is time consuming and poses the risk of degradation as samples are exposed to heat for extended periods of time. Due to this limitation, pure lycopene is often expensive (Ascenso et al., 2013). Improvements in extraction efficiency or reduction in extraction time may reduce the processing costs while producing a high value color.

In its natural form, lycopene is heat resistant and present in a thermodynamically stable, all-*trans*, crystal within the chromoplasts of plant cells (Harris & Spurr, 1969). Conventional extraction often requires heat to facilitate the migration of solvent to extract pigment compounds. Although increased temperatures correspond with improved solubility and organelle membrane disruption, heat exposure should be limited when possible due to the thermolabile nature of carotenoids once they are in solvent (Rodriguez-Amaya, 2001). Although lycopene has been shown to be more stable in general against isomerization and degradation compared to β-carotene (Nguyen & Schwartz, 1998) previous studies have demonstrated that heat treatments, longer than 1 hour, favored the *trans*-to-*cis* isomer conversion of lycopene while light irradiation induced *cis*-isomer degradation over time in tomato products (Chen, Shi, Xue, & Ma, 2009; Shi, Dai, Kakuda, Mittal, & Xue, 2008).

Microwave-assisted extraction (MAE) may provide a solution for this since this technology induces rapid heating primarily within polar constituents due to dipole rotation and ionic drifting (Neas & Collins, 1988). In theory, superheating of polar cellular components will improve migration of lycopene into the extraction solvent, while the short treatment times limit heat exposure of the nonpolar components. Previously, MAE has been used to enhance extraction of catechins, anthocyanins and curcuminoids (Baiano, Bevilacqua, Terracone, Contò, & Del Nobile, 2014; Dandekar & Gaikar, 2002; Zou et al., 2012) among others has improved efficiency compared to conventional extraction. Although MAE of various phytochemicals has been investigated, limited research has been done on the effect of MAE on *cis* vs. *trans* isomer yield. Thus, the objectives of this study were to 1) determine the optimal MAE conditions for lycopene from tomato peels using response surface methodology and 2) evaluate the effect of treatment on *cis*- and *trans*- lycopene yields.

2. Materials and methods

89 *2.1 Reagents and Standards*

90 All-*trans*-lycopene standard and all reagents were purchased from Sigma Chemical Co.
91 (St. Louis, MO). Solvents were purchased from J.T. Baker (Phillipsburg, NJ). Tomato peels
92 were generously donated by a Red Gold Co. (Elwood, IN). To prevent light-induced degradation
93 of lycopene, all extractions were done in yellow light and extraction solvents contained butylated
94 hydroxytoluene (BHT) to limit oxidation occurring during the centrifugation and handling of the
95 extracts.

96

97 *2.2 Raw Materials and Sample Preparation*

98 Tomato peels were obtained from a local processing facility as a byproduct of tomato
99 paste. During the tomato processing, caustic lye was used to remove peels. Consequentially,
100 received tomato peels were collected in bulk and neutralized with hydrochloric acid until a pH of
101 7 was obtained. Excess moisture was removed by squeezing peels with a cheesecloth prior to
102 storage. All samples were flushed with nitrogen and stored at -20°C until further processing.
103 Since smaller particle sizes better facilitate extraction, the peels were further processed
104 prior to microwave treatment. Frozen peels were ground using a spice grinder until a particle
105 size of < 0.5 cm was achieved. The moisture content of the ground peels was analyzed with a
106 MAX2000 Computrac Moisture Analyzer (Arizona Instruments, Chandler, AZ USA). Ideally,
107 the moisture content of each sample should be quantified, however, due to the destructive nature
108 of moisture analysis, the frozen supply of ground tomato peels were sampled from ten different
109 locations within the sample stock. The mean value (70.345 ± 1.405) was later used to calculate
110 the extraction yield of lycopene per weight of tomato peel on a dry weight basis. Although using
111 the mean moisture content is not the best way to express the data, the variability between
112 sampled portions was low (<2%).

Peels were not dried as the water present increased polarity, which could aid in selective heating during microwave irradiation. Ground peels were stored in glass, screw top bottles, flushed with nitrogen, and stored at -20°C until treated.

2.3 Experimental Design

Response surface methodology (RSM) was used to determine the effect of extraction parameters on lycopene yield. Initially, RSM was conducted to assess four factors, solvent ratio (X_1), solid-liquid ratio (X_2), microwave power (X_3), and energy equivalents (X_4), which were varied by adjusting treatment time, with a Box-Behnken design comprising of 3 center points (Table 1). A secondary RSM was employed to investigate solvents containing a higher ethyl acetate (EA) percentage. For this only two factors, solvent ratio (X_1) and microwave power (X_2), were studied with a central composite design (CCD) with two center points (Table 1). A second-order polynomial equation (Eq. 1) was used to express the response yield of all-*trans*-lycopene and *cis*-lycopene (Y_i) as a function of the experimental factors (X_i) for each RSM design:

$$Y_i = b_0 + \sum_{i=1}^{n} b_n x_n + \sum_{i=1}^{n} b_{mn} x_n{}^2 + \sum_{i=1}^{n} b_{nm} x_n x_m \quad (1)$$

where b_0 is a constant, b_n, b_{mn}, and b_{nm} are the linear, quadratic, and interaction coefficients, respectively. The multiple regression models were analyzed separately for each Y_i, such that one response was a function of four (low EA) or two (high EA) independent variables. The model was predicted using regression analysis and analysis of variance (ANOVA).

2.4 Microwave-assisted Extraction of Lycopene

Ground tomato peels were thawed to room temperature and weighed into teflon-lined extraction vessels at 1, 2, or 4 g. Precisely 20 mL of corresponding solvent was added with a magnetic stir bar prior to capping. A Mars Xpress microwave extraction system (CEM Corp., Matthews, NC) was used at 400, 800, and 1600W at varying times to achieve delivered energy

equivalents of 24, 36, and 48 kJ. Within the closed microwave system, 8 extraction vessels were arranged in a carousel following CEM Corp. protocol. Although 8 vessels were irradiated, only three vessels were sampled and analyzed as the triplicates per treatment.

Approximately 10 mL of saturated sodium chloride in water was added to the treated samples to facilitate partitioning and to break emulsions formed at the interface. This was then transferred to a 50 mL polypropylene tube and centrifuged in a 5804 centrifuge (Eppendorf, Hamburg, Germany) at 4,472*g*. The organic phase was collected and centrifugation was repeated with additional solvent two more times to ensure collection of all extracted lycopene. All organic phases were pooled, filtered through anhydrous sodium sulfate to remove residual water and adjusted to 50 mL prior to drying 2 mL aliquots under nitrogen and freezer storage, at -20°C. Although direct injection would be more efficient, hexane was removed to prevent solvent effects during analysis.

