

DPPH FREE RADICAL SCAVENGING ACTIVITY OF TOMATO, CHERRY TOMATO AND WATERMELON: LYCOPENE EXTRACTION, PURIFICATION AND QUANTIFICATION

TEHNIAT SHAHZAD, IJAZ AHMAD*, SHAHNAZ CHOUDHRY, MUHAMMAD K SAEED, MUHAMMAD N KHAN

Department of Biotechnology, Kinnaird College for Women, Lahore, Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Lahore, Pakistan-Applied Chemistry Research Centre, PCSIR Laboratories Complex, Lahore, Pakistan.
Email: ijazft@hotmail.com

Received: 08 Oct 2013, Revised and Accepted: 20 Jan 2014

ABSTRACT

Objective: The present study was aimed to evaluate the antioxidant activity and nutritional quality of tomato (*Lycopersicum Esculentum*), cherry tomato (*Solanum Lycopersicum var. cerasiforme*) and watermelon (*Citrullus Lanatus*) fruits. Lycopene was extracted, purified and characterized.

Methods: Antioxidant evaluation was carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. Lycopene was extracted with two extraction methods: acetone-petroleum ether and hexane extraction. Lycopene was purified on grade II alumina by column chromatography, which was evaluated by TLC. Elucidation of functional groups in lycopene was done by FTIR Spectroscopy.

Results: The results showed variation in nutritional quality and antioxidant potential among these fruits. Tomato and cherry tomato exhibited high antioxidant activity which was closely followed by watermelon. In DPPH assay, all fruit samples exhibited better antioxidant activity in water than in methanol. Hexane extraction gave better yield of lycopene for all the three fruits. The highest lycopene content was recorded in cherry tomato. The pure lycopene content was calculated to be 55.84mg/kg, 74.53mg/kg and 88.87mg/kg in tomato, watermelon and cherry tomato, respectively. Elucidation of functional groups in lycopene was done by FTIR Spectroscopy.

Conclusion: The results of the study showed that tomato, watermelon and cherry tomato, possess significant antioxidant activity. These results showed that the potential of these fruits should be used as medicine against the diseases caused by free radicals.

Keywords: Antioxidant activity, DPPH free radical scavenging assay, lycopene, TLC, FTIR Spectroscopy.

INTRODUCTION

Tomato (*Lycopersicum Esculentum*) is one of the world's major fruits. It is an excellent source of many nutrients and secondary metabolites including folate, potassium, vitamins C and E, flavonoids, β -carotene and lycopene which are essential for human health [1].

Cherry tomato (*Solanum Lycopersicum var. cerasiforme*) is small in size, has a sweeter taste and offers several significant nutritional benefits. [2] noted that cherry tomatoes have intense color and flavor, generally round in shape and weighing 10 to 30 g. Cherry tomato (*Solanum Lycopersicum*) contains ascorbic acid, vitamin E, flavonoids, phenolic acids and carotenoids [3]; also, is a major source of vitamins, minerals and fiber, important for nutrition and human health [4], and is the main source of lycopene [5]. Cherry tomato contained significantly ($p \leq 0.05$) higher nutrients including fiber [6].

Watermelon (*Citrullus Lanatus*) is an important fruit vegetable in the warmer regions of the world [7]. It has a juicy, sweet, usually red interior flesh. [8] noted that red fleshed watermelons are an excellent source of lycopene. Lycopene is the compound that is responsible for red color of watermelon [9]. Watermelon is a key source of lycopene [10].

The presence of carotenoids in the diet and their role in human health has become a subject of extraordinary interest. Carotenoids are beneficial in cardiovascular health [11]. The foods containing lycopene may be beneficial in cardiovascular disease [12]. Carotenoids, due to their unique structure, protect tissues against oxidative and photooxidative damage by free radicals or reactive oxygen species produced as a result of the metabolic and pathological processes [13].

Lycopene has unique structural and chemical features that contribute to specific biological characteristics [14]. Carotenoids have antioxidant properties and potential health benefits, selecting varieties with high concentration of carotenoids is important for breeding lines [9]. Carotenoids may be available from the diet and absorbed, metabolized, or utilized by the human body [15].

