Food Chemistry 188 (2015) 84–91

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Optimization of extraction of high purity all-*trans*-lycopene from tomato pulp waste

Mahesha M. Poojary, Paolo Passamonti *

University of Camerino, School of Science & Technology, Section Chemistry, Via S. Agostino 1, 62032 Camerino, Italy

ARTICLE INFO

Article history:
Received 19 January 2015
Received in revised form 22 April 2015
Accepted 28 April 2015
Available online 29 April 2015

Chemical compound studied in this article:
Lycopene (PubChem CID: 446925)

Keywords:
Lycopene extraction
All-*trans*-lycopene
Tomato processing waste
Optimization
Factorial design

ABSTRACT

The aim of this work was to optimize the extraction of pure all-*trans*-lycopene from the pulp fractions of tomato processing waste. A full factorial design (FFD) consisting of four independent variables including extraction temperature (30–50 °C), time (1–60 min), percentage of acetone in n-hexane (25–75%, v/v) and solvent volume (10–30 ml) was used to investigate the effects of process variables on the extraction. The absolute amount of lycopene present in the pulp waste was found to be 0.038 mg/g. The optimal conditions for extraction were as follows: extraction temperature 20 °C, time 40 min, a solvent composition of 25% acetone in n-hexane (v/v) and solvent volume 40 ml. Under these conditions, the maximal recovery of lycopene was 94.7%. The HPLC–DAD analysis demonstrated that, lycopene was obtained in the all-*trans*-configuration at a very high purity grade of 98.3% while the amount of *cis*-isomers and other carotenoids were limited.

1. Introduction

Lycopene, a bright red carotenoid pigment, is found in many natural products such as tomato, watermelon, pink grapefruit and papaya, and has several applications as nutraceutical, pharmaceutical, cosmeceutical and food supplements. It is a C40 polyisoprenoid compound containing 11 conjugated double bonds, which are responsible for its deep red color and potent antioxidant property. It has a singlet-oxygen quenching ability twice as high as that of β-carotene and ten times higher than that of α-tocopherol (Agarwal & Rao, 2000; Di Mascio, Kaiser, & Sies, 1989). Several epidemiological studies suggest that lycopene may have anti-carcinogenic and anti-atherogenic effects (Omoni & Aluko, 2005). Considerable evidence suggests that it has significant role in preventing cardiovascular (Arab & Steck, 2000; Cuevas-Ramos et al., 2013) and coronary heart diseases (Clinton, 1998). It also exhibits potent anti-inflammatory activity (Rafi, Yadav, & Reyes, 2007).

In the food industry, lycopene is used as a food additive to enhance storage stability and nutritional benefits. Due to its strong color, non-toxicity and fat solubility, it is also used as a natural food colorant (Naviglio, Caruso, Iannece, Aragòn, & Santini, 2008). A number of studies have been carried out using lycopene or lycopene enriched matrices as an additive in raw meat and meat products such as mortadella, frankfurters, fresh sausages, fermented sausages, hamburgers, minced meat, beef patties (Doménech-Asensi et al., 2013) etc. These studies, in general, have shown that the presence of lycopene leads to a better color in the food products, enhanced nutritional quality, reduced lipid oxidation and increased stability during the shelf-life period (Doménech-Asensi et al., 2013).

Tomato pulp waste, a by-product obtained during the processing of tomato juice, is considered as a rich source of lycopene (Chiu et al., 2007; Wang & Chen, 2005). Tomatoes contain about 30–400 mg/kg of lycopene in pulp and about 20–30 mg/kg in peels (Naviglio, Caruso, et al., 2008). According to Sharma and Le Maguer (1996), the lycopene content present in the pulp fraction which is rich in fiber, is more (42.3 mg/100 g) compared to the water soluble fraction (4.0 mg/100 g). However, the amount in the by-products may vary depending on the variety of tomato and the industrial processing techniques. The concentration of lycopene being highest in the pericarp of tomato, it is advantageous to use it as a source of this pigment (Wang & Chen, 2005). The quantity of tomato by-products derived from industrial processes is growing annually. It has been estimated that Italy produces about 200,000 t of tomato processing waste annually, while the worldwide production is more than 1,200,000 t (Zuorro, Fidaleo, & Lavecchia, 2011). Presently, the tomato

* Corresponding author.
E-mail address: paolo.passamonti@unicam.it (P. Passamonti).

http://dx.doi.org/10.1016/j.foodchem.2015.04.133
0308-8146/

processing waste is either used as animal fodder or simply discarded, but high abundance of lycopene in such waste indicates the prospect of utilizing it as a sustainable, alternative and low-cost source of this pigment (Poojary & Passamonti, 2015).

In fresh tomatoes, *l*ycopene is found predominantly in the all-trans configuration, the most thermodynamically stable form (Singh & Goyal, 2008). The extraction and processing methods, however, cause lycopene to undergo isomerization (*trans* to *cis* forms), which in turn leads to its degradation (Shi, Le Maguer, Kakuda, Liptay, & Niekamp, 1999; Xu & Pan, 2013). Undesirable isomerization and degradation of lycopene not only affects its color, stability and nutritive properties but also its biological activity (Eh & Teoh, 2012; Shi et al., 1999; Singh & Goyal, 2008; Xu & Pan, 2013). In addition, from an industrial point of view, it is very essential to achieve pure isomers of lycopene for using them in analytical, drug or food applications. Consequently, the implementation of appropriate extraction technique and process conditions is very important to preserve purity, stability and bioactivity of lycopene.