2.5 Conventional Extraction of Lycopene

Conditions used for the conventional extraction were selected to emulate the optimum conditions determined by response surface methodology. Conventional extraction was conducted with 1 g of ground tomato peels and 20 mL of a 1:1 (mL:mL) mixture of hexane (1 mg mL^{-1} BHT)-ethyl acetate in a 50 mL polycenrifuge tube. The tube was placed in a shaking water bath for 15 seconds at 45 °C, which falls within the temperature ranges observed for the optimal MAE treatment. Since conventional solvent extraction typically involves a longer heating time, another treatment was done following the same conditions, except the heat treatment was extended to 30 minutes. The conventional methods used for high EA treatment (0:1 solvent ratio, 1:20 solid-liquid ratio, 400 W, 24 kJ equivalents for 1 minute) comparison followed the same protocol, except 20 mL of ethyl acetate (1 mg mL^{-1} BHT) was used as the

161 solvent and heated for 1 minute and 30 minutes. Following heat treatment, the extraction
162 process was the same as that done for MAE after microwave irradiation.
163
164 *2.6 Quantification with High Performance Liquid Chromatography (HPLC-DAD)*
165 Carotenoid analysis was done using reversed phase HPLC-DAD based on the method
166 used by Kean, Hamaker, and Ferruzzi (2008) using an Agilent 1200 Series HPLC, equipped with
167 a diode array detector and a YMC Carotenoid S-3 C-30 column (2.0 × 150 mm, 3 μm particle
168 size). A binary mobile phase of methanol with 2% aqueous ammonium acetate (pH=4.5) and
169 ethyl acetate was used at a flow rate of 0.37 mL/min with a gradient as follows: 0% B (0
170 minutes), 80% B (6 minutes), 100% B (12 minutes), 0 % B (14 minutes). Precisely 10 μL of
171 sample was injected and lycopene was quantified at 470 nm. Peak spectra were collected within
172 the 200-600 nm range and analyzed with Chemstation software (Agilent Technologies, Santa
173 Clara, CA).
174 Chromatograms of all-*trans*-lycopene standard yielded a peak at a retention time of ~11
175 minutes. Three *cis*-isomers of lycopene were separated and all-*trans*-lycopene and isomers were
176 identified by comparing retention times with carotenoid profiles of a test salad containing known
177 carotenoids (Goltz, Campbell, Chitchumroonchokchai, Failla, & Ferruzzi, 2012) to rule out
178 extraction of non-lycopene carotenoids. Since only *trans*-lycopene is readily available as a
179 standard, *cis*-isomers were collectively quantified from the calibration curve of the all-*trans*-
180 lycopene. The 5-*cis*-isomer, which was observed as a pronounced shouldering peak off of *trans*-
181 lycopene, was quantified along with the other *cis*-isomers.

182 For calibration, a small amount of all-*trans*-lycopene standard was solubilized in
183 petroleum ether to make a stock lycopene solution with an absorbance ~0.8. The absorbance of
184 this solution and subsequent dilutions were read using a UV-Vis DU 800 spectrophotometer
185 (Beckman and Coulter, Inc., Brea, CA) at 470 nm. The stock solution was diluted to

concentrations between 6.0-0.04 μM. The absorbance, done in triplicate, was then used to calculate the concentration of the stock and six dilutions with a molar extinction coefficient of 1.85 x 10^5 L*mol^{-1}*cm^{-1}. Each lycopene dilution was dried under nitrogen and analyzed with HPLC-DAD to correlate peak area with lycopene concentration. The coefficient of determination (R^2) of the calibration curve was 0.999, with a limit of detection (LOD) and limit of quantitation (LOQ) of 0.31 and 0.94 μM, respectively. The LOD and LOQ were calculated based off of the standard deviation of the intercept and slope, based on Validation of Analytical Procedures Methodology Q2B ICHHT (2005).

2.7 Electron Microscopy Imaging

Transmission electron microscopy (TEM) was used to assess the effect of treatment on tomato peel structure. A non-extracted ground tomato peel sample, optimally treated samples (low EA) and 30-minute conventionally extracted samples were imaged by the Purdue Life Science Microscopy Facility (West Lafayette, IN). Processed tomato peels were received in acetone, transferred to fresh acetone containing 0.01 g mL^{-1} osmium tetroxide. After several rinses in fresh acetone they were embedded in EMbed-812 resin. Thin sections were cut on a Reichert-Jung Ultracut E ultramicrotome and stained with 0.02 g mL^{-1} uranyl acetate and lead citrate. Images were acquired on a FEI Tecnai T20 electron microscope equipped with a LaB_6 source and operating at 200 kV.

Since the ground tomato peels used for this experiment were previously processed, fresh tomatoes were sampled from a local grocery store and used as a reference for tomato structure. These were fixed in 0.025 g mL^{-1} glutaraldehyde in 100 moles mL^{-1} sodium cacodylate buffer, post-fixed in buffered 0.01 g mL^{-1} osmium tetroxide containing 0.008 mg mL^{-1} potassium ferricyanide, dehydrated with a graded series of ethanol, and embedded in EMbed-812 resin.

Thin sections were cut stained, and visualized following the same protocol as done for the ground tomato peels.

2.8 Statistical Analysis

Statistical analysis was conducted with JMP version 10 (SAS Institute Inc. 2012 Cary, NC). Data was subjected to analysis of variance (ANOVA) where factors and values were considered significant at $P<0.05$. Pairwise comparisons between control and optimized extraction yields were conducted post-hoc using the Tukey-Kramer method ($\alpha=0.05$). Lycopene content was expressed as mg/100g dry weight and each data point is represented by the mean values and standard deviation of three independent extractions.

3. Results and discussion

3.1 Lycopene Recovery of low EA extractions

In all MAE extractions, the primary compound was all-*trans*-lycopene (Figure 1). Only two parameter estimates, interaction effects solvent type*power and power*energy were significant at the $\alpha=0.05$ level, while the solid-liquid ratio did not appear to significantly ($P=0.330$) affect the all-*trans*-lycopene extraction yield. Based on the RSM the maximum predicted extraction yield of all-*trans*-lycopene was determined to be 10.362 mg/100g (Figure 2) with a solvent ratio of 1:1 treated for 15 seconds (24 kJ equivalents) at 1600W.

Statistical analysis of *cis*-isomer extraction yield indicated that the solid-liquid ratio and the interaction effect of solvent ratio*solid-liquid ratio were significant. This suggests that *cis*-isomer yields are increased as the solid-liquid ratio decreases and the EA proportion increases (Figure 3). In most cases (treatments 4 vs. 22, 3 vs. 23, 5 vs. 25, and 8 vs. 24), solvent ratio with a higher proportion of EA was shown to increase the *% cis* yield (Table 2). No parameters were found to be significant for affecting total lycopene yield.

234 Comparison between lycopene yields of the predicted optimized MAE treatment (1:1
235 solvent ratio, 1:0 solid-liquid ratio, 1600 W, 24 kJ equivalents done for 15 seconds) vs.
236 conventional treatment conducted at the same time and temperature in a shaking water bath
237 indicated that MAE exhibited a significantly greater all-*trans*-lycopene yield compared to the 15-
238 second but not the 30-minute conventional extraction (Figure 4). However, no differences were
239 found between *cis*-isomer and total lycopene yield.
240 It should be pointed out that when the predicted optimal conditions were actually tested,
241 the all-*trans*-lycopene yield obtained (Figure 4) was less than the value predicted by the model
242 (Figure 2). Statistical analysis indicated that the model for all-*trans*-lycopene had a significant
243 lack of fit with *P*-value=0.0143 and a low coefficient of determination (R^2=0.58). Treatment 11
244 (1:1 solvent ratio, 2:20 solid–liquid ratio, 1600 W, 36 kJ equivalents with a 30 second treatment)
245 had the greatest all-*trans*-lycopene yield at 9.279 $\pm$ 0.864 mg/100 g (Table 9). This may be due
246 to the need for additional energy, more than 24kJ, to extract lycopene at the given solvent and
247 power level. A statistical drawback of the Box-Behnken design is that over interpretation due to
248 extrapolation towards the corners of the response surface can occur. Thus, a second RSM
249 experiment (high EA) using a CCD was conducted focusing on only two factors.
250
251 *3.2 Lycopene Recovery of high EA extractions*
252 Since RSM demonstrated increasing all-*trans*-lycopene yields with increasing EA
253 concentrations (Figure 2), this second set of experiments employed solvent mixtures with lower
254 hexane-to-EA ratios (2:8, 1:9, 0:1) and fixed all treatments at 24 kJ equivalents with 400 W (1
255 minute), 800 W (30 seconds), or 1600 W (15 seconds). Solid-liquid ratio (1:20) was fixed
256 because a limited supply of sample was available. Surface plots also indicated that adjustments
257 in power and energy could improve yields, however, the Mars Xpress microwave extraction
258 system has only three power settings, 400, 800, and 1600 W, thus preventing the ability to

increase or decrease power. Energy was also limited as preliminary testing demonstrated that high-energy inputs caused solvent evaporation, which would effectively shut down the system for safety reasons. Since a higher proportion of EA increases the polarity of the solvent and the rate of heating, a fixed low energy equivalent (24 kJ) was chosen for the high EA experiments.

