PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 14 (12): 942-956, 2015
ISSN 1680-5194
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Lycopene Extraction from Tomato Pomace with Supercritical Carbon Dioxide: Effect of Pressures, Temperatures and CO_2 Flow Rates and Evaluation of Antioxidant Activity and Stability of Lycopene

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Abstract: Tomato fruits used in the production of tomato pomace were analyzed for the chemical composition such as total soluble solids, pH, titratable acidity, ash, calcium, magnesium, phosphorus, ascorbic acid, total sugar, reducing sugars, fat content, total polyphenolic content; flavonoid content, lycopene content and total carotenoid. The experiments were carried out at pressures, temperatures and CO_2 flow rates ranging from 1000 to 40000 psi, 40 to 80°C and 4 to 12 mL/min, respectively. The extracts were analyzed by high performance liquid chromatography and UV-visible spectroscopy. The results showed that with optimized operating conditions, the maximum recovery of 82.50% was obtained at 4000 psi, 80°C and 8 mL of CO_2/min. The recovery of lycopene increased with increases in pressure, temperature and CO_2 flow rate. The decrease in the amount of lycopene as a function of storage time was followed and the effect of storage conditions on the recovery (%) of lycopene was tested with stability (%) 85 and 74% at 4°C and at room temperature, respectively.

Key words: Lycopene, tomato pomace, supercritical CO_2 fluid extraction, antioxidant capacity, stability

INTRODUCTION

Besides its economic importance, the nutritional value of the tomato fruits (Lycopersicon esculentum, Mill), one of the most important worldwide agricultural crops, is rich in a plethora of natural antioxidant compounds. It is one of the most versatile and widely used food plants, being consumed both in raw and as a constituent of other products and dishes (Ilahy *et al*., 2011; Navarro-Gonzalez *et al*., 2011).

The tomato is a reservoir of diverse antioxidant molecules, such as ascorbic acid, vitamin E, carotenoids, flavonoids and phenolic acids (Martinez-Valverde *et al*., 2002; Periago *et al*., 2009). The chemical composition of the fruit depends upon its genetics, ripeness and the conditions under which it was cultivated (Martinez-Valverde *et al*., 2002).

Lycopene is a carotenoid hydrocarbon (also called carotene). It is a powerful *natural* antioxidant. This compound can be found in nature in the cis and trans forms, comprehending up to 72 geometrical isomers (Chasse *et al*., 2001). The trans form is the most stable and consequently, it is the most frequent structure found in tomato, corresponding to more than 90% of the total lycopene (Tavares and Amaya, 1994).

Lycopene, an open-chain hydrocarbon with 11 conjugated double bonds, has the highest degree of unsaturation among carotenoids and is the pigment principally responsible for the characteristic deep-red color of ripe tomato fruits and tomato products. Although, it has no provitamin A activity, lycopene dose exhibit physical quenching rate constant for singlet oxygen almost twice as high as that of B-carotene. However, the human body cannot produce this module and needs to obtain it from foods such as tomatoes and tomato products (Topal *et al*., 2006).

Lycopene in fresh tomato fruits occurs essentially in the all-trans configuration. However, undesirable degradation is unavoidable owing to isomerization of trans-lycopene to the cis form induced by thermal and oxidation during processing (Shi and Le Maguer, 2000). This results in some loss of color and biological activities. Oxidative degradation, the principal cause of extensive losses of lycopene, depends on the availability of oxygen and is stimulated by light, enzymes, some metal ions and co-oxidation with lipid hydroperoxide. Conditions necessary for isomerization and oxidation of lycopene exist during preparation, processing and storage of food. Lycopene is also subject to isomerization and oxidation during analysis and preventative measures must be taken to guarantee the reliability of analytical results (Lee and Chen, 2002; Topal *et al*., 2006). The concentration of lycopene in tomato varies from 30 to 200 mg/kg in the fresh fruit and from 430 to 2950 mg/kg on a dry basis and represents more than 85% of the total carotenoid content (Vasapollo *et al*., 2004).

According to Focus on Pigments (2007), total world consumption of lycopene was tripled to 15,000 tons in

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2004 compared to 5000 tons in 1995. Thus, alternative sources for the production of natural lycopene are warranted. Previously, *in vitro* and *in vivo* studies exhibited that lycopene has a beneficial role in chronic diseases such as cardiovascular disease, atherosclerosis, cancer and neurodegenerative disorders. However, some studies reported contrasting outcomes (Kong *et al*., 2010).

Since lycopene is highly soluble in organic solvents, it is extracted with organic solvents, which are usually toxic, expensive and hazardous to handle and may remain in the product. Consumer concern about health and environment has resulted in increasing interest in clean technologies and alternative and reliable extraction methods for lycopene and other carotenoids (Choudhari and Singhal, 2008; Phelps *et al*., 1996; Sabio *et al*., 2003).

Supercritical fluid extraction (SFE) with carbon dioxide as a solvent has provided an excellent alternative to the use of chemical solvents. The most significant advantages of using supercritical CO_2 are (i) its chemical inertness; (ii) its non-toxic, non-hazardous and non-inflammable characteristics; (iii) it leaves no solvent residue in the product and (iv) the process generates no waste, therefore it is environment benign technology. Also, there are many other advantages of using supercritical fluid extraction instead of conventional solvents. These include achieving higher purity, single step processing, reducing operating costs, selective fractionation, faster separation, physiologically compatible, in addition the oxygen free operating system prevents oxidation and low temperature minimize thermal degradation of sensitive materials and the resulting extract is microbially sterile as microorganisms and their spores are not soluble in the supercritical CO_2 (Raventos *et al*., 2002; Shi *et al*., 2009a, b). In this respect, supercritical fluids (Chester *et al*., 1994) have already proved their usefulness for performing extractions of high value added compounds from organic matrix (Blanch *et al*., 1994; Blanch *et al*., 1999). In these cases, adequate optimization of experimental variables (e.g., pressure, temperature, CO_2 flow rate and co-solvent) at which both the extraction and the subsequent depressurization are carried out results in a wide range of solvent strength's, enabling the selectivity of the extraction to be increased (Castillo *et al*., 2003). Manipulating the temperature and pressure above the critical points affects the properties of supercritical fluid such as: density, viscosity, diffusivity, heat capacity and thermal conductivity and enhances the ability of the supercritical fluid to penetrate and extract targeted molecules from source material (Raventos *et al*., 2002; Shi *et al*., 2009a, b). The supercritical fluid can penetrate into porous solid materials more effectively than liquid solvents resulting in faster mass transfer and extractions (Shi *et al*., 2009a, b). Recently, Rozzi *et al*. (2002) have examined the effects of temperature, pressure and CO_2 flow rate on supercritical fluid extraction of lycopene from tomato. It was shown that both temperature and pressure had an effect on the extraction of lycopene and that an optimum temperature and pressure combination (359 K and 34.47 Mpa) resulted in extraction of 61% of the lycopene.