Lycopene is a red-colored carotenoid with antioxidant properties and potential health benefits [16]. Lycopene is a primary carotenoid in human plasma, present naturally in greater amounts than β -carotene and other dietary carotenoids. This perhaps is an indication of its biological importance in the human defense system [17]. Lycopene is major carotenoid pigment found in ripe tomato and watermelon fruit [18]. A diet containing whole tomato powder and dietary restriction inhibited the development of prostate cancer, however a diet containing pure synthetic lycopene supplement could not [19]. Lycopene may inhibit growth of prostate tumor [20], provide protecting effects to maintain prostate health [21] and lower the risk of lung cancer in human [22]. Lycopene is a bioactive red colored pigment naturally occurring in plants. Lycopene-rich food is inversely associated to diseases such as cancers, cardiovascular diseases, diabetes and others diseases [23]. Lycopene, the predominant carotenoid found in tomatoes, showed high antioxidant ability and may prevent cancer. *In vivo* studies have revealed that lycopene inhibits tumor growth in the liver, lung, prostate, breast, and colon. Clinical studies have shown that lycopene protects against prostate cancer [24].

The higher level of antioxidants in the skin effectively reduces skin roughness [25]. Tomato paste containing lycopene provides protection against photo damage [26]. Diets rich in carotenoids can prevent cell damage, premature skin aging, and skin cancer. Topically applied antioxidants have shown an increase in radical protection after VIS/NIR irradiation [27]. Lycopene is fat-soluble [14] and well-absorbed if applied externally (e.g. in a cream or lotion). Thus, lycopene is an essential nutraceutical compound that provides significant health and medical benefits. Lycopene is generally stable during processing as long as it is within the plant tissue. The data about lycopene bioavailability, tissue distribution, metabolism, and biological actions in experimental animals and humans is being accumulated. However, much additional research is still needed [14].

The extraction and purification of lycopene is essential to use it in medicines, supplements, food ingredients and skin care creams etc. The local varieties of tomato and watermelon are easily available in the market. Keeping in view the nutraceutical importance of

lycopene, this study was undertaken with the objective to evaluate the contents and quality of lycopene in tomato, cherry tomato and watermelon to generate useful information on qualitative and quantitative aspects of lycopene from these fruits.

MATERIALS AND METHODS

Materials

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and BHT were purchased from Sigma Chemical Co (St Louis, MO, USA). All other chemicals used, including solvents, were of analytical grade. Tomato and watermelon were purchased from the local market. Cherry tomato (imported from France) was also purchased from local market.

Preparation of samples

The fruit samples were chopped and homogenized in a laboratory homogenizer to a paste-like consistency. To prepare powder, samples were chopped and dehydrated in cabinet dryer (70°C) with circulating hot air and ground in laboratory grinder.

Nutritional analysis

The nutritional analysis of samples were carried out for moisture content, total ash, crude protein (nitrogen x 6.25), crude fat, crude fiber, carbohydrate and energy value according to standard methods of [28].

Antioxidant activity (DPPH assay)

The solubility of dehydrated samples (tomato, watermelon and cherry tomato powders) was checked in distilled water, methanol and distilled water-methanol solution (50% v/v). The samples were more soluble in water and in methanol. So these were used for DPPH assay. The purple colored DPPH is a stable free radical, which is reduced to 2, 2-diphenyl-1-picrylhydrazine (yellow colored) by reacting with an antioxidant [29]. A concentration of 1mg/ml was prepared by adding 0.02 g sample in 20 ml distilled water or methanol. This spectrophotometric assay uses the stable radical 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) as a reagent [30]. Sample (100µl) was added to 3 ml of DPPH solution. After 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. The percentage inhibition of free radical (DPPH) was calculated as under:

$$\text{Inhibition \% (DPPH)} = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Where, A=Absorbance.

Acetone-petroleum ether extraction

Sample (1.0-1.5 g powder) was extracted with 10mL acetone-petroleum ether (50% v/v). The upper lycopene-containing organic layer was removed by means of a pipette and collected in test tube. Extraction was repeated. The extracts were combined, washed with 15mL saturated aqueous sodium chloride (NaCl) and removed the aqueous wash with a micropipette. The extract was washed with 10mL of 10% aqueous potassium carbonate (K₂CO₃) and removed the aqueous wash. The lycopene-containing organic layer was dried with a drying agent (calcium chloride). The excess solvent was allowed to evaporate at room temperature for a few minutes in the dark. The tubes containing lycopene extracts were covered with aluminum foil and stored in freezer until further analysis [31].