ANOVA of high EA MAE indicated that there was a significant difference amongst treatments(P=0.0164) for all-*trans*-lycopene extraction. In this case, only solvent ratio was found to be a significant factor influencing the extraction yield of all-*trans*-lycopene. The model did not exhibit a significant lack of fit (P=0.1624), and predicted a maximum yield of 13.872 mg/100g (Figure 5) for an extraction with ethyl acetate at a power of 400 W, which for a 24 kJ equivalent had a treatment time of 1 minute. For *cis*-isomer extraction, no significant difference was found amongst treatments. However, the % *cis* of the extracts was greatest for treatments 1 and 9, which were the only ones using a solvent ratio of 2:8 (Table 3).

The actual optimal all-*trans*-lycopene yield was determined as 13.592 mg/100 g (Figure 6), which was statistically greater than the 1 minute (P=0.0006) and 30-minute conventional extraction (P<0.0001). Similarly, the total lycopene yield was significantly greater for the optimized MAE treatment compared to the 30-minute control (P<0.0001) and conventional control (P=0.006). Significant differences were not observed amongst treatments for *cis*-isomer yield (P>0.05). However, the proportion of *cis*-isomers to extracted *trans*-lycopene is dramatically higher for the 30-minute control compared to the 1-minute control and the optimized MAE treatment. The relative increase in *cis*-isomers may be due to the longer treatment time.

The literature reports different lycopene recoveries depending on the extraction method and type of raw material. Enzyme assisted extraction, was found to be extremely efficient with 440 mg/100g of lycopene from tomatoes (Lavecchia & Zuorro, 2008), although the process can be costly. It should also be noted that whole tomatoes may contain more lycopene since they

have not been previously processed. Studies done on tomato peels reported yields ranging from 0.639-73.40 mg/100 g (Kaur, Wani, Oberoi, & Sogi, 2008; Knoblich, Anderson, & Latshaw, 2005; Shi et al., 2009). Kaur et al. (2008) found that a maximum recovery of 1.98 mg lycopene/100 g was attainable when tomato skins (0.15 mm particle size) were conventionally extracted with hexane:acetone:alcohol (2:1:1 mL:mL:mL) with 0.5 mg mL^{-1} BHT at a 1:30 solid-liquid ratio (w/v), at 50°C for 8 minutes four times. Specifically, lycopene yield increased as a function of extraction number (repeated on one sample) and decreasing particle size. The authors hypothesized that the extractions conducted at 50°C allowed for better breakdown of chromoplast membranes compared to cooler conditions, yet did not induce significant degradation compared to treatments done at 60°C. Shi et al. (2009) determined a higher total lycopene content in dried tomato skins at ~13.0 mg/100g when extracted with hexane overnight at 45°C. The authors compared this to supercritical fluid extraction and achieved a maximum recovery of 73.3% when ethanol and olive oil were used as modifiers at 75°C and 35 MPa, which is less efficient compared to the results in this study. Although the results presented in this study from MAE are likely an improvement over conventional solvent extraction and over supercritical fluid extraction, lycopene yields were still significantly lower than expected. Lower yields in this study may be partially due to previous processing (i.e. hot break) that peels underwent, which lowered the amount of extractable lycopene (Kaur et al., 2008) or due to differences in tomato variety (George et al., 2004).

Tomato peels following treatments (MAE and conventional) were still visibly orange, suggesting that lycopene remained in the peel as a non-extractable fraction. Calvo, Dado, and Santa-Maria (2007) encountered a similar result when they heated freeze-dried tomato peels in ethanol or ethyl acetate at temperatures ranging from 25-60° C. Ethanol was found to have a greater lycopene extraction yield, possibly due to its ability to better penetrate the peels compared to ethyl acetate, however, residual pigment remained in the sample following

309 treatment. Attempts were made to remove all the apparent color from the tomato peels (data not
310 shown), however none of the procedures were able to completely remove pigments. A well
311 established extraction procedure involving sonication of chloroform-soaked peels (Jun, 2006;
312 Naviglio, Caruso, Iannece, Aragòn, & Santini, 2008; Rozzi, Singh, Vierling, & Watkins, 2002)
313 was tested, however, the treated peels exhibited little noticeable color following treatment and
314 yielded low lycopene concentrations. Modifications were also made to the extraction procedure
315 following methods done previously by Goltz et al. (2012) by testing different solvent types and
316 ratios, further reducing particle size with a high shear mixer, and using caustic pretreatments
317 (done at various times and temperatures) with 0.4 g mL^{-1} potassium hydroxide in methanol.
318 However, the latter resulted in the pigment loss into the aqueous phase, due to either degradation
319 of the carotenoids (exhibited by lack of color or the development of a dark color). To determine
320 absolute lycopene content in tomato peels, future studies require treatments that can effectively
321 disrupt or degrade the physical cell structure barriers without affecting embedded lycopene.
322
323 *3.3 Transmission Electron Microscopy of Tomato Peels*
324 TEM micrographs were unable to display cell ultrastructure of byproduct tomato peels,
325 possibly due to the extent of processing the peels underwent prior to receiving. However, the
326 ultrastructure with lycopene bodies can be seen in the fresh tomato peel samples as the spherical
327 electron dense (dark) regions (Figure 7a). The non-treated byproduct tomato peel (Figure 7b)
328 appeared to be significantly less damaged compared to treated peels (Figure 7c & 7d). In
329 particular, the MAE (1:1 solvent ratio, 1:20 solid-liquid ratio, 1600 W, 24 kJ, for 15 seconds)
330 treated samples exhibited significantly more structural disruption as fissures and gray wholes
331 appeared to be more prevalent. This suggests that the MAE was better able to disrupt cellular
332 structure to reduce physical extraction barriers.
333 **4. Conclusions**

Optimization data indicated that solvent ratio and microwave power in relation to energy equivalents significantly affected the all-*trans*-lycopene extraction yield. *Cis*-isomer extraction was primarily affected by the solvent ratio and solid-liquid ratio. The maximum all-*trans*-lycopene yield of ~13 mg/100 g was obtained with ethyl acetate at 400 W, with a 24 kJ equivalent (1 minute). Significantly more all-*trans*-lycopene was extracted with ethyl acetate via MAE compared to a 1-minute and 30-minute conventional treatment. TEM suggested that selective, physical disruption occurs in the tomato peels during MAE. All-*trans*-lycopene has been of interest for food and pharmaceutical industries since it is the most stable isomer. Additionally, all-*trans*-lycopene exhibits greater color intensity compared to *cis*-isomers due to a hypsochromic shift and smaller extinction coefficient of the latter (Schieber & Carle, 2005). However, interest in *cis*-lycopene is growing as there is some evidence indicating that these isomers are more bioavailable compared to the all-*trans* form (Boileau, Boileau, & Erdman, 2002). Although certain limitations to MAE exist (i.e. consumer preference against solvent use and challenges with scaling up) the findings of this study offer applicable information that could steer other extraction techniques towards *cis* or *trans*-isomer recovery, depending on the application.