As it is clear, natural products are good sources of antioxidant and evaluation of economic costs and simplicity and availability of extraction methods are necessary (Aghel *et al*., 2011). These natural, economical sources provide an excellent opportunity for new food development in the global market. An interesting approach to this concept is the use of nutritional supplements to supply lycopene and carotenoids by way of foods and beverages. These are known nutraceutical or functional foods (Castillo *et al*., 2003). Lycopene or carotenoids extracted from tomato byproducts is in high demand by different industries (e.g., cosmetic, pharmaceutical, food and feed industries), because its bioactivity is associated with several health benefits.

In Jordan, tomato production provides an excellent opportunity to establish a new industry to produce lycopene or carotenoids for food, feed, cosmetic and pharmaceutical industries. Also, Jordan can build a new industry to produce functional foods and natural food colorants based on the use of tomato-byproducts such as tomato pomace.

The objectives of this work were to (i) determine the chemical composition of tomato fruits used in the production of tomato pomace (ii) study the effect of the operational conditions on the recovery and yield of lycopene from tomato-pomace (iii) evaluate the antioxidant activity of the resulting extract (iv) investigate the potential risk of lycopene oxidative degradation (i.e., stability test of lycopene extract) under different storage conditions.

MATERIALS AND METHODS

Preparation of tomato pomace: Tomato fruits were chopped and the homogenized in laboratory blender. The tomato solids were separated from liquid using a cheesecloth. The tomato solids (skin, seeds and internal tissues) were dried in an oven under vacuum at 40°C for 24 h. The moisture content was found to be around 4.0%. The dry pomace was kept in glass container protected from light by covering it in aluminum foil and then flushed with nitrogen and stored in freezer at -18°C.

Chemicals: All chemical materials used were of analytical grade and were obtained from Sigma-Aldrich unless otherwise specified.

Tomato fruit analysis: Tomato fruits samples were bought in a local market. Special care was taken to

select the most mature samples. Samples were first chopped and homogenized in a laboratory homogenizer (Ultra-Turrax-T25, Germany). The filtrate was used to determine different chemical parameters.

Determination of total soluble solids (TSS): The TSS was determined using aliquot of tomato juice filtrate. Refractometer (ABBE Refractometer RL-3 Poland) at 20°C was used to determine TSS by placing 2 drops of clear filtrate on the prism.

Determination of titratable acidity and pH value: The titratable acidity was determined by titration with NaOH (0.10 N) solution to pH 8.2 and expressed as (%) citric acid. The acidity was measured according to AOAC (1995). The pH value of tomato filtrate was determined by pH-meter (Hanna Instrument-Model-HI 8519).

Calcium, magnesium, total phosphorus and ash content determination: Calcium and magnesium in tomato fruits were determined by titrimetric method presented by Metrohm (Information Issue 2/2007), where the sample was incinerated in muffle furnace at 550°C before analysis. Total phosphorus of tomato fruits was determined by the vanado-molybdate colorimetric method (Pearson *et al*., 1981). Ash was determined by muffle furnace (Carbolite-UK) at 550°C for 24 h (AOAC, 1995).

Determination of ascorbic acid: Ascorbic acid content was determined by titrimetric method with 2,6 dichloroindophenol with substitution of 10% metaphosphoric acid by 1% oxalic acid (AOAC, 1995).

Determination of total and reducing sugars: Reducing and total sugars were estimated by using the calorimetric methods as presented by Seyoum (2002).

Determination of fat content: The fat content of tomato fruits was determined according to AOAC (1995) procedures.

Determination of polyphenolic content: The concentration of total phenolics was determined by the Folin Ciocalteau colorimetric method (Singleton *et al*., 1999). Measurements were carried out in triplicate and calculations based on a calibration curve obtained with gallic acid (Extrasynthese, Genay, France). The total phenolics were expressed as milligram of gallic acid equivalents (GAE) per 100 g of fresh weight (FW).

Determination of flavonoids content: The amount of flavonoids was determined spectrophotometrically (Miliauskas *et al*., 2004). The results were expressed as milligram of rutin equivalent per 100 g fresh weight (F.W).

Determination of total lycopene content in tomato fruits: The total lycopene content of tomato fruits was determined according to Fish *et al.* (2002). Samples were first chopped and homogenized in a laboratory homogenizer. Samples of 0.5 g where weighed and 5 mL of 0.05% (w/v) BHT (antioxidant) in acetone, 5 mL of ethanol and 10 mL of hexane were added. The recipient was introduced in ice and stirred on a magnetic steering plate for 15 min. After shaking, 3 mL of deionized water where added to each vial and the samples were shaken for 5 min on ice. Samples were then left at room temperature for 5 min to allow the separation of both phases. The hexane layer (upper layer) was measured in a 1-cm-path-length quartz cuvette at 503 nm blanked with hexane using a double beam spectrophotometer (Jasco-V-530). The lycopene content of the samples was estimated using the following equation:

$$\text{Lycopene content (mg/kg)} = \frac{(A_{503} - 0.0007) \times 30.3}{g_{tissue}}$$

A_{503} = Absorbance of the sample at 503 nm

Determination of total carotenoids: The total carotenoids were extracted and partitioned in acetone and petroleum ether respectively as described by Thimmaiah (1999). Absorbance was measured at 452 nm and total carotenoids content (mg/100 g F.W) was calculated using a calibration curve prepared against a high purity standard β-carotene.