Hexane extraction

Sample (0.3 to 0.6 g powder) was weighed in a beaker, 5 mL BHT-acetone solution (0.05%, w/v), 5 mL ethanol and 10 mL hexane was added. The beaker was placed in a bowl of ice on a magnetic stirring plate, stirred for 15 min and added 3 mL distilled water. It was shaken for 5 min on ice and incubated at room temperature for 5 min to allow the separation of both phases. The upper layer containing lycopene was isolated by means of a pipette and collected in a test tube. The tubes containing lycopene extracts were covered with aluminum foil and stored in the freezer until further analysis [32].

UV-VIS Spectrophotometry

The absorbance value at 503nm was used for the determination of lycopene content in tomato, cherry tomato and watermelon [33]. In one quartz cuvette (1 cm optical path), hexane was use as blank. Three absorbance values were obtained. The results were calculated by using the formula (Beer-Lambert law) [32]. Absorbance values of four fractions obtained by column chromatography were noted at 360, 443, 471 and 503nm [34].

$$\text{Lycopene content (mg/kg)} = \text{Absorbance}_{503} \times 31.2$$

Column chromatography

Lycopene obtained by acetone-petroleum ether extraction from tomato, cherry tomato and watermelon was purified by column chromatography using Plastic column (20ml). Alumina (Grade II) was used as adsorbent. Lycopene sample was added to the prepared column. The column was filled with the solvent and the sample was eluted from the column. Firstly, the yellow carotene band was eluted by hexane. The eluting solvent was switched to 10% acetone-hexane to elute the lycopene from the column [31].

Thin Layer Chromatography (TLC)

TLC was performed on crude lycopene (obtained by extraction) and pure lycopene (obtained by column chromatography). Silica plates (MERCK) were prepared by drawing a pencil line 1 cm from the bottom of the TLC plate. Samples were spotted using glass spotters. The organic solvent (9:1 petroleum ether and dichloromethane) was used [35]. TLC plate was placed in the tank for 5-10 min. The edge of plate was marked to indicate how far the solvent traveled up the plate. TLC plate was dried in hood, the pigments were marked with a pencil and the plate was analyzed under UV lamp.

$$\text{Rf value} = \text{distance from origin to component spot (cm)} / \text{distance from origin to solvent front (cm)}$$

Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared (FTIR) Spectroscopy was performed on purified lycopene samples. FTIR Spectrometer (BRUKER, Germany) was used. The liquid samples are sandwiched between two plates. The plates are transparent to the infrared light and do not introduce any lines onto the spectra. Standard spectra of the samples were collected individually [36].

RESULTS

Nutritional analysis

The proximate analysis of edible fruit and vegetables plays a crucial role in assessing their nutritional significance [37]. The nutritional tests were performed on fresh sample of whole fruits of tomato and cherry tomato. However, watermelon pulp, excluding the seeds was used. The result of nutritional analysis showed variation in concentration/ proportions of different constituent. Tomato contained moisture 92.0%, ash 0.56%, protein 1.98%, fat 0.95%, crude fiber 0.76% and carbohydrate 3.75% (Table 1). Cherry tomato contained moisture 91.78%, ash 0.70%, proteins 1.77%, fat 0.55%, crude fiber 0.40% and carbohydrate 4.80%.

Watermelon exhibited moisture 90.95%, ash 0.41%, proteins 0.71%, fat 0.45%, crude fiber 0.96% and carbohydrate 6.52%. The energy value was found to be 31.47, 31.23 and 32.97 Kcal/100g in tomato, cherry tomato and watermelon, respectively.

Antioxidant activity

Antioxidant capacity DPPH radical was used as a stable free radical to determined antioxidant activity of natural compounds. According to [38] scavenging of the stable radical (DPPH) is considered a valid and easy assay to evaluate scavenging activity of antioxidants. The antioxidant activity was determined in terms of the ability of the antioxidants in the fruit to inhibit oxidation. The comparison of antioxidant activity of fruit extracts is presented in (Table 2).