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Table 1 with heading

Table 1 Response Surface Methodology Parameters

Factor	Low EA Experiments			High EA Experiments		
	Coded Value			Coded Value		
	-1	**0**	**1**	**-1**	**0**	**1**
Solvent ratio (mL Hexane : mL Ethyl acetate)	1:0	1.5:0.5	1:1	2:8	1:9	0:1
Solid-liquid ratio (g/mL)	1:20	2:20	4:20	N/A; Fixed at 1:20 g/mL		
Power (W)	400	800	1600	400	800	1600
Energy (kJ)	24	36	48	N/A; Fixed at 24 kJ		

Table 2 with heading

Table 2 *Cis*, *trans*, and total lycopene yields from low EA MAE

Treatment No.	Coded factors (X_1-X_4)	Lycopene Yield mg/100 g									
		***Cis* Isomers**			***% cis***	***Trans***			***% trans***	**Total**		
1	0+0-	1.429	±	0.04	15.339	7.727	±	0.251	82.943	9.316	±	0.289
2	0000	1.701	±	0.555	21.247	6.615	±	1.207	82.626	8.006	±	1.193
3	-00+	2.128	±	0.102	19.460	8.499	±	1.033	77.723	10.935	±	0.994
4	--00	0.537	±	0.363	8.894	5.501	±	1.08	91.106	6.038	±	1.399
5	-0-0	1.791	±	0.39	17.949	7.877	±	1.7	78.944	9.978	±	2.094
6	0000	1.838	±	0.428	20.696	6.73	±	0.668	75.780	8.881	±	1.088
7	00+-	1.675	±	0.328	20.269	6.278	±	0.533	75.968	8.264	±	0.359
8	-00-	1.878	±	0.223	22.128	6.295	±	0.568	74.172	8.487	±	0.774
9	0++0	1.27	±	0.026	16.920	6.079	±	1.451	80.989	7.506	±	1.479
10	0000	2.039	±	0.05	23.442	6.339	±	1.474	72.879	8.698	±	1.445
11	+0+0	2.38	±	0.031	19.883	9.279	±	0.864	77.519	11.97	±	0.898
12	0-0-	3.012	±	1.652	27.253	7.397	±	0.67	66.929	11.052	±	2.279
13	00-+	2.065	±	0.197	21.907	7.041	±	0.743	74.698	9.426	±	0.632
14	0+-0	1.178	±	0.177	18.134	5.16	±	0.468	79.433	6.496	±	0.537
15	0-0+	3.946	±	0.245	34.111	6.999	±	1.45	60.503	11.568	±	1.608
16	0-+0	1.676	±	1.747	22.881	5.043	±	0.546	68.846	7.325	±	2.211
17	0+0+	1.103	±	0.19	19.546	4.383	±	0.431	77.671	5.643	±	0.459
18	0--0	2.209	±	1.38	30.269	4.473	±	0.914	61.291	7.298	±	2.289
19	-0+0	1.295	±	0.672	24.160	3.752	±	0.693	70.000	5.36	±	1.357
20	-+00	1.192	±	0.037	21.214	4.268	±	0.198	75.957	5.619	±	0.222
21	00++	0.873	±	0.069	27.758	2.802	±	0.525	89.094	3.145	±	0.631
22	+-00	4.174	±	0.495	34.160	7.435	±	0.276	60.848	12.219	±	0.266
23	+00+	1.721	±	0.152	23.057	5.43	±	1.031	72.749	7.464	±	1.131
24	+00-	1.546	±	0.097	19.513	6.064	±	1.509	76.537	7.923	±	1.608
25	+0-0	1.677	±	0.506	20.137	6.344	±	0.84	76.177	8.328	±	1.155
26	++00	1.009	±	0.069	20.100	3.855	±	0.093	76.793	5.02	±	0.157
27	00--	1.391	±	0.454	26.225	3.594	±	0.097	67.760	5.304	±	0.451

* Lycopene yields represent means ± SD (n=3)

Table 3 with heading

Table 3 *Cis*, *trans*, and total lycopene yields from high EA MAE

Treatment No.	Coded factors (X_1-X_4)	Lycopene Yield mg/100 g										
		***Cis* Isomers**			***% cis***	***Trans***			***% trans***	**Total**		
1	--	3.909	±	0.243	43.991	4.633	±	1.944	52.138	8.886	±	1.817
2	00	3.857	±	2.336	39.490	5.562	±	2.956	56.947	9.767	±	0.621
3	+-	3.44	±	0.636	19.799	12.195	±	0.884	70.187	17.375	±	0.253
4	-0	3.409	±	0.436	35.378	5.891	±	0.41	61.135	9.636	±	0.851
5	0-	3.288	±	0.898	25.980	9.028	±	0.178	71.334	12.656	±	0.867
6	+0	2.624	±	0.295	17.344	12.175	±	1.611	80.475	15.129	±	1.892
7	0+	2.263	±	0.366	22.493	7.454	±	0.346	74.088	10.061	±	0.379
8	00	2.824	±	0.998	30.799	6.002	±	0.857	65.460	9.169	±	0.205
9	-+	3.753	±	0.082	42.287	4.788	±	0.461	53.949	8.875	±	0.42
10	++	3.577	±	0.806	24.470	10.711	±	0.671	73.273	14.618	±	1.471

* Lycopene yields represent means ± SD (n=3)

Figure 1

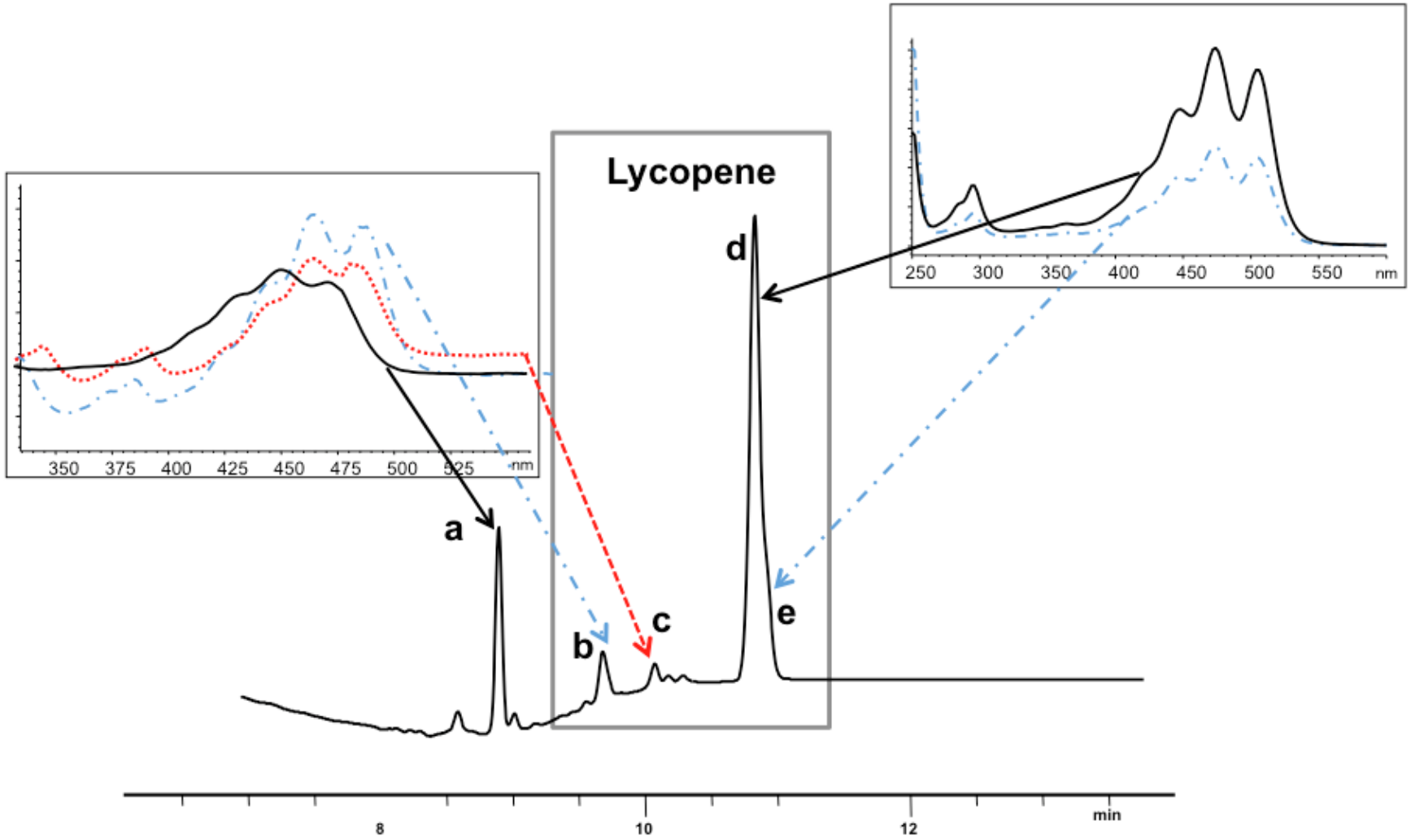
Lycopene
a
b
c
d
e
350 375 400 425 450 475 500 525 nm
250 300 350 400 450 500 550 nm
8
10
12
min