Dry pomace lycopene content: The lycopene content of the dry pomace was determined by two methods. The first method the spectrophotometric method as it was discussed before and reported by Fish *et al.* (2002). The dry pomace was ground before being extracted and 0.5 g of dry pomace was used in the determination of total lycopene spectrophotometrically. The second method used in the determination of lycopene in the dry pomace sample was the HPLC method (Epler *et al*., 1992). The amount of lycopene was determined in an HPLC apparatus equipped with a UV-visible detector (Tasco:Model:875, Japan), isocratic pump (Jasco-Model:880-PU, Japan) and column C-18-RP (Promosil-C-18, 25cm x 4.6 mm) positioned in a column cabinet (Jasco-Model:865-CO) to control the temperature around the column at 30°C. The system is connected with an integrator (Shmitzo-C-R6A, Japan). A mixture of methanol and tetrahydrofuran (90:10) (HPLC grade-May and Baker) as the mobile phase, with a flow rate of 1.5 mL/min, detection was set at 470 nm for lycopene. The peak of trans-lycopene was identified by comparing the retention time with that of standard lycopene. Lycopene standard was purchased from Sigma-Aldrich at a certified purity grade of 90-95%. The dry pomace was analyzed to determine acidity, ash, total

carotenoids, total lipids, crude protein, total sugar, calcium, magnesium and phosphorus by the methods reported previously.

Determination of lycopene standard purity (FAO-JECFA, 2006): Lycopene is a very sensitive compound in term of its stability and for that purity of standard was determined before being used in the analysis. The purity of lycopene standard determination method started with weighing 20 mg of lycopene standard was dissolved in 10 mL methylene chloride (HPLC-grade) and hexane (HPLC-grade) was added to volume. The absorbance of the solution was measured in a 1 cm optical cell at 470 nm, where hexane was used as the blank. The purity of the standard was calculated from following equation:

$$P_{st} = \frac{Amax \times 10000}{345 \times W_{st}}$$

Where:

P_{st} : The purity of the lycopene standard

A_{max} : The absorbance at the wavelength of maximum absorption

W_{st} : The weight of the standard (mg)

345 : The absorptivity of lycopene in hexane A purity of 96% was estimated

Preparation of lycopene standard curve: To calculate the concentration of lycopene in the extract, a calibration curve was established from five solutions of known concentration in tetrahydrofuran (THF, HPLC-grade). The concentrations of the standard lycopene solutions were 1, 2, 3, 4 and 5 ppm lycopene and 20 µL of each concentration were injected on duplicate base in the HPLC instrument to determine retention time and the area under the peak for each concentration. The coefficient of correlationship was 0.99.

Supercritical fluid extraction: Instrumentation and procedure: The extraction with supercritical CO_2 was performed with apparatus made by Supercritical Fluid Technologies, Inc., DE, USA (Model: SFT-100).

The SET-100 consists of two modules. The pump module is the bottom section. It controls CO_2 pressure and flow through the system. The oven module is the top section. It set on top of the pump module controls all temperature functions. The system has an extraction vessel of 100 ml placed in a column oven that controls the temperature. Gas requirement for this system is CO_2 with a purity of 99.99% in cylinder with a dip tube (Approx. 800-900 psi). The SFT-100 incorporates a Peltier cooler for CO_2 to prevent supercritical CO_2 from vaporizing. The dry tomato pomace was ground into a very fine particles immediately prior to extraction and the vessel was filled with a 25 g of tomato pomace. Before the liquid CO_2 passed into the extractor vessel, the pressure vessel sealed and connected to the CO_2 lines before the main valve on CO_2 cylinder was opened. The main valve on the CO_2 cylinder was opened to allow the system to equilibrated at tank pressure. The LED displayed on the pump module the vessel pressure 750-800 psi which is the CO_2 tank pressure. The CO_2 was pressurized to the desire pressure and heated to the specified temperature by means of the pump to reach the supercritical state. The pressure of the system was controlled to within 100 psi. Once the system was equilibrated at the selected pressure and temperature, the static/dynamic valve was opened on the oven cabinet. The restrict or valve was slowly opened to achieve the desired flow of CO_2 liquid through sample. After the completion of the extraction, the pressure was set to 0 psi and the temperature to a few degrees below ambient. The extraction vessel was allowed to vent completely to 0 psi before removing the sample.

The extracted substances were recovered in a vial connected to restrict or. This vial was protected by covering it with aluminum foil to prevent oxidation and degradation of the lycopene. The amount of lycopene in the extract was determined by HPLC and spectrophotometric methods as reported previously. The samples were blanketed with nitrogen and kept in the dark at -18°C prior to analysis to prevent any degradation by heat, air and light.

Operational conditions: Different parametric operational conditions were used to optimize the supercritical fluid extraction of lycopene from tomato pomace, these were pressure, temperature and flow rate of CO_2.

Effect of pressure: The effect of pressure on the extraction of lycopene from tomato pomace was investigated by conducting the experiments at pressures of 1000, 2000, 3000 and 4000 psi. The flow of CO_2 was kept 5 mL/min throughout the experiment. The low temperature of 40°C was chosen because of the thermally labile characteristics of lycopene.

Effect of temperature: The effect of temperature on the extraction of lycopene from tomato was investigated by conducting the experiments at temperatures of 40, 50, 60, 70 and 80°C. Both the extraction pressure and flow of CO_2 were kept constant at 4000 psi and 5 mL/min, respectively.

Effect of flow rate: The effect of flow rate on the extraction of lycopene from tomato pomace was investigated by conducting the experiments at flow rate of 4, 8 and 12 mL. The temperature and pressure were the optimum conditions found in previous experiments. The extraction time was 60 minutes for all experiments. The efficiency of the extraction process (Recovery %) was the ratio between the amount of lycopene estimated

Table 1: Chemical composition of fresh tomato used in the production of tomato pomace

Parameter	Content
Total soluble solid (%)	4.25±0.10
pH	4.10±0.05
Titratable Acidity as citric acid (%) of F.W	0.39±0.01
Ash (%) of F.W	0.70±0.04
Calcium (mg/100 g D.W)	145±5.03
Magnesium (mg/100 g D.W)	126±5.25
Phosphorus (mg/100 g D.W)	410±10.00
Ascorbic acid (mg/100 g F.W)	15.50±1.00
Total sugar (g/100 g F.W)	1.84±0.03
Reducing sugar (g/100 g F.W)	0.75±0.05
Fat content (%) of F.W	0.10±0.02
Total polyphenolic content, expressed as Gallic acid equivalent mg/100 g F.W	2.35±0.04
Flavonoid content expressed as equivalent Rutin mg/100 g F.W	1.09±0.01
Total lycopene content mg/100 g F.W	5.80±0.10
Total carotenoids expressed as β-carotene, mg/100 g F.W	8.36±0.15

Results are a means±SD of triplicate analysis
F.W: Fresh weight of tomato fruit
D.W: Dry weight of tomato fruit

in the extract to initial amount of lycopene determined by chemical solvent multiplied by 100.

