IJFANS
International Journal of Food And Nutritional Sciences

Volume 7, Issue 3, July 2018, www.ijfans.com e-ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

IJFANS International Journal of Food and Nutritional Science

e-ISSN 2320 -7876 www.ijfans.com
Vol. 7, No. 3, July 2018

Research Paper ***Open Access***

ANTIOXIDANT CONTENT IN SKIN AND SEEDS OF THREE TOMATO VARIETIES (Solanum lycopersicum) GROWN IN 2 REGIONS OF MEXICO

Diana Itzel Rodríguez Durán[1], Sofía Arellano Cárdenas[1], Patricia Rosales Martinez[1], Felipe García Ochoa[1] and Ma. del Socorro López Cortez[1]*

*Corresponding Author: Ma. del Socorro López Cortez, ✉ socolc@prodigy.net.mx

Received on: 23rd May, 2017 Accepted on: 25th October, 2017

Several studies have shown that tomato (*Solanum lycopersicum*) is an excellent source of antioxidant compounds such as lycopene and other carotenoids. More recent studies have revealed the presence of different polyphenolic compounds in the skin of this fruit, such as resveratrol, quercetin and rutin, which have shown positive effects in the prevention and treatment of some types of cancer, such as colon, stomach and prostate cancer. The present study identified the main polyphenolic compounds in three tomato cultivars (Saladet, Bola and Cherry) harvested in Mexico. Physicochemical properties showed that samples were of optimal quality, and antioxidant activity was found to be highest in the skin of the tomatoes, regardless of the variety or geographical origin. Fruits from the Cherry cultivar showed the highest concentration of phenolic compounds (98.59 mg GAE/100 g), with an antioxidant activity of 234 µm TE/100 g. Chlorogenic acid, rutin, naringin, resveratrol and quercetin were identified by HPLC-RP in the skin and seeds of Mexican tomatoes, and their concentrations depend on the variety and geographical origin of the fruits.

Keywords: Tomato, Phenolic, Antioxidants, *Solanum lycopersicum*

INTRODUCTION

Worldwide, non-infectious diseases (cancer, chronic respiratory diseases and diabetes mellitus) are currently the main cause of mortality (63%) (Cause-specific mortality, 2009). Given the above, five main risk factors have been identified: high blood pressure, high cholesterol levels, obesity, physical inactivity, and insufficient intake of fruits and vegetables (World Health Organization, 2009). As such, the World Health Organization (GINA/OMS, 2010) promotes an increase in the intake of fruits and vegetables for the prevention of disease, since these foods are rich in vitamins, essential micronutrients, fiber, vegetable proteins and phytochemicals, being an essential part of a healthy diet (Boeing *et al.*, 2006).

One of the vegetables considered an excellent source of antioxidant compounds, such as lycopene, is tomato (Vitale *et al.*, 2010). Recent studies have shown the presence of polyphenolic compounds – e.g., chlorogenic acid, rutin, naringin, resveratrol, quercetin – in both the skin and the seeds of this fruit (Ragab *et al.*, 2006). Polyphenols have been demonstrated to aid in the treatment of some chronic diseases such as colon, stomach and prostate cancer (Liu *et al.*, 2008). They also help regulate LDL cholesterol in cardiovascular disease (Saleem *et al.*, 2005) and insulin concentration in diabetes mellitus (Bravo, 1998).

Lycopene is the most abundant and studied antioxidant in tomato (Slimestad and Verheul, 2009). Several studies were conducted to identify other phytochemicals, and these studies led to innovations in the fruit, such as transgenic tomatoes with increased levels of resveratrol and other

[1] Escuela Nacional de Ciencias Biológicas-IPN. Departamento de Biofísica. Prol. de Carpio y Plan de Ayala, Col. Casco de Sto. Tomás, C.P. 11340, México D.F.

phytonutrients (Paradiso *et al.*, 2013) to improve the characteristics of the fruit. These bioactive compounds have been quantified in several foods of vegetable origin, such as grape, peanuts, nuts and wine (Wu *et al.*, 2013; Rosales-Martínez *et al.*, 2014; and Acuña-Avila *et al.*, 2016).

On the other hand, production of tomatoes in Mexico is a very important horticultural activity, and this country is the main producer worldwide, mostly exporting to Canada and the United States (SAGARPA, 2010; and Ríos *et al.*, 2014). However, in the last few years, tomato overproduction has caused considerable waste of crops and therefore of bioactive compounds (SAGARPA, 2010; and Bastida, 2012).

In the present study, antioxidant compounds other than lycopene, such as chlorogenic acid, rutin, naringin, resveratrol and quercetin, were analyzed and quantified in three tomato cultivars from Mexico, with the aim of proposing the use of surplus fruit as a source of phytochemicals to produce functional foods.

MATERIALS AND METHODS.

Raw Material

Three types of tomato (*Solanum lycopersicum*) at different ripening stages were collected in the field: *Saladet, Cherry* and *Bola*. The harvest dates for each region were as follows: San Luis Potosí, June 16, 2013; Estado de México, September 18, 2013. Cherry tomato samples were obtained from a greenhouse in each region in October 2012. All the samples were transported under refrigeration to the laboratory, where they were selected and washed. The fruits were stored at room temperature until they reached the "Red" maturation stage as specified by the Official Mexican Standard, which establishes that over 90% of the surface of the fruit must be red in color (NMX-FF-031-1997-SCFI, 1997).

