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Optimization of supercritical fluid extraction of lycopene from tomato skin with central composite rotatable design model

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Abstract

Response surface methodology using the central composite rotatetable design (CCRD) model was used to optimize parameters for supercritical carbon dioxide extraction of lycopene ($C_{40}H_{56}$) from dried tomato skin. The CCRD consisting of three-factored factorial design with two levels was used in this study. The factors used were temperature of the extraction chamber (40 and 70 °C), pressure of the extraction fluid (25 and 45 MPa), and modifier concentration (5 and 15%). Judged by the lack-of-fit-test, coefficient of determination and the standard errors results from the analysis of variance have shown the model to be adequate. The linear, quadratic and cross-effects were 0.58, 0.28 and 0.05, respectively. The independent variables have significantly ($p < 0.05$) influenced the extraction of all *trans*-lycopene from tomato skin. Although, no significant ($p > 0.05$) individual effect of modifier concentration shown, a synergetic effect was observed. A second-degree polynomial equation was developed from a response surface analysis for all *trans*-lycopene yield and the highest yield was predicted at 62 °C, 45 MPa (450 bar) and 14% temperature, pressure and modifier concentration, respectively and the recovery of all *trans*-lycopene was 33%.

Keywords: Supercritical carbon dioxide; CCRD; Optimization; Extraction; Lycopene; Tomato

1. Introduction

The industrial processing of tomato products produces lots of wastes, even though it is a potential source for carotenoid (lycopene, α-, β-, γ-, ξ-carotene and also present in miniscule are phytoene, phytofluene, neurosporene and lutein). Ripe tomatoes are the most abundant source of lycopene with over 90% concentrated in the skin, which constitutes the greater part of the waste [1,2]. Lycopene a hydrocarbon by classification is apolar an acyclic aligomer of isoprenoid type of bioactive natural pigment with eight isoprene linked by chains of 13 double bond (11 conjugated and 2 non-conjugated) molecular structure [3]. Although, this carotenoid is more stable than other pigments like chlorophyll and anthocyanins but are thermolytic at high temperatures and also degrades when exposed to oxygen as well [2].

Epidemiological studies have shown the benefits of lycopene rich diets, its effect on minimizing the risk of cardiovascular ailments and different forms of cancers [4,5]. The question, whether the amount of carotenoid consumed directly in daily rations has any significant effects on the prevention of these ailments is yet to be answered [6]. However, the quantification of lycopene in food products and development of a safe extraction process to complement the fortification of functional foods is of public interest. Conventional organic solvent extraction process is nearly perfected, but consumers are wary of its safety in food products, since they had been proven carcinogenic when ingested.

Many researchers have successfully extracted lycopene from tomato by supercritical carbon dioxide (SC-CO_2) extraction, and have established the environmental soundness of the technology and complimentary safe solvent for extracting food grade bioactive components from agricultural products used as ingredients. One significant thermodynamic advantage of using supercritical fluid is its ease of separation from the extracted solutes by simply modifying the operation conditions either pressure or temperature. The supercritical fluids have liquid-like densities that give superior mass transfer characteristics compared to organic solvents and characterized as low-viscosity and high-diffusivity fluid. The low surface tension eases penetration of

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supercritical fluids into the porous biological matrix while releasing solutes [7].

Different independent variables such as pressure, temperature, flow rates and co-solvent concentration on lycopene yield using SC-CO_2 extraction process have been studied by many researchers. Baysal et al. [8] observed highest yield (54%) of lycopene at 55 °C, 30 MPa and a flow rate of 4 kg/h with 5% ethanol used as a modifier. Ollanketo et al. [10] observed an optimum condition at 40 MPa, 110 °C and recovered 94% of lycopene in 15 min, however the bioactivity of the lycopene may be compromised due to heat intensity considering the instability of lycopene at higher temperatures and Pol et al. [9] made a remarkable observations that pressure was independent of lycopene yield at 90 °C. Rozzi et al. [11] obtained a maximum yield (61%) at 8 °C, 35 MPa and 2.5 mL/min. Organic solvents are used to improve the solubility of the bioactive components and solvents like acetone, methanol, hexane, etc. are more effective modifiers than ethanol as reported in literature [11], but ethanol seemed to be favoured because of its non-toxic effect, which is ideal for food products. Data on the optimization of lycopene extraction process in tomatoes is very scarce in the literature. Central composite rotatable design (CCRD) is an ideal tool for process optimization [12,13], its rotatability characteristics enables it to identify optimum responses around its center point without changing the predicting variance. Several researchers have used the CCRD statistical model to optimize processes in food processing [14,15]. Ozkal et al. [16] used the model to optimize supercritical extraction of oil from apricot and lipid extraction from roasted pistachio nuts by Palazoglu and Balaban [17]. Began et al. [18] also optimized the solubility of crude soy lecithin in SC-CO_2 using this model while Adasoglu et al. [19] optimized the extraction of essential oil from Turkish lavender flower and carotenes was optimized from carrots [20]. The optimization of lycopene in tomato would provide a valuable data for process design and pilot and industrial scale-up applications and hence the objective of this study was to optimize the SC-CO_2 extraction of lycopene using the CCRD statistical model.