Figure 2

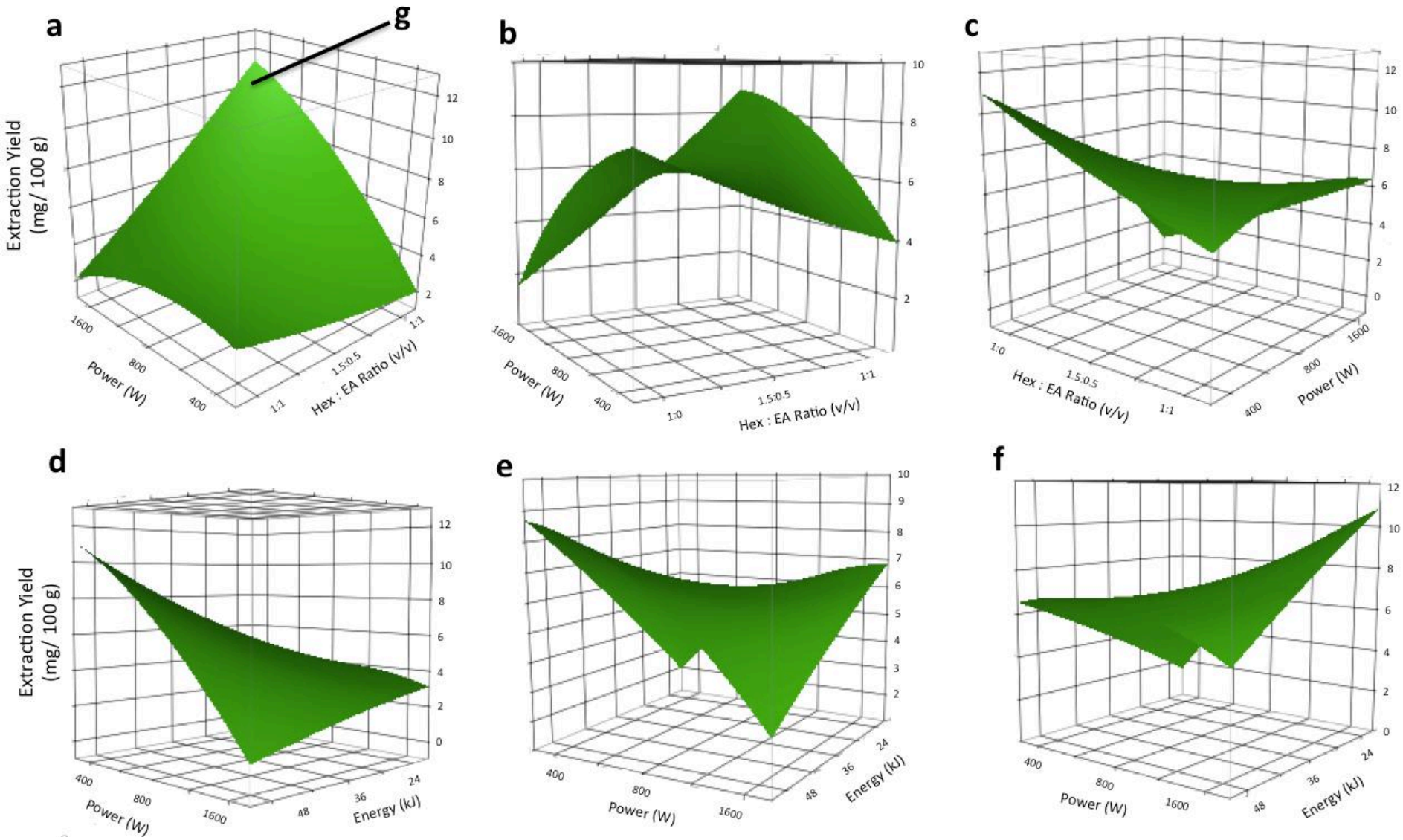
a
g
Extraction Yield (mg/ 100 g)
Power (W)
Hex : EA Ratio (v/v)
b
c
d
Energy (kJ)
e
f

Figure 2 (Black and White)