Table 1: Nutritional values of tomato, cherry tomato and watermelon

Sr. No.	Constituents	Tomato	Cherry tomato	Watermelon
1	Moisture %	92.00	91.78	90.95
2	Ash %	0.56	0.70	0.41
3	Protein %	1.98	1.77	0.71
4	Fat %	0.95	0.55	0.45
5	Crude fiber %	0.76	0.40	0.96
6	Carbohydrate %	3.75	4.80	6.52
7	Energy kcal/100g	31.47	31.23	32.97

Table 2: Percent inhibition values and antioxidant activity by DPPH assay

Sr. No.	Fruit (powder)	Solution	Mean absorbance	Antioxidant activity (% inhibition)
1	Tomato	In distilled water	1.1064	44.65
		In methanol	1.2457	37.68
2	Cherry tomato	In distilled water	1.2835	44.63
		In methanol	1.1068	35.80
3	Watermelon	In distilled water	1.1814	40.90
		In methanol	1.2571	37.11

Percent inhibition value of BHT (65.50%) was taken as reference point.

Table 3: Lycopene concentrations of tomato, watermelon and cherry tomato

Sr. No.	Fruit	Acetone-petroleum ether extraction		Hexane extraction	
		Absorbance at 503nm	Lycopene mg/kg	Absorbance at 503nm	Lycopene mg/kg
1	Tomato	2.340	71.68	1.110	34.65
2	Cherry tomato	3.433	105.17	2.435	76.01
3	Watermelon	2.788	87.03	1.833	57.22

The antioxidant activity was found to be greater in water (44.65%, 44.63% and 40.90%) than in methanol (37.6 8%, 35.80% and 37.11%) for tomato, cherry tomato and watermelon, respectively (Table 2).

Quantification of lycopene

Absorbance of the crude extracts was obtained at 503nm for determining the content of lycopene in tomato, cherry tomato and watermelon. Previously [33] also measured lycopene concentration in acetone extracts of tomato at 503nm. The quantitative spectrophotometric analysis (Table 3) showed that lycopene contents from tomato, cherry tomato and watermelon obtained by acetone-petroleum ether extraction were 71.68, 105.17 and 87.03 mg/kg, respectively; which were greater than those obtained by hexane extractions (34.65, 76.01 and 57.22 mg/kg, respectively). As higher lycopene concentration was obtained by acetone-petroleum ether extraction. Thus the samples obtained by acetone-petroleum ether extraction were purified by column chromatography for further study

The absorbance values of the four fractions (obtained by column chromatography) of each fruit at wavelengths 360, 443, 476 and 503nm are given in Table 4. The fraction 3 exhibited the maximum

absorbance (A) value at 476nm for all three fruits which is also a confirmatory test for lycopene. Hence, fraction 3 was identified to be the purified lycopene. The absorbance values of purified lycopene from tomato, cherry tomato and watermelon at 476nm were 2.088, 3.087 and 2.692, respectively (Table 4). The absorbance values of purified lycopene fraction at 503nm were used to determine the final concentration of lycopene in tomato, watermelon and cherry tomato. Cherry tomato exhibited higher Lycopene content 88.87 mg/kg, followed by watermelon 74.53 mg/kg and tomato 55.84 mg/kg (Figure 1)

TLC analysis

The results showed that crude extracts of tomato, watermelon and cherry tomato also contained pigments other than lycopene. The Rf values calculated for lycopene from tomato, cherry tomato and watermelon by acetone-petroleum ether extraction were 0.620, 0.616 and 0.610; however by hexane extraction were 0.603, 0.643 and 0.630, respectively (Table 5).

TLC was performed after column chromatography for confirming the purified nature of the lycopene. The Rf values calculated for purified lycopene from tomato, cherry tomato and watermelon were 0.608, 0.615 and 0.608, respectively (Table 5).

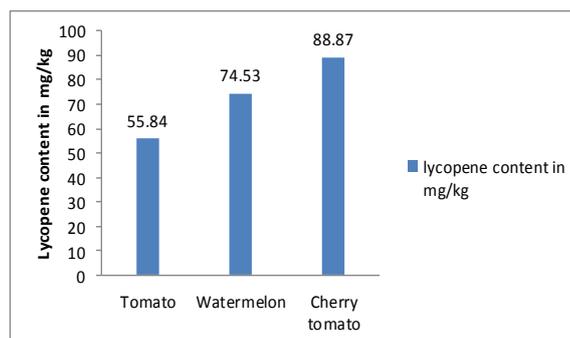
**Fig. 1: Purified lycopene contents in mg/kg in tomato, watermelon and cherry tomato**

Table 4: Absorbance values of tomato, cherry tomato and watermelon fractions (acetone- petroleum ether extraction) obtained by column chromatography at different wavelengths