Sample Preparation

The fruits were separated into three different fractions: skin, pulp and seeds. The pericarp (skin) was carefully detached from the pulp manually, and its thickness ranged from 0.15 to 0.25 mm. The seeds were separated from the locules, and the mucilage was removed using a strainer. Pulp was the remaining fraction of the tomatoes after these operations. Finally, 18 samples of the three different fractions from each variety and geographical origin were obtained and stored at -20 °C in dark glass flasks until analysis.

Physicochemical Parameters

The percentage of moisture was measured according to (AOAC, 1975). Total evaporation of water from the whole fruit, pericarp and seeds was carried out in a BINDER stove at 110 °C until constant weight was reached (3 h).

The acidity of the juice from the tomato pulp was measured by volumetric titration using 0.01 N NaOH according to (NMX-F-102-S, 1978). The total acidity was calculated in terms of the predominant acid, in this case citric acid, according to equation 1:

$$\%\ citric\ acid = \frac{(P-T)*N*P\ Eq.*V*100}{m*a} \quad ...(1)$$

where P = volume of 0.01 N NaOH (mL) used in the titration of the sample; T = volume of 0.01 N NaOH (mL) used in the titration of the blank; N = normality of NaOH (meq/ml); P Eq = equivalent mass of citric acid (0.064 g/meq); V = calibrated volume (mL); m = mass of the sample (g); a = aliquot (mL).

The *pH* of every sample of fresh tomato juice was measuring using a pH 21 HANNA potentiometer, according to the Official Mexican Standard (NMX-F-317-S, 1978). *Relative density* was measured with a pycnometer (NMX-V-032-S, 1980). The *soluble solids* (°Brix) were measured using an ATAGO "Abbe" refractometer according to (NMX-F-103, 1982), quantifying the percentage of solids dissolved in freshly extracted tomato juice.

Methanol Extraction of Phenolic Compounds

Soluble phenols were extracted from fresh skin, pulp and seeds according to the method described by Singleton and Rossi (1965) with some modifications. Two grams of sample were weighed and mixed with 10 mL of methanol solution (100%) and stirred for 24 h in the dark at room temperature. The solution was filtered under vacuum using *Whatman* number 5 filter paper. The extractions were performed in triplicate. The extract was stored in a freezer for no more than four days before analysis.

Total Phenols (TP)

The total phenol contents were quantified following the method of Singleton and Rossi (1965). One hundred microliters of the polyphenolic extract and 100 µL of Folin-Ciocalteu reagent were mixed and maintained in the dark for 3 min at room temperature; afterwards, 2 mL of a 5% sodium bicarbonate solution and 2.8 mL of distilled water were added to obtain a final volume of 5 mL, and the mix was kept for 1 h in the dark at room temperature. Then, absorbance was measured at 725 nm using 100% methanol as a standard.

The TP contents of samples were obtained by comparison with a standard calibration curve prepared using gallic acid in concentrations of 0 to 500 µg/mL, with $R^2 = 0.9945$.

Antioxidant Capacity

Antioxidant capacity was determined by chelation of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams *et al.*, 1995). A 0.1-mL aliquot of the methanol extract of each sample was mixed with 3.9 mL of a 0.004% (w/v) DPPH methanol solution. Absorbance of DPPH was immediately measured at 517 nm. Samples were left to react with DPPH for 30 min the dark, and then the absorbance was measured using pure methanol as the blank. The percentage of inhibition compared to the initial absorbance of the DPPH-methanol solution was expressed as equivalent µmoles of 6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid (TROLOX) per 100 g of fresh sample (molesTE/100 g mf). A reference curve was made, including the regression equation relating the concentration of TROLOX to the inhibition percentage for each methanol solution. The percentage of discoloration of DPPH was calculated according to (Equation 2).

$$Percentage\ of\ inhibition = \frac{Absorbance\ of\ blank - Absorbance\ of\ sample}{Absorbance\ of\ blank} * 100 \quad ...(2)$$

Analysis of Phenolic Compounds by HPLC

This procedure was carried out according to the method of Nicoletti *et al*. (2007) with some modifications. Before the analysis, the methanol extracts described above were filtered with nylon syringe filters (0.45 µm in diameter). For the separation of Individual Phenols (IP), a *Beckman-Coulter* High-Performance Liquid Chromatography (HPLC) system was used, equipped with 168 DAD, 120 solvent modules, manual injection in reverse Phase with Diode Arrangement (PDA), and a controller and detector of UV/visible light. Chromatographs showing absorbance at 280, 306, 320 nm were obtained; these correspond to the wavelengths of maximum absorbance for naringin, resveratrol and chlorogenic acid respectively and 370 nm was used for rutin and quercetin. Separation was performed on a reversed-phase C18 Waters Spherisorb ODS column with a length of 250 mm, a diameter of 4.6 mm, and an internal particle size of 5 µm. The separation column was kept at a temperature of 30 ± 1 °C, and the flow rate of the mobile phase was 1 mL/min. Phenolic compounds were separated by a gradient obtained using a mixture of 0.5% formic acid in water (Phase A) and acetonitrile (Phase B). Both phases were filtered using vacuum and inorganic membrane filters (0.2 µm thick) in an environment that was strictly free of lint and dust particles. The amount of both samples and standards injected was 20 µL. The elution program was as follows: 8 min linear gradient increasing from 10 to 18% of acetonitrile in water acidified with formic acid at 0.5% (v/v); 2 min isocratic with 18% acetonitrile; 5 min linear gradient increasing from 18 to 25% acetonitrile; 3 min linear gradient increasing from 25 to 35% acetonitrile; 10 min isocratic with 35% acetonitrile; 2 min increasing the gradient to 60% acetonitrile to conclude the elution; 5 min to return to the 10% initial condition.