Antioxidant activity of lycopene extracting using the DPPH method: It was the Sanchez-Moreno *et al*. (1998) method to determine the antioxidant activity of the extracts. A 0.1 mL of an ethanolic solution of the extract (obtained at 80°C and 4000 psi) at several concentrations was added to a 2 mL of methanolic DPPH solution (6 x 10^5 mol/L). The mixture was sonicated for 30 sec and its absorbance at 515 nm was measured right away (ABC_0). The absorbance was measured again at the same wavelength after 30 and 60 min (ABS_f) of reaction carried out in the absence of light. The percentage of DPPH reduction (G_R) in 60 min was calculated using the following equation:

$$AC = 100\,(1 - \frac{ABS_{(t)}}{ABS_{(c=0)}})$$

The antioxidant capacity (AC) is defined as the absorbance measured at any time ($ABS_{(t)}$) divided by the absorbance of the solution without extract:

Stability test for lycopene

Extracts up to 9 months: The stability of lycopene in tomato pomace extract (obtained at 80°C and 4000 psi) was tested. The extract was distributed into brown vials under N_2 and stored at 4°C in a low temperature and at room temperature. The concentration of lycopene extract was determined by spectrophotometric and HPLC methods as described previously. Two tests were carried out for lycopene extract stability, at 0, 1, 3 and 9 months. The stability of lycopene extract obtained from tomato pomace was calculated from the following equation:

$$\text{Stability (\%)} = \frac{\text{Lycopene concentration at 0, 1, 3 and 9 months of storage}}{\text{Initial lycopene concentration}} \times 100$$

Statistical analysis: Mean±standard deviation (SD) and were tested by one-way analysis of variance using SPSS computer programme (version 15). Differences between the means of treatments were tested using Least Significant Differences (LSD) test at $p<0.05$.

RESULTS AND DISCUSSION

Chemical composition of tomato fruit: The level of total soluble solids, pH, titratable acidity (as citric acid) and ascorbic acid in the fresh tomato fruits that were used in the production of tomato pomace (Table 1), were within the levels reported in the literatures (Davies and Hobson, 1981; Gupta *et al*., 2011; Meaza *et al*., 2007; Naz *et al*., 2011; Pila *et al*., 2010; Radzericius *et al*., 2012).

The difference with respect to total soluble solids of tomato fruits might be due to varietal difference (Thakur *et al*., 2011). The amount of total soluble solids are similar to that reported by Radhakrishnaish *et al*. (1987); Nainwal *et al*. (1992) and Ereifej *et al*. (1997) who reported 4.2 to 6.00 in different varieties of tomatoes. The acidity expressed as percent anhydrous citric acid in fruit was 0.39 which is within the range of titratable acidity (0.1-0.5%) reported by Ereifej *et al*. (1997).

The pH value of the tomato fruits was 4.20 and comparable with that reported by Pila *et al*. (2010) other pH values reported by many authors were ranged between 3.12 and 4.60 reported by Adubofuor *et al*. (2010) and Dobricevic *et al*. (2007), respectively. The pH values of tomato fruits are influenced by many factors such storage conditions (Moneruzzaman *et al*., 2009), which lead to change in total titratable acidity during storage due to the metabolic activities of living tissues during which depletion of converted into sugars and their derivatives or their utilization in respiration (Bhullar *et al*., 1981). Also, Rathore *et al*. (2007) indicated that the fluctuation of pH might be due to the variations in the titratable acidity is attributed due to increased activity of citric acid glyoxylase during ripening or reduction in acid content may be due to their conversion into sugars and further utilization in metabolic process during postharvest conditions. The cultivar effect has a strong effect on pH values of tomato fruits (Pila *et al*., 2010) and pH titratable acidity vary during maturation stage of tomato fruits (Shibli *et al*., 1995). Young *et al*. (1993) reported that of tomato fruits pH values were influenced by malic and citric acids accumulation which are genetically controlled property.

Table 2: Effect of pressure (in psi) on lycopene extraction efficiency (%) at a flow rate of 4 mL/min and temperature at 40°C, by HPLC method

Pressure (psi)	Lycopene (mg/g D.W)	Recovery (%)
1000	0.06±0.01[a]	2.90
2000	0.27±0.02[b]	12.50
3000	0.50±0.05[c]	23.10
4000	0.74±0.02[d]	34.20

*Results are a means±SD of triplicate analysis
*Means within a column with a different super script letter are significantly at ($p \leq 0.05$)
*D.W: Dry weight basis
*Initial lycopene content = 2.16 mg/g D.W

Table 3: Effect of temperature (°C) on lycopene extraction efficiency (%) at a flow rate of 4 mL/min CO_2 and pressure of 4000 psi, by HPLC method

Temp. (°C)	Lycopene (mg/g D.W)	Recovery (%)
40	0.74±0.02[a]	35.40
50	0.90±0.02[b]	43.10
60	1.08±0.04[c]	51.60
70	1.27±0.02[d]	60.80
80	1.60±0.03[e]	76.60

Results are a means±SD of triplicate analysis. Temp: Temperature
*Means within a column with a different super script letter are significantly at ($p \leq 0.05$)
*Initial lycopene content = 2.09 mg/g D.W

The ascorbic acid content of tomato fruits was 15.5 mg/100 g fresh weight, was lower than the range reported by Pokluda (2006) (31.4 to 39 mg/100 g F.W) and higher than that reported by Pila *et al.* (2010) (9.11 to 14.76 mg/100 g F.W). Esteves *et al.* (1984) indicated that an increase in ascorbic acid content in tomato fruit is thought to be an indication that the fruit is still in the ripening stage, while a decrease indicates a senescent fruit. To maintain the level of ascorbic acid in tomato is by lowering of respiration of fruits or oxidation of ascorbic acid content (Mapson, 1970). The variations in ascorbic acid content of tomato fruit reflect a range of genetic and environmental factors that result in quantitative variation across varieties (Dumas *et al.*, 2003). Similar conclusion was reached by Radzericius *et al.* (2012) concerning the amount of ascorbic acid in which it mainly depends on tomato genotype and less influence had fruit ripening stage. Radzericius *et al.* (2012) reported that the average vitamin C content of fully ripen tomato fruits varies from 10 to 20 mg/100 g F.W, in which the result of this research is positioned within this range.