Wavelength (nm)	Fraction 1	Fraction 2	Fraction 3 (lycopene)	Fraction 4
Absorbance values of tomato				
360	0.110	0.163	1.434	0.151
443	0.043	0.155	1.634	0.069
476	0.038	0.153	2.088	0.066
503	0.036	0.123	1.823	0.065
Absorbance values of cherry tomato				
360	0.141	0.255	1.521	0.147
443	0.076	0.494	1.940	0.087
476	0.073	0.574	3.087	0.084
503	0.072	0.410	2.901	0.081
Absorbance values of watermelon				
360	0.154	0.195	1.296	0.165
443	0.078	0.185	1.905	0.091
476	0.072	0.184	2.692	0.090
503	0.070	0.122	2.433	0.088

Table 5: Rf values for tomato, watermelon and cherry tomato

Fruit	Before purification		After purification
	Acetone-petroleum ether extraction (Rf)	Hexane extraction (Rf)	Acetone petroleum-ether extraction (Rf)
Tomato	0.620	0.603	0.608
Cherry tomato	0.616	0.643	0.615
Watermelon	0.610	0.630	0.608

Fourier Transform Infrared Spectroscopy (FTIR)

Functional group analysis of the extracts from tomato, cherry tomato and watermelon was performed via FTIR spectroscopy. The IR spectra of extracted lycopene of tomato, cherry tomato and watermelon showed typical bands arising from CH stretching (3000-2800 cm^{-1}). Other bands occurred at 1477-1400 cm^{-1} (C-H bending) and 1400-1100 cm^{-1} (C-C and C-C-H stretching). Strong and broad absorption bands of water were shown in the 3700-3500 cm^{-1} .

DISCUSSION

Nutritional analysis of Pakistani tomato and watermelon, and imported cherry tomato available in the Pakistani market revealed that moisture contributed more to the fresh fruit weight. Cherry tomato exhibited higher ash contents, indicating that it is relatively rich in minerals. The nutritional analysis of tomato was found to be in close conformity with the data already reported by [39]. They found moisture 92-94%, protein 1-2%, fiber 0.4-0.7%, ash 0.4-0.6% and fat 1-2% in tomato. Tomatoes are low in fat and rich source of dietary fiber, minerals, and vitamins. Therefore, intake of tomato in human diet is advised in cholesterol controlling and weight reduction programs. The minor differences in the nutritional composition may be due to the difference in the fruit variety, origin and growth conditions. Antioxidants through their scavenging power are useful for the management of various diseases. The antioxidant activity was determined in terms of the ability of the antioxidants in the fruit to inhibit oxidation. Percentage inhibition of tomato and cherry tomato was found to be at par followed by watermelon. Furthermore, better antioxidant activity was exhibited in aqueous solutions than in organic solvent (methanol).

Acetone-petroleum ether extraction resulted in higher crude lycopene yield than hexane extraction. During purification by column chromatography, lycopene had more affinity with alumina due to its high degree of unsaturation and was eluted after yellow-orange carotene pigments. A typical carotenoid such as lycopene displays maximum absorbance at 476nm [40]. Spectrophotometry results revealed that lycopene showed maximum absorbance at 476nm, followed closely by absorbance at 503nm. The purified lycopene content was found to be the maximum in cherry tomato (88.87 mg/kg) as compared to Pakistani tomato (55.84 mg/kg) and watermelon (74.53 mg/kg) (Fig 2). Lycopene content in tomato ranges from 55 to 181 mg/kg [41], 4.31 to 5.97 mg/100 g fw [42].

Lycopene content varied significantly among the tomato varieties, with cherry tomato having the highest lycopene content [3] and depends on the variety, geographic location, technique of cultivation, climatic conditions and degree of ripeness of tomato fruit [3, 41]. The results for lycopene contents in watermelon are also in agreement with those reported by [43]. According to [44] 'Classica' a Roma type of organically grown tomato, was highest in lycopene content (106 mg/kg) and the other cultivars had 50-60mg/kg lycopene in soft red fruit. Cherry tomatoes was analyzed by [45] for antioxidant activity. They found significant differences among different cultivars with respect to lycopene. The antioxidant activity is mainly due to lycopene, and other pigments such as B-carotene and xanthophylls also contribute to this activity [38]. Lycopene was found in significant quantities in tomato, cherry tomato and watermelon. These fruits also exhibited powerful antioxidant activity indicating that lycopene is a major contributor of antioxidant capacities of these fruits.