Quantification of single phenols was made according to the external standard method, drawing HPLC standard calibration curves for chlorogenic acid (5-100 mg/L), rutin (20-160 mg/L), naringin (5-20 mg/L), resveratrol (0.1-5 mg/L) and quercetin (5-35 mg/L).

Statistical Analysis

All data are reported as the mean ± standard deviation of three repetitions. A one-way analysis of variance (ANOVA) was performed and interpreted using a lowest significant difference of $p < 0.05$, followed by a Tukey test ($p < 0.05$). Both analyses were made using the statistics software Minitab version 16.

RESULTS AND DISCUSSION.

Physicochemical Parameters

Table 1 shows the physicochemical parameters of the three selected tomato varieties: *Cherry* presented the highest value for acidity, followed by *Saladet* and *Bola*. All are within the acidity intervals reported by Nuez *et al*. (2001) of 0.3-0.4% and by Zambrano *et al*. (1996) of 0.20-0.40%. It has been reported that acidity decreases significantly as the fruit ripens, due to the degradation of polymeric carbohydrates (starch, pectic compounds and hemicellulose), increasing the concentration of monosaccharides. The differences between samples are due to the variety and origin (Zambrano *et al.*, 1996). The mean pH values were 3.92, 4.34 and 4.44 for *Cherry, Saladet* and *Bola*, respectively. These values are also within the intervals of 4-4.6 reported by Guzmán (2004) and 4.34-4.36 reported by Odriozola *et al*. (2008) in tomatoes from the varieties *Rambo, Durinta, Bodar, Pitenza, Cencara* and *Bola*. Nuez *et al*. (2001) reported pH values of 4.2 and 4.4 in tomato pulp, as well as 4.5 °Brix, which are similar to the values observed in the present study. Zambrano *et al*. (1996) reported values of 4.4-4.9 °Brix and mentioned a

Table 1: Physical-Chemical Properties of Tomato Juice (Mean ± Standard Deviation, n = 3)

Variety/Región	Titratable Acidity (Citric Acid %)	pH	Soluble Solids (°Brix)	Relative Density (g/mL)
Cherry				
San Luis Potosí	0.35 ± 0.02^{a}	3.80 ± 0.04^{d}	3.7 ± 0.05^{c}	1.017 ± 0.0016^{c}
Edomex	0.33 ± 0.01^{ab}	4.03 ± 0.05^{c}	3.8 ± 0.04^{c}	1.005 ± 0.0230^{c}
Saladet				
San Luis Potosí	0.28 ± 0.02^{b}	4.39 ± 0.01^{a}	4.5 ± 0.01^{b}	1.023 ± 0.0002^{bc}
Edomex	0.22 ± 0.02^{c}	4.28 ± 0.01^{b}	4.8 ± 0.24^{b}	1.025 ± 0.0012^{abc}
Bola				
San Luis Potosí	0.21 ± 0.01^{c}	4.42 ± 0.01^{a}	5.7 ± 0.21^{a}	1.051 ± 0.0068^{ab}
Edomex	0.19 ± 0.01^{c}	4.46 ± 0.01^{a}	5.6 ± 0.08^{a}	1.057 ± 0.0010^{a}

Note: ^{a,b,c} Different letters in the same column indicate significant difference (Tukey method) ($p<0.05$).

significant increase in soluble solids during the ripening of the fruit.

The relative densities of tomato juice (1.0196-1.0288) from *Saladet* and *Bola* are within the interval established by the Mexican Official Standards, unlike *Cherry*, which showed a lower relative density, probably due to the lower content of soluble solids of this variety, as indicated by a low Brix value.

All physicochemical values agree with those reported by the National Medical Sciences and Nutrition Institute Salvador Zubirán (INCMNSZ) in Mexico (Muñoz *et al.*, 2010), as well as (FAO, 1985), indicating that the raw materials selected for the present study had good quality and were at the optimal ripening stage.