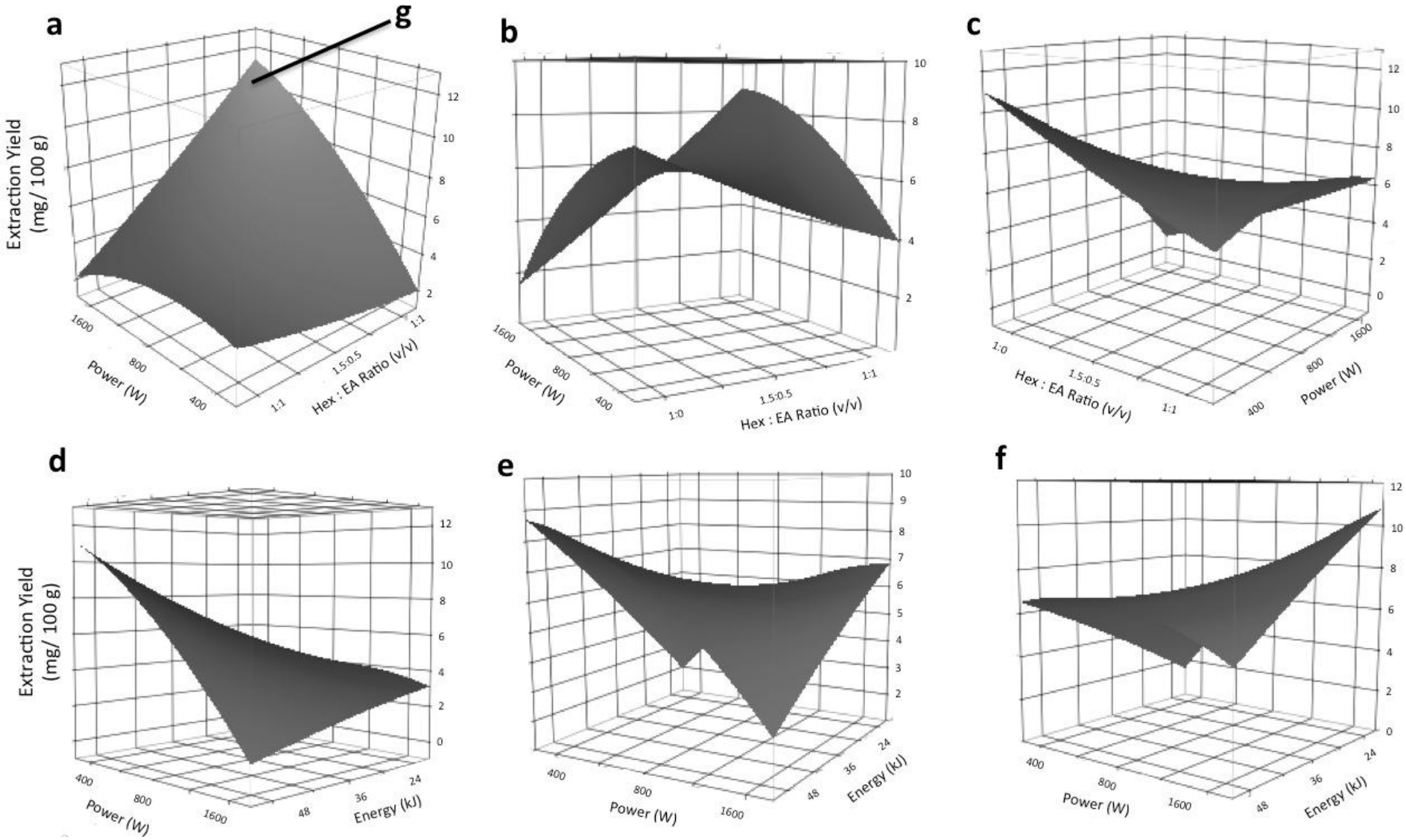

Figure 3

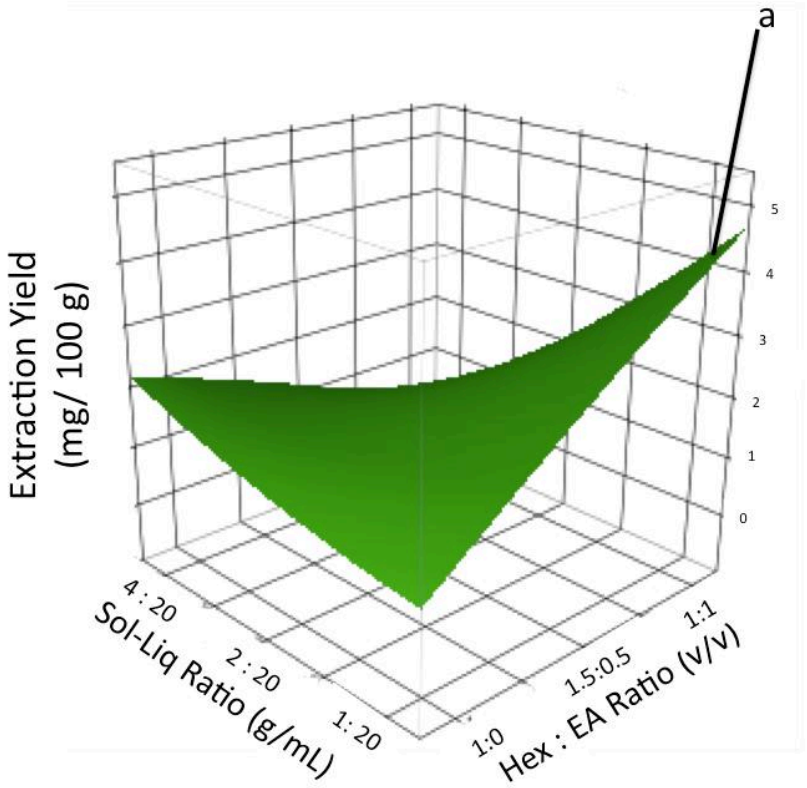
a
Extraction Yield
(mg/ 100 g)
5
4
3
2
1
0
4 : 20
2 : 20
1: 20
Sol-Liq Ratio (g/mL)
1:0
1.5:0.5
1:1
Hex : EA Ratio (v/v)

Figure 3 (Black and White)

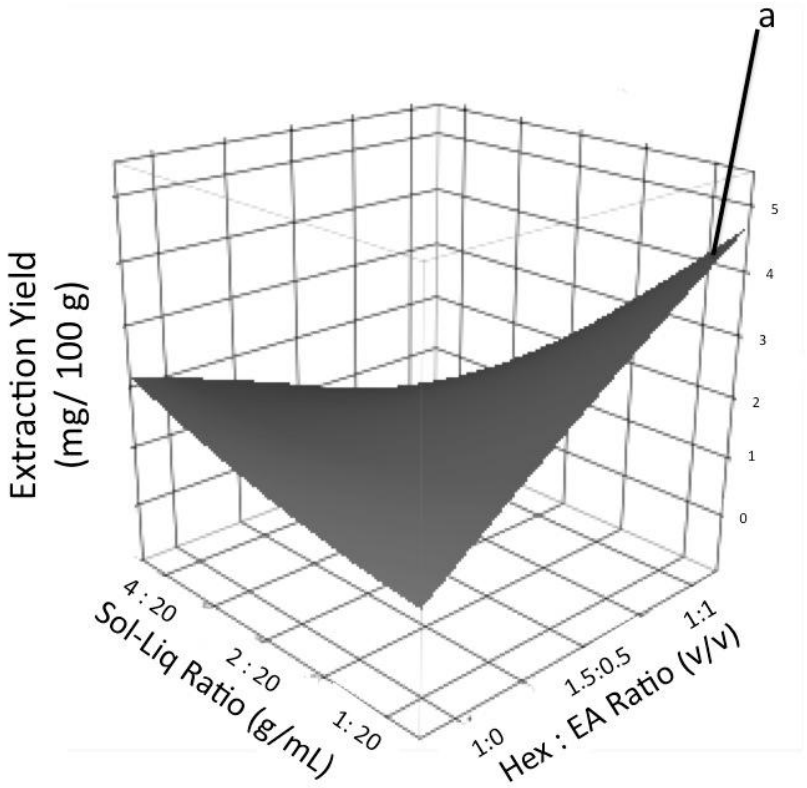

Figure 4

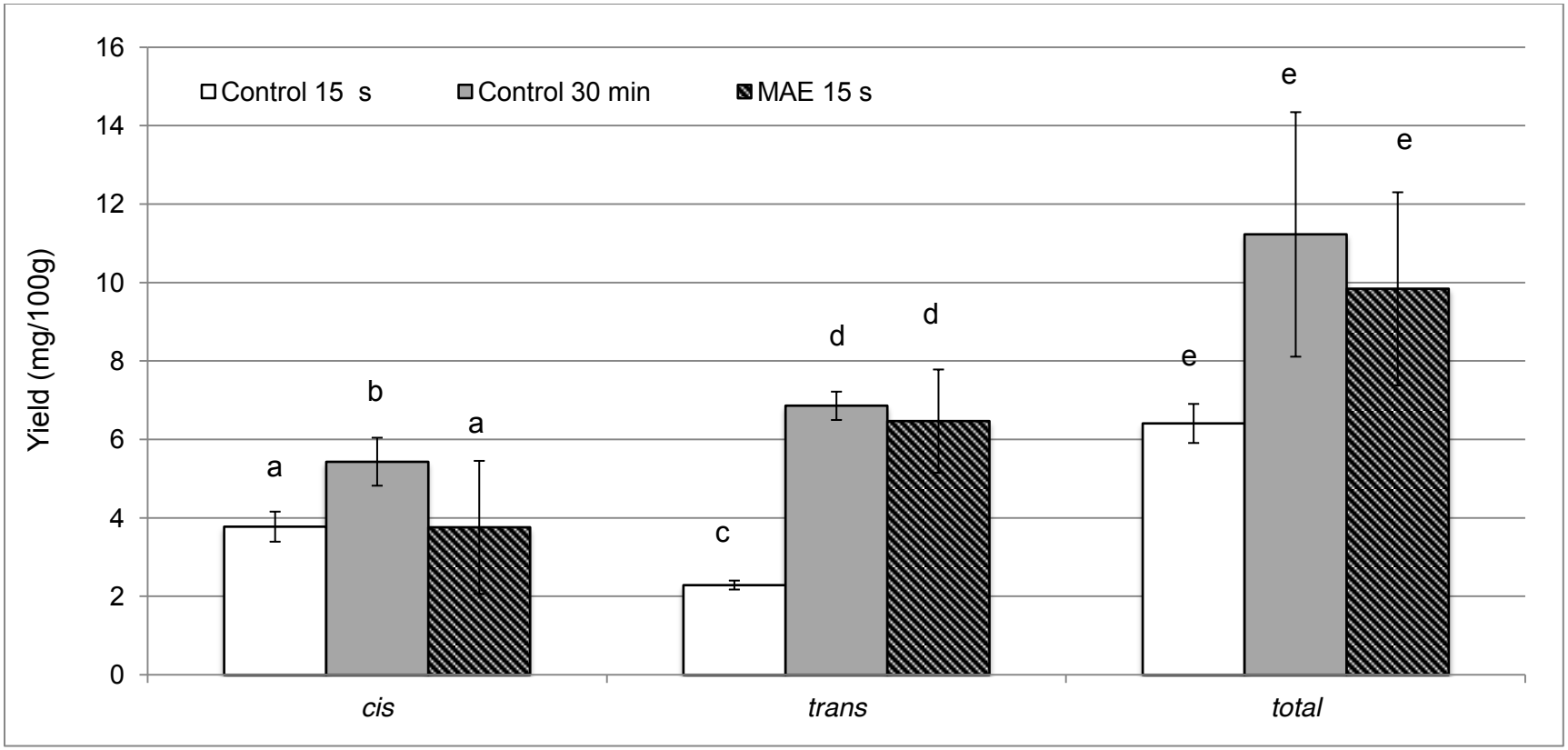
Control 15 s
Control 30 min
MAE 15 s
Yield (mg/100g)
16
14
12
10
8
6
4
2
0
a
b
a
c
d
d
e
e
e
cis
trans
total