The ash content of tomato fruit was 0.7% on fresh weight basis (Table 1). This obtained value is in close conformity to those obtained by Ereifej *et al.* (1997), who reported 0.5 to 0.7% ash in tomato on fresh weight basis. Hernandez-Suarez *et al.* (2008) obtained ash content, for different cultivars, was between 0.59-0.65. The mean ash content obtained in this work was similar to other data reported in the literature (Oke *et al.*, 2005). Shibli *et al.* (1995) reported that most tomato cultivars have ash percent fall in the range of 0.5 to 0.7% on fresh fruit weight basis.

The calcium, magnesium and phosphorus contents results presented in Table 1, were 145, 126 and 410 mg per 100 g dry weight basis, respectively. The calcium content was within the range reported by Shibli *et al.* (1995); Velisek *et al.* (1999) and Pokluda (2006). The calcium content in tomato fruits was found to be influenced by many factors such as tomato cultivars, growing year and harvest data and these effects were variable and no trend was possible (Pokluda, 2006).

The content of magnesium was 126 mg/100 g DW on average and was lower than the range found by Pokluda (2006). Similar to calcium content, magnesium is strongly influenced by tomato cultivars, growing year and harvest data (Pokluda, 2006). Many investigations showed that the growing time, which is connected mostly with light and thermal conditions, significantly affects the quality of tomato including its chemical composition (Thybo *et al.*, 2006; Hernandez-Suarez *et al.*, 2008).

The value of phosphorus content was 410 mg/100 g of dry weight (Table 1), which is within the range of phosphorus in tomato fruits found by Kallo (1985) where he indicated that tomato fruit contained 0.2 to 0.8 g phosphorus/100 g of dry fruit weight. The phosphorus content of tomato fruits is also influenced by the same factors that affecting calcium content as previously mentioned.

Results on total sugar, reducing sugar and fat content are presented in Table 1. The total sugar content was found to be 1.84 g/100 g on fresh weight basis. Similarly, Loiudice *et al.* (1995); Thakur and Kaushal (1995) and Gupta *et al.* (2011) reported very close range of total sugar in tomato fruits. One of the major factors that affect the total sugar contents is the genotype and varieties of tomato fruits (Hussain *et al.*, 2010). The reducing sugar content (0.75% of F.W) was much lower than that reported by Hussain *et al.* (2010), who found that reducing sugar content of different tomato varieties were 2.39 to 2.74% of fresh weight. Total sugar varied independently of the fruit maturity (Radzericius *et al.*, 2012). They reported that the results obtained for total sugars and reducing sugars were 1.13 to 2.63 g/100 g F.W and 0.55 to 0.77 g/100 g F.W, respectively. Kowalczyk *et al.* (2011) showed that sugar content range was 1.76 to 3.66 g/100 g F.W, they also reported that tomato date of harvest had strong effect on sugar content, with a highest content in July than in September. Shi *et al.* (1999) stated that the content of sugars increased simultaneously with tomato fruit ripening, because ripening of tomato is characterized by the synthesis of more sugars. The overall average content of reducing sugars in fresh tomato for five cultivars was

between 0.4 to 4.4% of fresh weight with a significant effect of the cultivation method but not sampling periods (Hernandez-Suarez *et al*., 2008).

The fat content of the tomato fruits used in preparing the pomace was 0.1% on fresh weight basis (Table 1). This result is in accordance to the results of Gupta *et al.* (2011) and Anita (1997) when reported that the crude fat content in different tomato genotypes varied from 1.54 to 1.89% on dry matter basis. Many researchers found that tomato fruits contain traces of fat Splittstoesser (1990) and Shibli *et al.* (1995).

The total phenolic content of fresh tomato fruits was 2.35 mg per 100 g on fresh weight basis (Table 1). This result is in agreement with results obtained by Marsic *et al.* (2011) but lower than that reported by Helmja *et al.* (2007). Polyphenols to lycopene in tomato fruits to have antioxidant capacity, which repeatedly exceed the capacity of ascorbic acid. George *et al.* (2004) reported a large variations among the polyphenol content of different tomato cultivars. The polyphenol content in tomato fruits is influenced by many factors such as the genotypes, temperature, in which the thermal stress induces the accumulation of phenolic compounds. At 35°C, the polyphenol level is double that produced at 25°C (George *et al*., 2004).

The total flavonoid content of tomato fruits is 1.09 mg/100 g fresh weight basis (Table 1), which is lower than the results obtained by Martinez-Valverde *et al.* (2002) and Slimestad *et al.* (2008) and higher than that obtained by Jacob *et al.* (2010) of about 0.53 mg/100 g fresh weight basis. The quantity of flavonoids present in tomato is influenced by genotype, environmental conditions, extraction procedure and cultural practices. The effect of these factors give a wide variation in the flavonoids content in tomato fruits (Slimestad and Verheul, 2009). Flavonoids are potent antioxidant *In vitro* and epidemiological studies suggest a direct correlation between high flavonoid intake and decreased risk of cardiovascular diseases, cancer and other age-related diseases (Colliver *et al*., 2002).