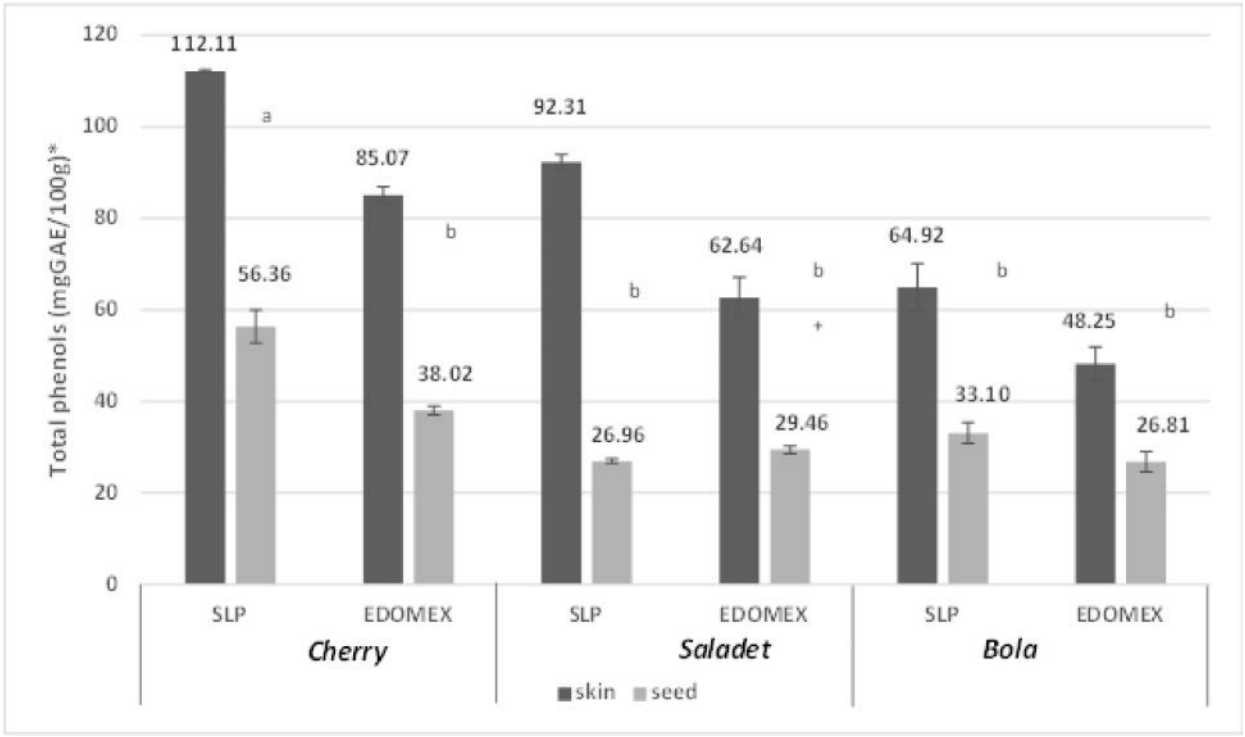
Moisture contents in the three cultivars were 90.47 ± 1.13 and 95.93 ± 0.09%, agreeing with the values reported by Muñoz *et al*. (2010) from INCMNSZ and FAO (1985), who reported values of 92.8 and 94.2%, respectively; as well as Guil-Guerrero and Rebolloso-Fuentes (2009), who studied eight cultivars from Spain and reported moisture contents of 92.5-96%. Values may vary according to the climatic conditions, ripening stage and cultivar. The percentages of moisture for tomato skin samples were 83.48 ± 1.13 and 89.53 ± 0.83%; for seeds, these values were 82.99 ± 0.28 and 89.20 ± 0.01%; which are lower those reported by Toor and Savage (2005), who observed values between 94.33 ± 0.1% and 93.67 ± 0.12% in skin and seeds, respectively. Differences in skin values may be due to thickness, since this parameter varies considerably according to the peeling method (with hands or using a knife). In the study by Toor and Savage (2005), no peeling method or thickness is mentioned.

Total Phenolic Compounds

Figure 1 shows the results obtained for content of total phenols in each cultivar, fraction and region. Analyses of total phenolic compounds, antioxidant activities and individual phenols were carried out only in skin and seeds, since preliminary tests showed very low values in the pulp. The results are discussed by fraction, cultivar and region of origin, as follows.

Fraction. A significantly higher content ($p < 0.05$) of phenolic compounds was observed in the skin than in the seeds, regardless of the cultivar or geographic origin (Figure 1). Values from the present study were higher than those reported by Toor and Savage (2005) (skin 26.9 to 30.3 mg GAE/100 g and seeds 15.8 to 28.8 mg GAE/100 g) for three tomato cultivars. Navarro *et al*. (2011) reported a higher content of phenolic compounds in tomato skin (158.10 ± 7.70 mg GAE/100 g); however, this study does not specify skin thickness or whether the sample is fresh or dried. Strack (1997) notes that the highest value observed in the skin may be due to stress suffered by the plant, which may be biotic or abiotic, such as UV rays, pesticides, pathogens and predators. Such stress triggers defense mechanisms in the form of synthesis of secondary metabolites, such as phenolic compounds. These tend to accumulate in the external parts of the plant to protect the seeds, whose main function is to originate a new plant. Therefore, a higher

Figure 1: Total Phenolic Compounds in Mexican Tomato, Mean ± Standard Deviation, n = 3



Note: * GAE, Gallic acid equivalents in 100 grams fresh sample. a, b, c Different letters between the same fraction and region indicate significant difference with respect to the variety. $^{+,++}$ Different signs in the same variety and fraction indicate significant difference with respect to the region. Determined by Tukey method ($p<0.05$).

concentration of phenols is found in the skin than in the pulp.