Figure 5

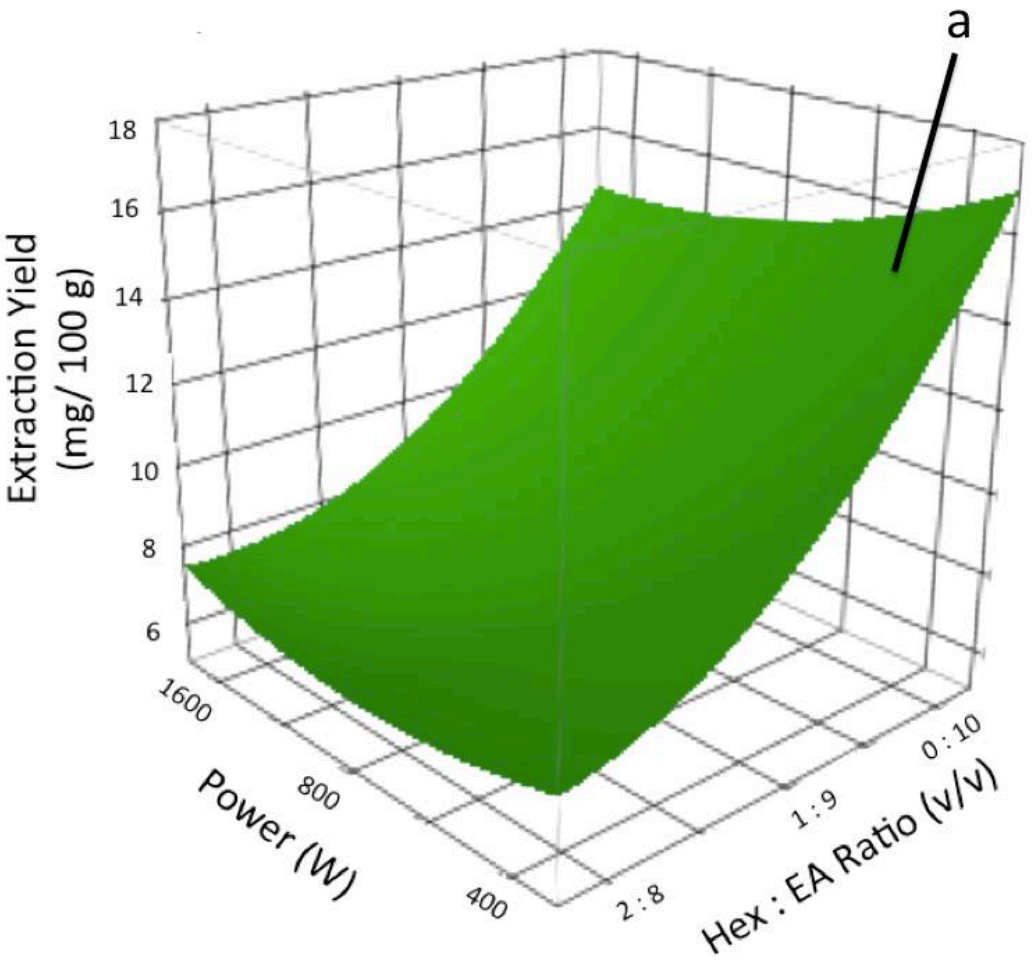
a
Extraction Yield
(mg/ 100 g)
18
16
14
12
10
8
6
1600
800
400
Power (W)
2 : 8
1 : 9
0 : 10
Hex : EA Ratio (v/v)

Figure 5 (Black and White)