2. Materials and methods

2.1. Sample preparation

Tomato skin sample was supplied by the Heinz Company of Canada LTD's tomato processing plant at Leamington, Ontario, Canada. The moisture content was 3% wet basis and contained about 30% tomato seeds. The sample was ground to fine particle sizes (Ca. 0.5–1 mm) using a coffee grinder (SmartGrind Deluxe, CBM7B electric burr grinder, Black & Decker, Mirama, FL). The ground samples were enclosed in opaque containers and placed in the refrigerator until needed. The sample that required treatment with a modifier were treated with ethanol (ethyl alcohol 95%) (Commercial Alcohol Inc., Brampton, Ontario) an hour prior to the commencement of the extraction process base on the experimental requirement as outlined in Table 2.

2.2. Supercritical fluid extraction

A supercritical extraction unit (Spe-ed SFE NP, Model 7013, Applied Separations Inc., Allentown, PA) equipped with a pump unit (Model 7103; Applied Separations Inc., Allentown, PA) was used to extract lycopene and other carotenoid. A bone-dry CO_2 gas cylinder (Praxair Product Inc., Kitchener, Ontario) was connected to the input source and cooled to 5 °C with a refrigerated bath (RB-5, Techne Cambridge Ltd., England) before pumping. The pump module is connected to a central compressed air system of the Guelph Food Research Center. The SC-CO_2 flow rate was kept constant at 3.5 LPM. The oven module houses the 10 mL extraction vessel equipped with an automatic temperature control. Each time 1.2 g sample was placed in the extraction vessel sandwiched with defatted glass wood pieces forming a fixed bed in the vessel. Once the vessel was assembled in place the system was charged with SC-CO_2 at different alternative operation condition as shown by the experimental design as outlined in Table 2. The static and dynamic extraction period were kept constant for treatments at 20 and 10 min, respectively. The extract (oleoresin) was collected through the control and collection module into a sample vial (20 mm autosampler vials, Fisher Scientific Ltd., Nepean, Ontario), the temperature of which was controlled at 100 °C. The collection vials are wrapped in aluminium foil and stored in a freezer at −20 °C prior to quantitative analysis.

2.3. Chemical extraction

Chemical extraction of lycopene from tomato skin was also conducted to serve as a reference for the percent total recovery from the samples. One gram of sample was placed in an extraction tube and 30 mL of chloroform was added and placed in a temperature (45 °C) controlled shaker overnight and subsequently sonicated for 30 min. The sample was centrifuged for 20 min at 3220 × g. The aliquot was decanted for HPLC analysis of lycopene content. To recover the residual lycopene the procedure was repeated by the addition of 10 mL of chloroform, sonicate and proceeded as outlined above.

2.4. Sample preparation for HPLC analysis

Lycopene contents were quantified by a high-pressure liquid chromatography (HPLC) (Agilent 1100 Series, Agilent, Germany), equipped with an auto injector and UV photo diode array detector, which was set at 475 nm. Fig. 1 shows a visible absorption spectrum of the extract in hexane. Three maxima wavelengths (λ = 446, 475 and 500 nm) were noted for both the SC-CO_2 extract and the lycopene standard, therefore, judging by the spectrum in Fig. 1, the wavelength used in this experiment is adequate.

An alphaBond C_{18}, 10 μm, 125 A, 3.9 mm × 300 mm column (Alltech, Deerfield, IL) was used for the separation and quantitation of all *trans*-lycopene from the oleoresin. A gradient elution method was developed with a mobile phase of acetone and water ratio of 75:25 and 90:5 for 10 and 20 min, respectively, and a flow rate of 1 mL/min. A 50 μL injection