Cultivar

Cherry showed the highest phenolic content, followed by *Saladet* and *Bola*. Differences proved to be significantly ($p < 0.05$) (Figure 2). *Cherry* may suffer greater stress to adapt to the environment that the other varieties, since it is obtained by artificial selection on traditional tomatoes planted outdoors. This leads to the production of more secondary metabolites as an adaptation mechanism; among these are phenolic compounds.

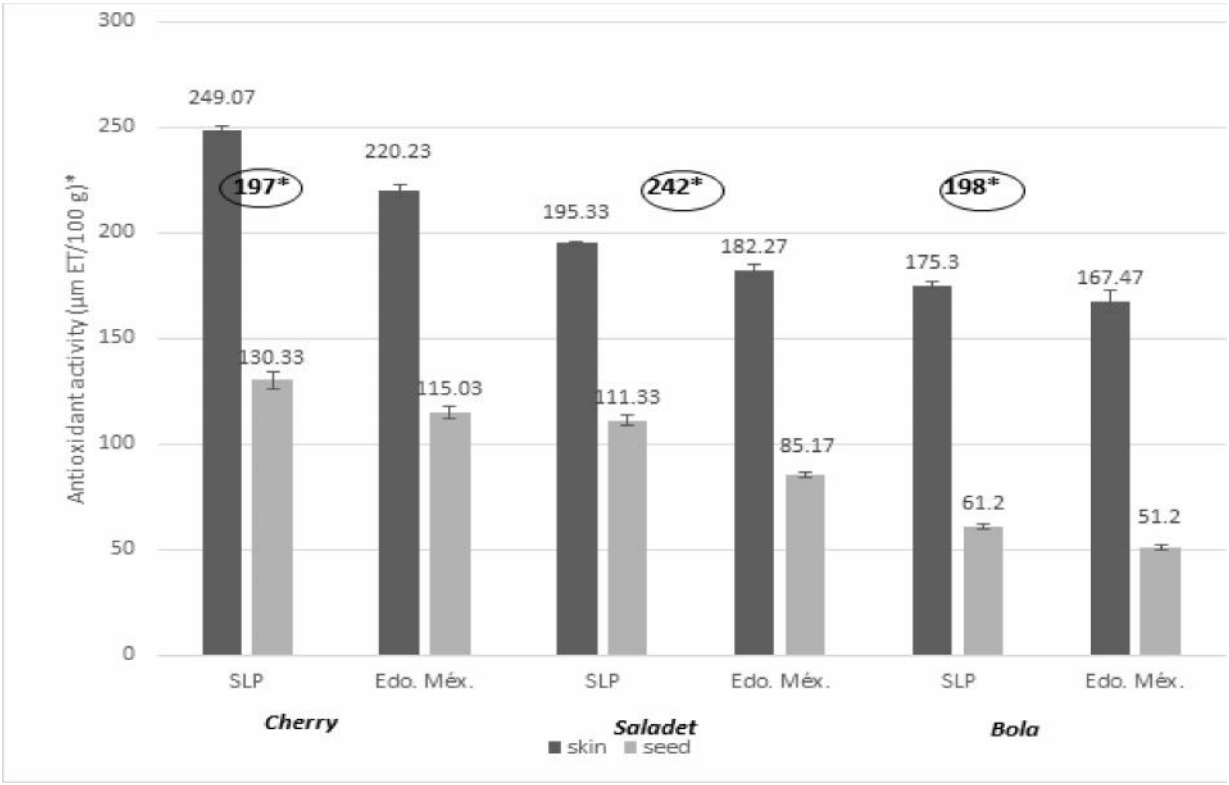
Toor and Savage (2005) report values of 30.3 mg GAE/100 g in the skin and 28.8 mg GAE/100 g in the seeds, which are significantly lower to those obtained in the present study for the skin and similar for the seeds. For *Bola*, the same authors found values of 30.3 mg GAE/100 g in skin and 15.8 mg GAE/100 g in seeds, both significantly higher than those from the present study. The same authors reported a total phenol content of 26.9 mg GAE/100 g in fresh *Cherry* skin and 21.6 mg GAE/100 g in seeds of the same sample, values significantly lower than those of the present study.

Geographic Origin

Samples of the three cultivars obtained in the State of San Luis Potosí showed higher contents of total phenolic compounds in both fractions. These differences may be due to climate and soil conditions, as mentioned by Elbradawy and Sello (2016). Tomato is a plant better adapted to semi-arid climates such as the region of San Luis Potosí, where soils are rich in clay and salts and contribute to the activation of defense mechanisms. In contrast, climate conditions in the State of México offer less saline soils with a more alkaline pH and higher relative humidity. This probably causes the plants to have a less stressful growing environment and therefore less need to employ defense mechanisms, hence a lower content of phenolic compounds (Mondragón, 2007).

Differences in the contents of phenolic compounds may be due to the cultivar, type of soil and geographic origin, among other factors (Giuntini *et al.*, 2005).

Figure 2: Antioxidant Activity in Mexican Tomato, Mean ± Standard Deviation, n = 3



Note: * Mmol trolox equivalents/100 g fresh sample. a,b,c Different letters between the same fraction and region indicate significant difference with respect to the variety. $^{+,++}$ Different signs in the same variety and fraction indicate significant difference with respect to the region. Determined by Tukey method ($p<0.05$). * Values of Toor *et al.* (2006).