TLC of the purified samples resulted in only one pigment on the TLC plate. Rf values calculated for the purified lycopene were 0.608, 0.615 and 0.608 for tomato, cherry tomato and watermelon, respectively. The Rf values for lycopene are comparable with [46]. Fourier-transform infrared (FTIR) spectroscopy is a well-established, nondestructive technique for analyzing agricultural and food products. It enables the identification of the functional groups in the compounds. This method allows at least partial identification of a compound's chemical structure [36]. All these three spectra showed typical bands arising as a result of CH stretching (3000-2800 cm^{-1}). Other bands occurred at 1477-1400 cm^{-1} (C-H bending) and 1400-1100 cm^{-1} (C-C and C-C-H stretching). Strong and broad absorption bands of water were shown in 3700-3000 cm^{-1} . Purified lycopene in all the three fruits displayed similar spectra. Thus FTIR revealed that lycopene purified in all the three cases possessed the same chemical entities.

CONCLUSIONS

The antioxidant activity of tomato, cherry tomato and watermelon was measured in water and methanol extracts. These fruits vary in antioxidant activity; however, high activity was recorded in tomato and cherry tomato followed by watermelon. The aqueous solution was found to be better solvent than methanol for antioxidant activity of these fruits. Acetone-petroleum ether extraction was found better for high lycopene yield than hexane extraction. Purified lycopene

can be easily obtained through single column purification. Tomato, cherry tomato and watermelon also exhibited high lycopene contents indicating that lycopene is major contributor of antioxidant capacities of these fruits. The results of the present study suggests that these fruits contained potential antioxidant bioactive compounds particularly lycopene, which if properly utilized could provide source of biologically active nutraceutical ingredient/medicine application. It also shows its titanic importance as therapeutic agent in preventing or curing the diseases caused due to oxidative stress.