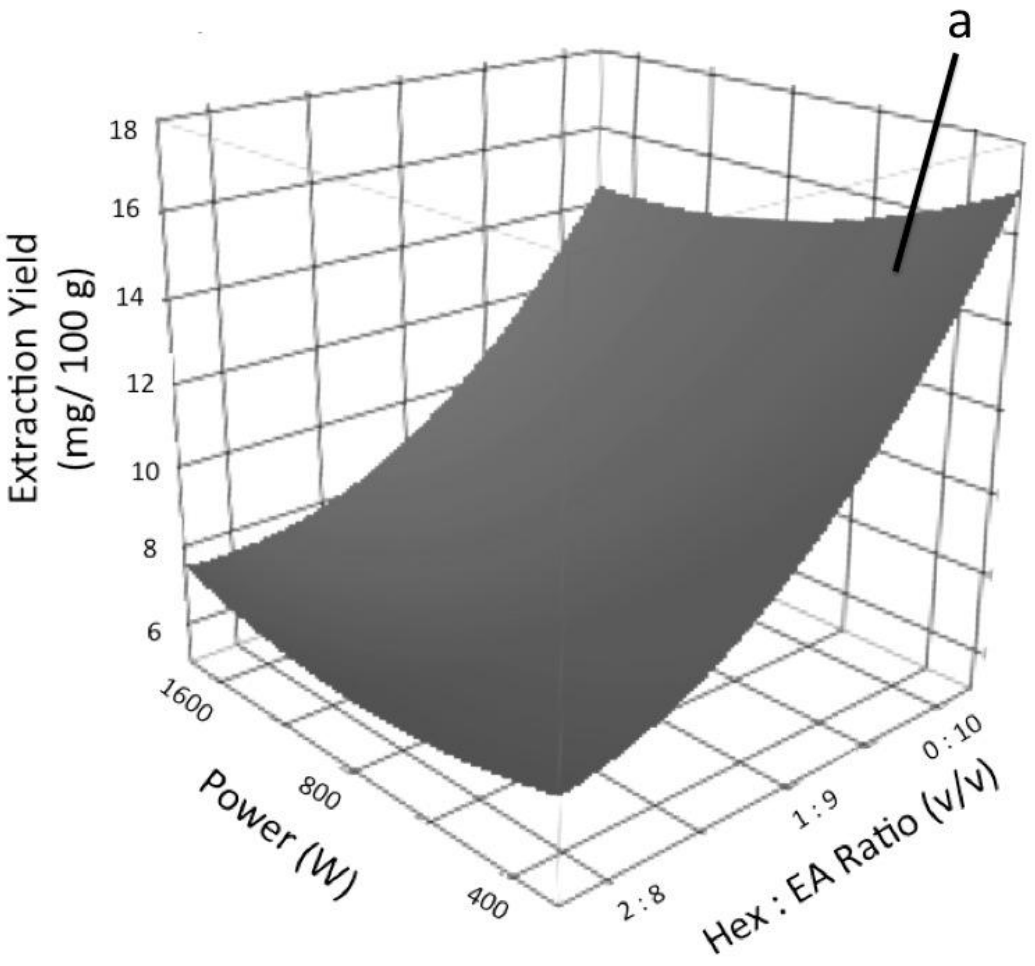

Figure 6

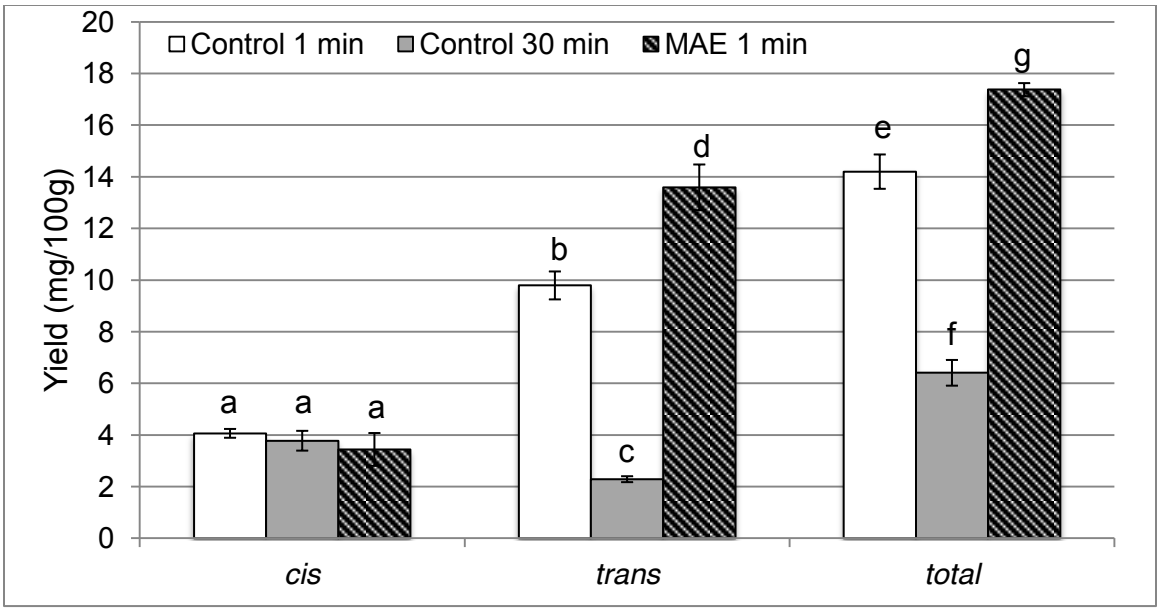
Control 1 min
Control 30 min
MAE 1 min
Yield (mg/100g)
20
18
16
14
12
10
8
6
4
2
0
a
a
a
b
c
d
e
f
g
cis
trans
total

Figure 7

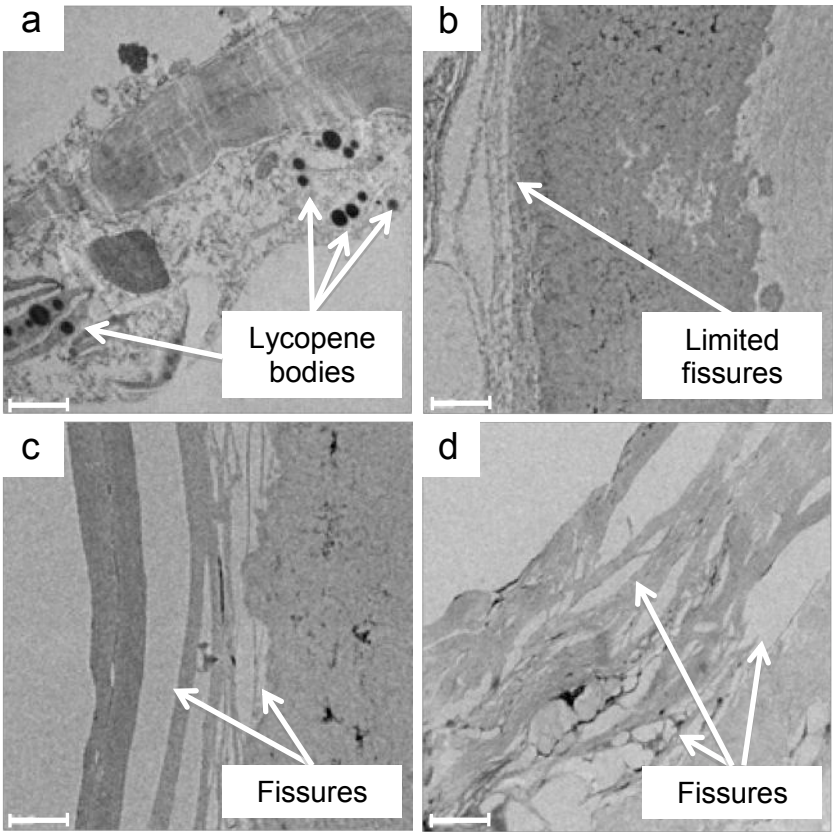

Figure Captions

Figure 1. Representative chromatogram of carotenoid extract from MAE of tomato peels at 470 nm. Suspected peak identies are as follow: (a) β-carotene, (b) *cis*-lycopene isomer, (c) *cis*-lycopene isomer, (d) all-*trans*-lycopene, (e) 5-*cis*-lycopene.

Figure 2. Response surface plots for all-*trans*-lycopene yield from low EA MAE with solvent ratio vs. power (top row) and energy vs. power (bottom row) plotted. Power levels are fixed at (a) 24kJ, (b) 36 kJ, and (c) 48 kJ and solvent ratios are fixed at (d) 1:0 hexane:EA, (e) 1.5:0.5 hexane:EA, and (f) 1:1 mL hexane : mL EA solvent ratio. The maximum predicted extraction yield was (g) 10.362 mg/100g with a treatment comprising of: 1:1 mL hexane : mL EA solvent ratio, 1600 W, 24 kJ. Plotted response values represent predicted values from the model.

Figure 3. Response surface plot for *cis*-lycopene yield from low EA MAE. The maximum *cis*-isomer extraction yield was predicted to be (a) 4.450 mg/100g with a solvent ratio of 1:1 mL hexane: mL EA and a 1:20 solid-liquid ratio.

Figure 4. Comparison of control (conventional) methods vs. optimized low EA MAE. The MAE conditions used (1:1 solvent ratio, 1:20 solid-liquid ratio, 1600 W, 24 kJ equivalents for 15 seconds) were determined as optimal by RSM. Extraction yields of *cis, trans,* and total lycopene are shown where same letters denote values that are not significantly different at the α=0.05 level based on the Tukey Kramer method for pairwise comparisons. Response values shown represent the mean $\pm$ SD (*n*=3).

Figure 5. Response surface plot for all-*trans*-lycopene yield from high EA MAE. The maximum all-*trans*-extraction yield was predicted to be (a) 13.872 mg/100g with a full EA solvent and when treated at 400 W. Solvent ratio significantly affected the extraction yield (*P=0.004)* while power did not (*P=0.210*). Plotted response values indicate mean $\pm$ SD (n=3).

Figure 6. Comparison of control (conventional) methods vs. the high EA MAE treatment with the highest all-*trans*-lycopene yield. The MAE conditions used (0:1 solvent ratio, 1:20 solid-liquid ratio, 400 W, 24 kJ equivalents for 60 seconds) were determined as optimal by RSM. Extraction yields of *cis, trans,* and total lycopene are shown where same letters denote values that are not significantly different at the α=0.05 level based on the Tukey Kramer method for pairwise comparisons. Response values shown represent the mean $\pm$ SD (*n*=3).

Figure 7. TEM images of tomato peels following (a) fresh tomato peel with no extraction, (b) byproduct tomato peel with no extraction, c) control extraction for 30 minutes, and (d) MAE (1:1 solvent ratio, 1:20 solid-liquid ratio, 1600 W, 24 kJ, for 15 seconds). Visibly more holes and fissures are present in extracted samples, thus suggesting that MAE, and to some extent conventional extraction, cause structural disruption. Scale bars indicate 1 μm.