Figure 2 shows the values obtained for antioxidant activities of the samples. The results are expressed as Trolox Equivalents (µmol TE) in fresh samples and were analyzed according to the variables of fraction, cultivar and geographic region. As shown in Figure 2, antioxidant activities of the samples vary according to the cultivar, being highest for *Cherry*, followed by *Saladet* and *Bola* samples. Tomatoes with the highest antioxidant activities were from San Luis Potosí.

Fraction

A trend similar to that observed for total phenols was observed for fraction: antioxidant activity is significantly higher ($p < 0.05$) in skin than in seeds, regardless of the variety and region.

Cherry had the highest values for antioxidant activity in both skin and seeds, followed by *Saladet* and finally *Bola*. Ilahy *et al.* (2011) reported similar values for antioxidant activities in four tomato varieties cultivated in North Italy (129, 208, 271 and 166 µmol TE/100 g of fresh sample). Published work has also stated that the antioxidant activity of tomato skin is 2.5 times higher than that of pulp (George *et al.*, 2004), due to the presence of different antioxidant compounds such as vitamins (ascorbic acid and vitamin E), phenolic compounds (flavonoids, phenolic acids, tannins and stilbenes), and carotenoids such as lycopene and β-carotene, among others, which are synthesized to protect against different threats from the environment in which the plant develops (Raffo *et al.*, 2006). This may explain the need for higher antioxidant activity in skin than in seeds. On the other hand, Al-Wandawi *et al.* (1985) reported that tomato seeds contain essential amino acids and particularly high amounts of minerals (Fe, Mn, Zn and Cu), as well as mono-unsaturated fatty acids (such as oleic acid). However, other antioxidants have not been reported, as few articles have studied this fraction, even though it is relevant for total antioxidant activity of tomatoes.

Cultivar

Figure 2 shows that antioxidant activity is higher ($p < 0.05$) in *Cherry* than in *Saladet* and *Bola*. Toor and Savage (2005) observed higher antioxidant activity in *Saladet* (242 µmol TE/100 g of fresh sample) and B*ola* (198 µmol/100 g) than did the present work. These authors reported 197 µmol TE/100 g for *Cherry* samples, a value lower than that of our study. Differences are attributed to the contents of ascorbic acid, total flavonoids and hydrophilic phenolic compounds present in the skin of the fruits. Other authors note that the antioxidant properties of tomatoes depend greatly on lycopene content (Martínez-Valverde *et al.*, 2002); however, both studies agree that the concentration of ascorbic acid plays an important role in antioxidant activity. The variations observed for different cultivars also depend on environmental conditions, different substrates in the soil, genotype differences, and stress suffered by the plant.

Geographical Region

Antioxidant activities for samples obtained in Estado de México were lower than those for samples from San Luis Potosí. Wang *et al*. (1996) and Toor *et al*. (2006) reported that accumulation of antioxidant compounds in the pericarp is favored under higher solar radiation and temperature and that the highest antioxidant activities are observed when crops are harvested during spring and summer, as opposed to autumn and winter. The region of San Luis Potosí favors antioxidant synthesis due to its arid to semi-arid environmental conditions, with higher temperatures than Estado de México. Stewart *et al*. (2000) reported that growth temperature is related to the antioxidant activity of the crops. In greenhouses, UV radiation is filtered by glass or plastic, and the plants thus produced have less exposure to radiation and therefore lower antioxidant activity than those cultivated outdoors. Evidence indicates that UV light damages plant DNA and induces the accumulation of flavonoids and other phenolic compounds in the outer tissues with the aim of protection against damage. It has also been reported that tomato fruits exposed to direct sunlight during growth contain higher levels of carotenoids that those growing in shaded areas (McCollum, 1954).

In addition, different studies revealed that moderate saline stress on the soil improves tomato attributes (Luthria *et al.*, 2006). Wittwer and Castilla (1995) showed that sodium chloride in soils reduces titratable acidity, potassium and nitrogen in the fruits while increasing lycopene, b-carotene and ascorbic acid, as well as sweetness, flavor intensity and antioxidant capacity. The presence of salts in the soil reduces water absorption by the plant and leads to a decrease in growth rate and productivity. This effect is related to the osmotic effect produced by dissolved salts and to the damage produced by the specific toxicity of the particular compounds or ion excess. When large amounts of salts enter the plant via transpiration flow, they are transported towards the leaves, where they produce marginal burn and even impair growth and productivity (Martínez *et al.*, 2010). At this point, the plant begins defense mechanisms against salts by synthesizing several compounds with antioxidant characteristics.