REFERENCES

- Lumpkin HM. A comparison of lycopene and other phytochemicals in tomatoes grown under conventional and organic management systems. Technical Bulletin No. 34. AVRDC publication number 05-623. Shanhuu, Taiwan: AVRDC-the World Vegetable Center 2005; p. 48.
- Nuez F. Desarrollo de nuevos cultivares. In: F. Nuez (ed.). El cultivo del tomate 1999; p. 625-669.
- Kuti JO, Konuru HB. Effects of genotype and cultivation environment on lycopene content in red-ripe tomatoes. Journal of the Science of Food and Agriculture 2005; 85: 2021-2026.
- Razdan M, Mattoo AK. Genetic improvement of solanaceous crops. Vol. 2. Tomato. Science Publishers, Enfield, NH, USA 2007; p. 646.
- Candelas MG, Alanis MJ, Bautista M, Del Rio F, García D. Contenido de licopeno en jugo de tomate secado por aspersión. Revista Mexicana de Ingeniería Química 2008; 4 (3): 299-307.
- Opara UL, Al-Ani MR, Al-Rahbi NM. Effect of Fruit Ripening Stage on Physico-Chemical Properties, Nutritional Composition and Antioxidant Components of Tomato (*Lycopersicon esculentum*) Cultivars. Food and Bioprocess Technology 2012; 5 (8): 3236-3243.
- Paris HS, Daunay M-C, Janick J. Medieval iconography of watermelons in Mediterranean Europe. Annals of Botany 2013; 112: 867-879.
- Perkins-Veazie P, Collins J. Flesh quality and lycopene stability of fresh-cut watermelon. Postharvest Biology and Technology Journal 2004; 31 (2): 158-166.
- Davis AR, Collins JK, Fish WW, Webber III CL, Perkins-Veazie P, Tadmor YK. A rapid hexane-free method for analyzing total carotenoid content in canary yellow-fleshed watermelon. In: Cucurbitaceae: Proceedings, Asheville, North Carolina. September 17-21, 2006; p. 545-552.
- Vaughn KLS, Edgar CC, Jerry WK, Luke R, Julie C. Extraction conditions affecting supercritical fluid extraction (SFE) of lycopene from watermelon. Journal of Bio-resource Technology 2008; 99 (16): 7835-7841.
- Kraemer K, Packer L, Sies H, and Ute O-J. Carotenoids and Retinoids: Molecular Aspects and Health Issues. Illinois, USA: AOCS Publishing. AOCS Press Champaign; 2005.
- Peter L and Erdman Jr JW. Lycopene and risk of cardiovascular disease. Oxidants and antioxidants in biology: A conference organized by the Oxygen Club of California (OCC) and co-sponsored by the *Linus Pauling Institute*. Fess Parker's Double Tree Resort Santa Barbara, California. March 10-13, 2004.
- Dembinska-Kiec A. Carotenoids: risk or benefit for health. *Biochimica et Biophysica Acta* 2005; 1740: 93-94.
- Chauhan K, Sharma S, Agarwal N, Chauhan B. Lycopene of tomato fame: its role in health and disease. *International Journal of Pharmaceutical Sciences Review and Research* 2011; 10 (1): 99-115.
- Khachik F. 2004. Chemical and Metabolic Oxidation of Carotenoids. Oxidants and antioxidants in biology: A conference organized by the Oxygen Club of California (OCC) and co-sponsored by the *Linus Pauling Institute*. Fess Parker's Double Tree Resort Santa Barbara, California. 10-13 March, 2004.
- Davis AR, Fish WW, Perkins-Veazie P. A rapid Hexane-free method for analyzing lycopene content in watermelon. *Journal of Food science* 2003; 68 (1): 328-332.
- Wilcox JK, Catignani GL, Lazarus C. Tomatoes and cardiovascular health. *Critical Reviews in Food Science and Nutrition* 2003; 43 (1): 1-18.
- Shi J, Le Maguer M, Bryan M. Lycopene from Tomatoes in Functional Foods: Biochemical and Processing Aspects. CRC Press, Boca Raton. 2002.
- Boileau TW, Liao Z, Kim S, Lemeshow S, Erdman Jr JW, Clinton SK. Prostate carcinogenesis in N-methyl-N-nitrosourea (NMU)-testosterone-treated rats fed tomato powder, lycopene, or energy-restricted diets. *J Natl Cancer Inst.* 2003; 95: 1578-86.
- Obermuller-Jevic U, Olano-Martin E, Weerden WV, Kramer K, Cross CE, Schroder FH, Packer L. Lycopene and Prostate Health. Oxidants and antioxidants in biology: A conference organized by the Oxygen Club of California (OCC) and co-sponsored by the *Linus Pauling Institute*. Fess Parker's Double Tree Resort Santa Barbara, California. March 10-13, 2004.
- Stacewicz-Sapuntzakis M, Bowen PE. Role of lycopene and tomato products in prostate health. *Biochimica et Biophysica Acta* 2005; 1740: 202-205.
- Wang X-D. Biological activity of lycopene against smoke-induced lung lesions. Oxidants and antioxidants in biology: A conference organized by the *Oxygen Club of California (OCC)* and co-sponsored by the *Linus Pauling Institute*. Fess Parker's Double Tree Resort Santa Barbara, California. March 10-13, 2004.
- Kong KW, Khoo HE, Prasad KN, Ismail A, Tan CP, Rajab NF. Revealing the power of the natural red pigment lycopene. *Molecules* 2010; 15 (2): 959-987.
- Trejo-Solís C, Pedraza-Chaverrí J, Torres-Ramos M, Jiménez-Farfán D, Salgado AC, Serrano-García N, Osorio-Rico L, Sotelo1 J. Multiple Molecular and Cellular Mechanisms of Action of Lycopene in Cancer Inhibition. Evidence-Based Complementary and Alternative Medicine. Hindawi Publishing Corporation. Article ID 705121, 17 pages. Volume 2013.
- Darvin M, Patzelt A, Gehse S, Schanzer S, Benderoth C, Sterry W, Lademann J. Cutaneous concentration of lycopene correlates significantly with the roughness of the skin. *European Journal of Pharmaceutics and Biopharmaceutics* 2008; 69: 943-947.
- Rizwan M, Rodríguez-Blanco I, Harbottle A, Birch-Machin MA, Watson REB, Rhodes LE. Tomato paste rich in lycopene protects against cutaneous photodamage in humans in vivo: a randomized controlled trial. *British Journal of Dermatology* 2011; 164: 154-162.
- Meinke MC, Friedrich A, Tschersch K, Haag SF, Darvin ME, Vollert H, Groth N, Lademann J, Rohn S. Influence of dietary carotenoids on radical scavenging capacity of the skin and skin lipids. *European Journal of Pharmaceutics and Biopharmaceutics* 2013; 84: 365-373.
- AOAC. Official Methods of Analysis. The Association of Official Analytical Chemists. 18th Ed. Arlington, Virginia, USA: 2005.
- Ivanišová E, Tokár M, Mocko K, Bojňanská T, Mareček J, Mendelová A. Antioxidant activity of selected plant products. *Journal of Microbiology, Biotechnology and Food Sciences* 2013; 2: 692-1703.
- Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. *LWT Food Science and Technology* 1995; 28 (1): 25-30.
- Lehman JW. Operational organic chemistry. A problem-solving approach to the laboratory course. 4th ed. Prentice Hall. 2009.
- Ravelo-pérez LM, Hernández-borges J, Rodríguez-delgado MA, Borges-miquel T. Spectrophotometric analysis of lycopene in tomatoes and watermelons: A Practical Class. *The Chemical Educator* 2008; 13 (1).
- Souza MCD, Singha S, Ingle M. Lycopene Concentration of Tomato Fruit can be estimated from Chromaticity Values. *Plant and Soil* 1992; 27 (5): 465-466.
- Naviglio D, Pizzolongo F, Ferrara L, Arag A, Santini A. Extraction of pure lycopene from industrial tomato by-products in water using a new high-pressure process. *Journal of the Science of Food and Agriculture* 2008; 88 (14): 2414-2420.
- Vasta JD, Sherma J. Analysis of lycopene in nutritional supplements by silica gel high-performance thin-layer chromatography with visible-mode densitometry. *Acta Chromatographica* 2008; 20 (4): 673-683.
- Bunghhez IR, Raduly M, Doncea S, Aksahin I, Ion RM. Lycopene determination in tomatoes by different spectral techniques (UV-VIS, FTIR and HPLC). *Journal of Nanomaterials* 2011; 6 (3): 1349-1356.