The lycopene content of tomato fruit was determined as 5.80 mg/100 g on fresh weight basis (Table 1). This lycopene content was higher than the results obtained by Nainwal *et al.* (1992); Anita (1997) and Gupta *et al.* (2011) but lower than the range (8.55-13.6 mg/100 g F.W) reported by Radzericius *et al.* (2009). The level of lycopene in tomato is critically influenced with the environmental temperature and its production is inhibited at a temperature above 32°C (Farkas, 1994), lycopene content in tomato harvested in greenhouse grown (8.30 mg/100 g F.W) than in field-grown (5.92 mg/100 g F.W) at different harvesting time (Brandt *et al*., 2003). Lycopene forms more readily at lower temperatures 16 to 26°C being the most favorable temperature range (Helyes *et al*., 2006), also they found that lycopene contents determined fundamentally by the genetic nature of cultivars but is also strongly affected by environmental factors. Lycopene is one of the two most important carotenoids in fruits of tomato, which determined fruit red color and β-carotene, which accounts for approximately 7% of the tomato carotenoids. Therefore, tomato products and their quality can be well characterized by the content of these elements (Nguyen and Schwartz, 1999). Lycopene importance to human health is in its capacity to act by quenching the singlet oxygen and scavenging peroxyl radicals, which cause the oxidative modification of lipids, proteins, low density lipoprotein (LDL) particles and DNA (Oshima *et al*., 1996; Tapiero *et al*., 2004).

The total carotenoids content of the tomato fruits was 8.30 mg/100 g on fresh weight basis (Table 1). This value was higher than the range of the total carotenoids content reported by Pila *et al.* (2010) which is between 2.94 to 5.23 mg/100 g on fresh weight basis, where the tomato cultivars showed significant effect on the total carotenoids content. Similar results were obtained by Abushita *et al.* (2000), where tomato cultivars showed differences in carotenoids content Radzericius *et al.* (2012) found that the highest amount of β-carotene was found in fully ripen fruits.

The results indicated that tomato fruits contained good nutritional profile and physiochemical properties and can be used in tomato processing industry. Maturity at harvest is very important to composition and quality of tomatoes. Tomato fruit quality has been assessed by the content of chemical compounds such as total soluble solids, acidity, sugars etc. Based on the achieved results, it can be concluded that the tomato fruits used in the research satisfactory fruit quality.

Supercritical CO_2 Extraction of Lycopene from Tomato Pomace: To investigate the effect of pressure, temperature and CO_2 flow rate and this optimize the operating conditions, supercritical CO_2 extraction was carried out under the following condition: (1) pressure from 1000 psi to 4000 psi at constant temperature at 40°C and CO_2 flow rate at 4 mL/min (2) temperature at 40, 50, 60, 70 and 80°C at constant CO_2 flow rate at 4 ml/min and constant optimum pressure value determined in the first experiment of the optimization process (3) CO_2 flow rate of 4, 8 and 12 mL/min under a constant optimum pressure and temperature, all experiments at the same extraction period of 60 min. Table 2 indicates the effect of pressure on extraction yield (mg/g DW) and recovery (%) of the lycopene from tomato pomace at constant temperature of 40°C and CO_2 flow rate of 4 mL/min. it can be seen that increasing the operating pressure from 1000 psi to 4000 psi at 1000 psi intervals resulted in a gradual increase in the yield of extraction as well as in the recovery of lycopene. Similar results were obtained by Rozzi *et al.* (2002) and Huang *et al.* (2008).

It is well known that an increase in pressure at constant temperature enhances solvent density and at higher densities, molecular interaction between the solvent and the solute boosted, resulting in greater dissolution of the solute (Rozzi and Singgh, 2002; Turner *et al.*, 2001). But at constant temperature at 40°C, an increase in pressure from 1000 psi to 4000 psi did not improve the total amount of lycopene extracted, where the maximum lycopene extracted was 34.20 and 35.80%, for HPLC and spectrophotometric methods, respectively (Table 2). The reason for this might be that the increased pressure caused compacting of the sample and channeling of the CO_2 flow, resulting in the restriction of CO_2 movement into and out of the tomato pomace sample.

The efficiency of the extraction depends on numerous conditions including polarities of solvent and solute and the phase equilibria beside pressure and temperature conditions (Hauthal, 2001; King, 1995; Rozzi and Singgh, 2002; Turner *et al.*, 2001). To achieve maximum extraction to lipid soluble materials, such as lycopene, experimentally optimizing the procedure by varying the pressure, temperature and flow rate conditions can determine the most efficient extraction conditions for the system being used (Montanari *et al.*, 1999; Spiricigo *et al.*, 1999).

Kassama *et al.* (2008) reported that the lycopene content tend to peak with increased pressure at moderate temperature, this change reflects the solubility behaviour of lycopene in supercritical CO_2 as density affected by the relative change of pressure. Vasapollo *et al.* (2004) found that the extractions of lycopene at constant temperature, the density of the fluid, therefore, the solubility of lycopene increases with the increasing of pressure. Similar conclusion was received by Gomez *et al.* (2002) in which the enhancement of solvating powers can be reached as the result of increasing pressure and consequently the CO_2 fluid density. Wei *et al.* (2005) reported that the solubility of carotenoid in supercritical CO_2 is proportional to the density of supercritical CO_2 but it was not a linear relationship. King (1990) explained that there is a pressure region where significant increase occurs in the solubility of the solute in supercritical fluid. The most relevant process parameter is the extraction pressure that can be used to tune the selectivity of the supercritical fluid (CO_2) (Reverchon and De Marco, 2006). The same authors concluded that the general rule is: the higher is the pressure, the larger is the solvent powers and the smaller is the extraction selectivity. Frequently, the solvent power is described in term of the supercritical CO_2 density at a given operating conditions. Therefore, using pure supercritical CO_2 at higher pressure can be sufficient to compensate the absence of co-solvent and has the advantage of producing a solvent free extract (Reverchon and De Marco, 2006). Nobre *et al.* (2009) reported that the recovery of lycopene depended on the content of the compound, like lycopene, in the starting material and increased with increase in pressure and solvent flow rate and with a decrease in the particle size (Nobre *et al.*, 2009). At low pressure, less than 4000 psi, the poor recovery of lycopene could be probably be due to the fact that lycopene solubility is much lower at these lower pressure values (De la Fuente *et al.*, 2006; Gomez *et al.*, 2002) and also because at lower pressure, there could possibly exist a competition between carotenoids and lipids for supercritical solvent and so the extraction of lycopene occurred only after the major part of lipids which have higher solubility in CO_2 were extracted. An increase in the pressure level above 200 bar led to an unexpected reduction in the extraction yield (Bimakr *et al.*, 2009, 2011). This unexpected result can probably be related to the reduced diffusion rates of the extracted compound from the plant matrix to the supercritical fluid medium (Rezaei and Temelli, 2000).