Cherry samples from San Luis Potosí showed the highest antioxidant activity (Figure 2). Raffo *et al*. (2006) reported values of 0.170 and 0.420 mM TE/100 g for fresh sample in two different crops of *Cherry* and reported that antioxidant activity of the hydrophilic fraction is significantly affected by genotype and treatment, regardless of the ripening stage, due to the presence of rutin, naringin, ascorbic acid and chlorogenic acid. The authors also mention that the highest antioxidant activity is found in fruits harvested during the months of March and April. Antioxidant activity depends on several environmental factors before and after harvest (Lenucci *et al.*, 2006). It has also been documented that *Cherry* tomato crops that suffered water stress showed improved sugar levels, titratable acidity, vitamin C and antioxidant activity (Sánchez *et al.*, 2011). In brief, concentration of secondary metabolites in tomatoes is influenced by several factors, such as cultivar, geographic area (climate and soil), ripening stage, time of harvest, agronomic practices (irrigation and fertilization), and storage conditions (Leonardi *et al.*, 2000).

Analysis of Phenolic Compounds by HPLC

Table 2 shows the retention time obtained for each standard injected individually. Figure 3 presents the chromatograms of the selected standards. Figures 3 and 4 show the chromatograms of the skin samples from the three cultivars and both geographical regions. Chromatograms for seeds were obtained in the same manner, and only results for *Cherry* are shown in Figure 5, where the five standards of the study are identified: chlorogenic acid, rutin, naringin, resveratrol and quercetin. Naringin was only found in seeds from *Cherry*. The presence of different phenolic compounds, such as chlorogenic acid, rutin and resveratrol, agrees with Nicoletti *et al*. (2007), who analyzed samples of transgenic tomatoes and identified caffeic acid, naringerin, kaempferol,

Table 2: Phenolic Compounds Content in Mexican Tomato Fruit (Mean ± Standard Deviation, n = 3)

Variety	Región	Fraction	Poliphenol Compound (µg/100 g fm)				
			Clorogénic Acid	Rutin	Naringin	Resveratrol	Quercetin
		Rt (min)	7.68 ± 0.02	15.98 ± 0.03	17.02 ± 0.02	20.3 ± 0.01	22.04 ± 0.03
Bola	SLP	skin	5.80 ± 0.59^{bc}	22.14 ± 0.07^{c}	6.84 ± 0.07^{b}	2.09 ± 0.08^{b}	2.88 ± 0.10^{e}
		seed	7.24 ± 0.10^{cd}	7.98 ± 0.10^{b}	ND	0.4 ± 0.03^{c}	2.75 ± 0.24^{d}
	EDOMEX	skin	2.50 ± 0.07^{d}	17.86 ± 0.58^{c}	3.80 ± 0.01^{d}	0.94 ± 0.16^{b}	5.40 ± 0.14de
		seed	10.33 ± 0.94^{b}	ND	ND	0.38 ± 0.05^{c}	2.91 ± 0.34^{d}
Saladet	SLP	skin	4.17 ± 0.10^{bcd}	116.58 ± 4.55^{a}	1.79 ± 0.20^{e}	7.01 ± 0.94^{a}	11.18 ± 0.60^{b}
		seed	4.99 ± 0.13^{d}	14.78 ± 0.10^{a}	ND	ND	5.01 ± 0.10^{c}
	EDOMEX	skin	3.37 ± 0.50^{cd}	55.91 ± 0.4^{bc}	1.18 ± 0.10^{f}	1.18 ± 0.44^{b}	14.43 ± 0.10^{a}
		seed	8.51 ± 0.38^{bcd}	ND	ND	0.28 ± 0.10^{d}	3.34 ± 0.10^{d}
Cherry	SLP	skin	15.89 ± 0.37^{a}	70.49 ± 1.0^{ab}	15.40 ± 0.19^{a}	3.14 ± 0.10^{b}	10.71 ± 0.20^{bc}
		seed	18.74 ± 0.11^{a}	8.17 ± 0.17^{b}	1.62 ± 0.17^{a}	2.86 ± 0.10^{a}	6.17 ± 0.28^{b}
	EDOMEX	skin	6.82 ± 0.56^{b}	51.1 ± 1.66^{bc}	4.98 ± 0.04^{c}	1.74 ± 0.27^{b}	6.88 ± 0.78^{cd}
		seed	8.84 ± 0.62^{bc}	13.85 ± 0.10^{a}	ND	0.61 ± 0.10^{b}	8.31 ± 0.23^{a}

Note: * ND = Not detected. a,b,c,d,e,f Different letters between the same column and fraction, indicate significant difference with respect to the polyphenol compound. Determined by Tukey method ($p<0.05$). Rt (min): retention time in min. (Mean ± standard deviation, n = 3).

Figure 3: Separation of Polyphenolic Standards, Identification of Peaks: Chlorogenic Acid, Rutin, Naringin, Resveratrol and Quercetin