37. Saeed MK, Shahzadi I, Ahmad I, Shahzad K, Ashraf M, Viqar-un-Nisa. Nutritional analysis and antioxidant activity of Bitter gourd (*Momordica charantia*) from Pakistan. *Pharmacologyonline* 2010; 1: 252-260.
38. Suhaj M. Spice antioxidants isolation and their antiradical activity: a review. *Journal of Food Composition and Analysis* 2006; 19: 531-537.
39. Gupta A, Kawatra A, Sehgal S. Physical-chemical properties and nutritional evaluation of newly developed tomato genotypes. *Journal of Food Science* 2011; 2 (7): 167-72.
40. Aghel N, Ramezani Z, Amirfakhrian S. Isolation and quantification of lycopene from tomato cultivated in Dezfoul, Iran. *Jundishapur Journal of Natural Pharmaceutical Products* 2011; 6 (1): 9-15.
41. Garcia E, Barrett DM. Assessing lycopene content in California processing tomatoes. *Journal of Food Processing and Preservation* 2005; 30 (530): 56-70.
42. Kaur C, Walia S, Nagal S, Walia S, Singh J, Singh BB, Saha S, Singh B, Kalia P, Jaggi S, Sarika. Functional quality and antioxidant composition of selected tomato (*Solanum lycopersicon* L) cultivars grown in Northern India. 2013. *LWT - Food Science and Technology* 2013; 50: 139-145.
43. Tlili I, Hdider C, Lenucci MS, Riadh I, Jebari H, Dalessandro G. Bioactive compounds and antioxidant activities of different watermelon (*Citrullus lanatus* (Thunb.) Mansfeld) cultivars as affected by fruit sampling area. *Journal of Food Composition and Analysis* 2011; 24: 307-314.
44. Perkins-Veazie P, Roberts W, Collins JK. Lycopene content among organically produced tomatoes. *Journal of Vegetable Science* 2006; 12 (4): 93-106.
45. Lenucci MS, Cadinu D, Taurino M, Piro G, Dalessandro G. Antioxidant composition in cherry and high-pigment tomato cultivars. *Journal of Agricultural and Food Chemistry* 2006; 54 (7): 2606-13.
46. Mritunjay K, Mondal DB, Ananya D. Quantification of catechin and lycopene in *Calendula officinalis* extracts using HPLC method. *Asian Journal of Pharmaceutical and Clinical Research* 2011; 4 (2): 128-129.

Galley Proof