Finally, solubility enhancement at higher pressure was obtained in result of major role of diffusion in the mass transfer rates of the extractable materials from the sample matrix into the supercritical fluid. An increase of pressure can result in an increase in the fluid density which alter solute solubility.

Therefore, it is interesting to control the composition of the extract using pressure. There are other factors that are responsible for the result obtained, there are the volatility and polarity of the extracted analyte (Gomez *et al.*, 2007; Wang *et al.*, 2008).

Table 3 shows the effect of temperature on the extraction yield (mg/g DW) and recovery (%) of lycopene from tomato pomace at five temperature levels of 40, 50, 60, 70 and 80°C at 4000 psi constant pressure.

In the present study, the extraction yield (mg/g DW) and recovery of lycopene from tomato pomace increased with temperature and the highest yield and recovery was obtained at 80°C and 4000 psi pressure. These conditions resulted in the extraction of 76.20% and 78.20% for HPLC and spectroscopic methods, respectively. Similar results were reported by Rozzi *et al.* (2002) and Huang *et al.* (2008) where lycopene extraction was increased with an increase in the extraction temperature.

The density of CO_2 at constant pressure is reduced with increasing temperature and leading to reduce the solvent power of supercritical CO_2. However, the increase in temperature will accelerate mass transfer and improve the extraction yield (Wang *et al.*, 2008). The increase of temperature can increase the vapour pressure of the extractable compounds. Thus, the tendency of the compounds to be extracted is increased to pass in the supercritical fluid phase (Reverchon and De Marco, 2006), therefore, it is difficult to predict the effect of temperature (Bimakr *et al.*, 2009). In this manner, the solute vapour pressure played a key role leading to increase the extraction yield and recovery of lycopene due to temperature increase.

Table 4: Effect of CO_2 flow rate (ml/min) on lycopene extraction efficiency (%) at 80°C and 4000 psi, by HPLC method

CO_2 FR (mL/min)	Lycopene (mg/g D.W)	Recovery (%)
4	1.57±0.03^{ac}	76.60
8	1.70±0.0^{b}	82.50
12	1.62±0.04^{ac}	79.02

FR: Flow rate
*Results are a means±SD of triplicate analysis
*Means within a column with a different super script letter are significantly at ($p \leq 0.05$)
*Initial lycopene content = 2.05 mg/g D.W

Table 5: Results of DPPH analysis of the tomato pomace extract obtained at 4000 psi and at 80°C

EC (mg/mL)	DPPH Reduction G_R (%)
0.10	9.40
0.20	14.35
0.40	18.80
0.80	24.80
1.20	28.70

EC: Extract concentration

Table 6: Antioxidant capacity (AC%) of the tomato pomace extract obtained at 4000 psi and at 80°C

	---------- Antioxidant capacity AC (%) ---------		
EC (mg/mL)	0	30 min	60 min
0.10	3.5	14	17
0.20	5.5	24	26
0.40	6.0	30	34
0.80	8.0	41	45
1.20	12.5	48	52

EC: Extract concentration

An increase in the density of CO_2 is associated with an increase in its solvating power, the decrease in the diffusivity reduce the interaction between the supercritical fluid and the solute contained within the matrix, rendering poor recoveries in the extraction process (Macias-Sanchez *et al*., 2007). It can be concluded that the solubility of any supercritical solute depends on a complex balance between fluid density and solute vapour pressure, both controlled by temperature and pressure conditions employed. Generally; it can be concluded that below the critical pressure of CO_2, an increase in temperature decreases the solvency due to the decrease in fluid density. Above this pressure an increase in temperature can improve the extraction efficiency despite the decrease in fluid density, since the vapour pressure of the solute (lycopene) is increased. Table 4 shows the effect of CO_2 flow rate on yield (mg/g DW) and recovery (%) of lycopene extracted from tomato pomace. The results showed that the increase in flow rate of CO_2 from 4 to 8 mL/min increased the yield (1.57 to 1.70 mg/g DW) and recoveries (81.20 to 76.60%) of lycopene extracted from tomato pomace.

Increasing the flow rate of CO_2 from 8 to 12 mL/min showed a drop in the yield and recovery of lycopene extraction measured by the two analytical methods. The flow rate represents the amount of solvent whether it is enough or not to remove lycopene molecules from tomato pomace cells through the extraction vessel. Topal *et al*. (2006) reported that increasing the flow rate of CO_2 resulted in decrease in the amount of lycopene extracted at a later stages of extractions. One of the reasons for this may be the channeling effect, where by the solvent is formed through the sample at such a high flow rate that it passes around the solid matrix and does not diffuse through the pores within the sample (Tonthubthimthong *et al*., 2001). For low flow rate, insufficient amount of CO_2 led to reduction in the extraction yield and after a certain time, no more lycopene was recovered from the tomato pomace matrix even at higher flow rate (Topal *et al*., 2006).

The fact that the highest flow rate presented a drop in the lycopene recovery from tomato pomace could be due to channeling effects and the impossibility of reaching equilibrium between solid and liquid phase at such a high flow rate (Nobre *et al*., 2009). The CO_2 flow rate is a crucial parameter in supercritical CO_2. The proper selection of this parameter has the scope of producing the complete extraction of the desired compound in the shorter time. This parameter is connected to the thermodynamics (solubility) and the kinetics of the extraction process in the specific raw matter (Reverchon and De Marco, 2006). CO_2 flow rate is a relevant parameter that affects the overall process velocity specially when the process is controlled by an external mass transfer resistance or by equilibrium and consequently it determines the extraction rate.

Antioxidant activity of the tomato pomace extract and lycopene extract stability: The percentage of DPPH reduction (G_R) results in 60 min for the tomato extract obtained at 4000 psi and 80°C are presented in Table 5. The range of the percentage of DPPH reductions was between 9.40 to 28.70% for extract concentration between 0.1 to 1.20 mg/mL. It can be noticed that the G_R values increase with extract concentration. These results are in agreement with that obtained by Egydio *et al*. (2008) where the G_R values showed an increase with extract concentration.

