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

K.K.H.Y. Ho, M.G. Ferruzzi, A.M. Liceaga, M.F. San Martín-González*

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

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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.

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

* Corresponding author. Tel.: +1 765 496 1140; fax: +1 765 494 7953.
E-mail address: fsanmartin@purdue.edu (M.F. San Martín-González).

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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 h, 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

2.1. Reagents and standards

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

2.2. Raw materials and sample preparation

Tomato peels were obtained from a local processing facility as a byproduct of tomato paste. During the tomato processing, caustic lye was used to remove peels. Consequentially, received tomato peels were collected in bulk and neutralized with hydrochloric acid until a pH of 7 was obtained. Excess moisture was removed by squeezing peels with a cheesecloth prior to storage. All samples were flushed with nitrogen and stored at −20 °C until further processing.

Since smaller particle sizes better facilitate extraction, the peels were further processed prior to microwave treatment. Frozen peels were ground using a spice grinder until a particle size of <0.5 cm was achieved. The moisture content of the ground peels was analyzed with a MAX2000 Computrac Moisture Analyzer (Arizona Instruments, Chandler, AZ USA). Ideally, the moisture content of each sample should be quantified, however, due to the destructive nature of moisture analysis, the frozen supply of ground tomato peels were sampled from ten different locations within the sample stock. The mean value (70.345 ± 1.405) was later used to calculate the extraction yield of lycopene per weight of tomato peel on a dry weight basis. Although using the mean moisture content is not the best way to express the data, the variability between 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 1600 W 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

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		