Lycopene from tomatoes is usually extracted using organic solvents such as hexane, acetone, ethanol, chloroform, petroleum ether, tetrahydrofuran (Khachik et al., 1992; Naviglio, Caruso, et al., 2008; Sadler, Davis, & Dezman, 1990; Van Den Berg et al., 2000; Xu & Pan, 2013). A solvent mixture of hexane/acetone or hexane/acetone/ethanol solvent mixture is often used (Lin & Chen, 2003; Taungbodhitham, Jones, Wahlqvist, & Briggs, 1998) because the recovery and stability of lycopene extracted using these mixtures is superior compared to other solvents (Barba, Hurtado, Mata, Ruiz, & De Tejada, 2006; Taungbodhitham et al., 1998). In addition, some recent studies have shown that lycopene could also be extracted using various advanced methods such as ultrasound assisted extraction, ultrasound/microwave assisted extraction, supercritical fluid extraction, enzyme assisted extraction, selective inclusion in deoxycholic acid (Eh & Teoh, 2012; Fantin, Fogagnolo, Medici, & Perrone, 2007; Lianfu & Zelong, 2008; Zuknik, Nik Norulaini, & Mohd Omar, 2012; Zuorro et al., 2011) etc.

In view of enabling a cost effective production of high purity lycopene, this study was aimed at process optimization of its extraction, from tomato pulp waste by employing FFD. Emphasis has been given to enhance the productivity and selectivity of the all-*trans*-lycopene with less isomerization and degradation in extraction process and to provide insights on the mechanism of extraction of lycopene.

2. Materials and methods

2.1. Materials

Tomato pulp waste devoid of seed particles was obtained from Pezziol SPA, Parma, Italy. The water content of the sample was about 35%. Lycopene standard (⩾95%) and sodium chloride (⩾99.5%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). ACS grade acetone and n-hexane, HPLC grade methanol and acetonitrile were also purchased from Sigma–Aldrich, USA. Milli-Q ultrapure water with a resistivity of 18.3 MΩ cm was used in all the steps (Millipore, Germany).

2.2. Lycopene extraction

Lycopene extraction was carried out in a 35 ml screw-top vial, equipped with an external water circulating heating jacket connected through a thermostated water bath (±0.1 °C). 1.0 g of sample was added to the vial and agitated with a magnetic stirrer with required volume of acetone/n-hexane mixture under appropriate experimental conditions (Table 2). On completion of the extraction procedure, stirring was stopped and the extract was filtered and then washed by 10 ml of aqueous 1.0 M NaCl solution with vigorous shaking for 15 min. Later, the suspension was allowed to stand for 15 min for separation of polar and non-polar phases. The non polar phase was then taken and its absorbance was measured using a UV visible spectrophotometer (Beckman, Model: 640i, USA) at 472 and 503 nm. To minimize the interference from other carotenoids, the concentration of lycopene was calculated at 503 nm using a molar extinction coefficient of $17.2 \times 10^4\ M^{-1}\ cm^{-1}$ (Fish, Perkins-Veazie, & Collins, 2002). Lycopene yield ($Y_{mg/g}$) was expressed as mg/g fresh weight.

2.3. Total lycopene content and % recovery determination

Tomato pulp waste (1.0 g) was extracted repeatedly with 30 ml of 25% acetone in n-hexane (v/v) for 15 min each time until absorbance of the extract at 503 nm was lower than the instrumental noise (0.5 mAU). These extracts were then combined and the total lycopene content or maximum original mass of lycopene in tomato pulp waste was analyzed using UV–VIS spectrophotometry by following the procedure described above. Percentage recovery (percentage yield, $Y_{\%}$) was expressed as:

$$Y\% = \frac{\text{Amount of lycopene extracted in a single extraction step (mg/g)}}{\text{Maximum original mass of lycopene in tomato pulp waste (mg/g)}} \times 100$$

2.4. HPLC analysis

The purity of lycopene in the extracts was determined using an HPLC–DAD instrument (Agilent, Model: 1200, USA) equipped with a reversed phase Gemini C18 column (250 mm × 4.6 mm × 5 μm, Phenomenex, USA) and diode array detector (DAD). Pigment separation was carried out in gradient mode with mobile phase, composed of three solvents including acetonitrile, methanol and water at a flow rate of 1.00 ml/min, as described in our previous work (Poojary & Passamonti, 2015). The detection wavelength was 472 nm and the column temperature was maintained at 25 °C. The peak for *trans*-lycopene in the extract was identified by comparing the retention time with that of the lycopene standard, while other peaks were tentatively identified based on absorption spectrum characteristics as described in the literature (Eh & Teoh, 2012; Lin & Chen, 2003; Xu & Pan, 2013). The percentage purity of the extracted lycopene was calculated by the area normalization method.

2.5. Experimental design

A full factorial design was used to investigate the effects of four independent variables including extraction temperature (A), extraction time (B), percentage of acetone in n- hexane (C) and solvent volume (D) on the yield of lycopene ($Y_{mg/g}$ or $Y_{\%}$). The independent variables were coded at two levels as −1 and 1 (Table 1). The complete design consisted of 19 experimental points including three analyses as central point replications (coded as 0) is shown in Table 2. The 19 sets of experiment were performed in a random order in order to minimize the effects of the uncontrolled factors. A multiple regression analysis was performed on experimental data which corresponds to the following first-order equation (Chang, Tow, & Ismail, 2011):

$$Y_i = \beta_o + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j \quad (1)$$

where Y_i is the response function (lycopene yield, $Y_{mg/g}$ and $Y_{\%}$), X_i and X_j are input variables which influence the response variable