The antioxidant capacity (AC%) values of the tomato pomace extract obtained at 4000 psi and 80°C are presented in Table 6.

The results showed an increase in the values of antioxidant capacity with extract concentration and time up to 60 minutes. After 60 minutes of incubation the range for antioxidant capacity values was between 17 to 52% for 0.1 and 1.2 mg/mL, respectively.

The scavenging capacity of the extract to DPPH radicals is in a concentration-dependent fashion (Dorman *et al*., 2004). The results of the DPPH free radical scavenging assay suggest that the extracts with different lycopene concentration are capable of scavenging free radicals in

Table 7: Stability of lycopene concentration (%) in tomato pomace extract obtained at 4000 psi and at 80°C, under different storage conditions

Initial time t = 0		1 month		3 months		9 months	
4°C	Room temp.	4°C	Room temp.	4°C	Room temp.	4°C	Room temp.
(a) Lycopene concentration determined by spectrophotometric method							
100%	100%	96%	88%	93%	82%	85%	74%
(b) Lycopene concentration determined HPLC							
100%	100%	93%	84%	90%	80%	83%	70%

solution and thus may be able to prevent the initiation of free radical-mediated chain reactions by stabilizing reactive species before they can participate in deleterious reactions (e.g the abstraction of hydrogen from susceptible polyunsaturated fatty acids). The extract of lycopene from tomato pomace showed a similar results in scavenging capacity of the DPPH radical as that reported by Basuny *et al.* (2009) who found four levels of the concentrations (100, 200, 400 and 800 ppm) of tomato lycopene showed a very high antioxidant capacity of 40 and also reported that the reaction of grade lycopene and BHT with DPPH were similar to tomato lycopene with DPPH in term of antioxidant capacity. The results of this research showed that tomato lycopene extract was a good scavenger for interacting with the nitrogen-centered stable free radical, DPPH. The DPPH test provided direct information about the reactivity of lycopene with a stable free radical, indicating that lycopene extract acted as a potent and direct free radical scavenger. Similar conclusion was reached by Hsiao *et al.* (2004). It has been noted before that antioxidant properties of tomatoes depend largely on lycopene content (Martinez-Valverde *et al.*, 2002).

The results indicate that lycopene can be seen as a powerful antioxidant that protects the body from the harmful effect of the oxidant. Studies have concluded that dietary antioxidants such as lycopene reduced the risk of developing cancers (Khan *et al.*, 2008). The antioxidant capacity of lycopene offers protection against-radiation induced damage to cells (Srinivasan *et al.*, 2007) and to increase antioxidant enzyme activity (Bose and Agrawal, 2007), reduction of plasma cholesterol and C-reactive protein (Jacob *et al.*, 2008) and prevention of lipid and DNA damage (Matos *et al.*, 2006). This work can give us a good potential to start establishing a new industries in Jordan based on the use of a waste material such as tomato pomace to produce products like food supplements, cosmetic product to protect skin and food colorant with high antioxidant capacity.

The results of lycopene stability (%) in tomato pomace extract obtained at 4000 psi and 80°C under different storage conditions (i.e., 4°C and at room temperature 23-27°C) during a storage period of 9 months are presented in Table 7.

The stability of lycopene concentration (%) was determined by two methods (Spectroscopic and HPLC methods), where the results of the spectroscopic method showed a little bit higher values than that of HPLC method due to interference problem with spectroscopic method from other components of extract. The results indicated that the low storage temperature at 4°C maintained significantly higher stability to lycopene concentration than that at room temperature (i.e., 23-27°C). Similar results were obtained by Osterlie and Lerfall (2005), who showed that storage temperature (4 and 8°C) has little influence on the lycopene content. Lycopene was found to be sensible to an increase in the storage temperature and some metallic ions such Cu^{+2} and Fe^{+2} that catalyze its oxidation (Shi and Le Maguer, 2000). Different food components are considered a serious factors that have an effect on lycopene autoxidation and consequently the stability of lycopene in the extract (Xianquan *et al.*, 2005). Galicia *et al.* (2008) concluded that the retention of lycopene depends on illumination, temperature and storage time and whether the pigment is extracted from blanched or unblanched tomatoes. The stability of lycopene may be variable in different food systems because of the complex nature of food components (Anguelova and Warthesen, 2000).

Conclusion: The results of this study indicate that operating conditions (pressure, temperature and flow rate) are crucial points in supercritical carbon dioxide extraction of lycopene from dried tomato pomace. A total 1.70 mg of lycopene/g of sample of tomato pomace, 82.50% of the total lycopene content was recovered at optimum operating conditions which are as follows: pressure 4000 psi, temperature 80°C and CO_2 flow rate 8 mL/min.

Pure high quality lycopene can easily be extracted and recovered from tomato pomace without the use of modifiers by using supercritical fluid technology at the operating conditions. To make use of the highly functional substance lycopene, which is a natural antioxidant the protect people from a broad range epithelial cancers.

The results obtained confirm the potential of supercritical fluid extraction for developing environmentally friendly methods suitable to extract lycopene and suggest the possibility of taking advantage of very economical and natural sources like surplus of tomato production and waste during tomato processing in Jordan to obtain high-added value products.

Quantitative assessment of lycopene extract showed relatively high antioxidant power at low lycopene concentration. The lycopene extract showed, also, a high stability after 9 months of storage at 4°C and room temperature.

This part of the study is very critical in how to preserve lycopene during processing and storage specially with an increasing understanding of the health benefits of lycopene. Also, this research activity on lycopene stability will benefit the food industry by enhancing the quality of tomato pomace extracts and greatly improve the competitiveness and nutritional quality of food products containing lycopene extract. This type of work will also improve the quality of nutraceutical products. In addition, This work can be a model to establish an industry in Jordan based on the use of abundant surplus of tomato fruits and tomato processing waste in Jordan, a value-added product for food, pharmaceutical and cosmetic industries. This type of industry in Jordan will enhance the agricultural sector to obtain a new technology supporting this important sector for Jordan economy.

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