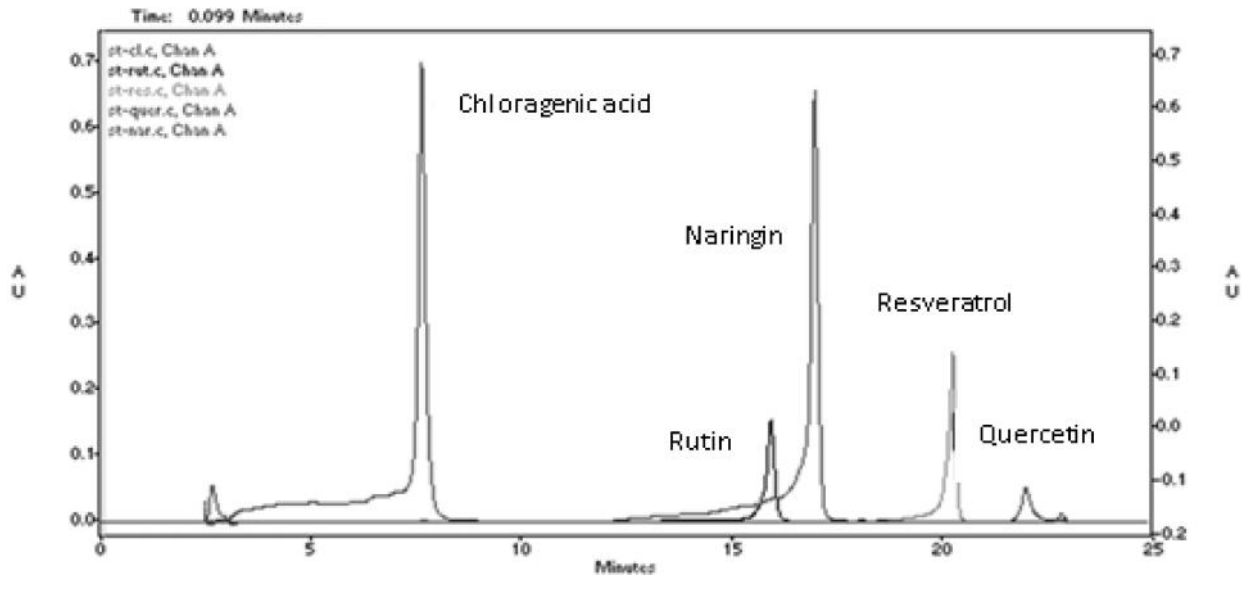
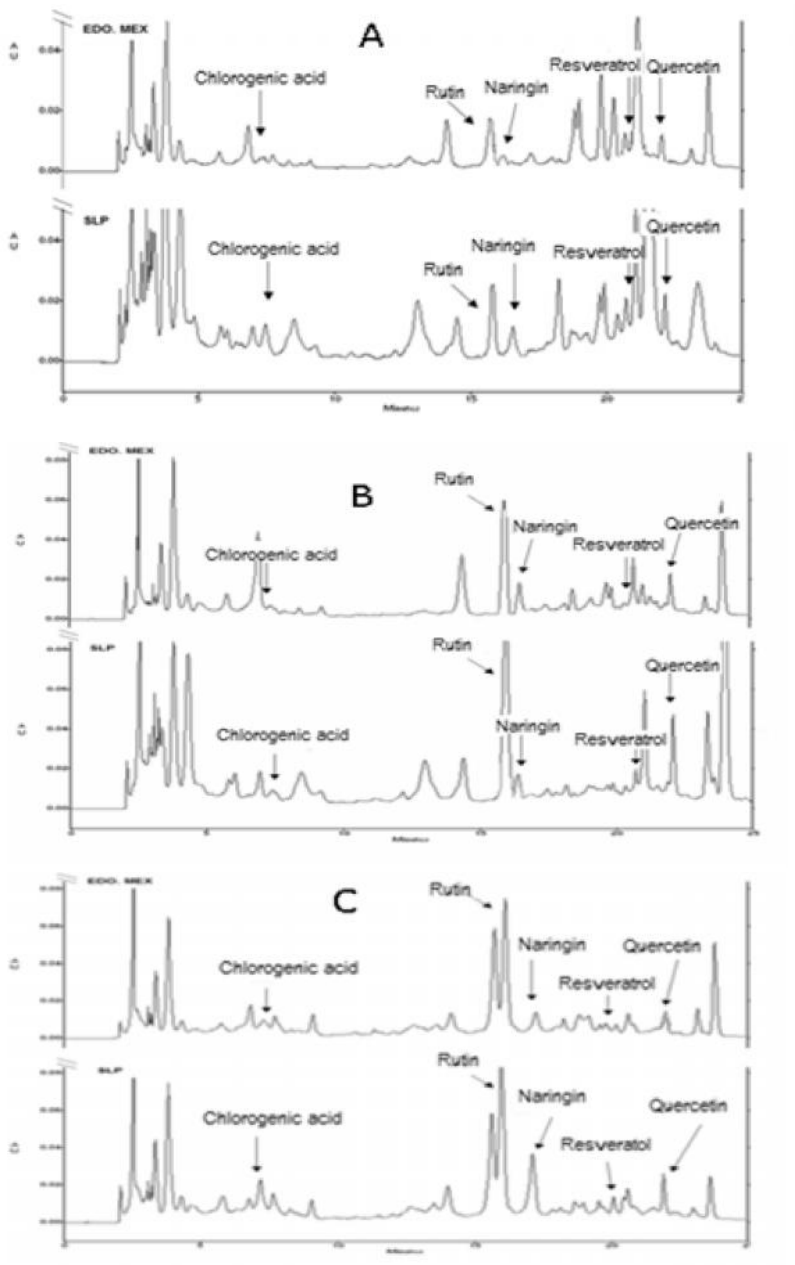


Figure 4: Chromatogram of Tomato Skin Variety A) Bola, B) Saladet and C) Cherry

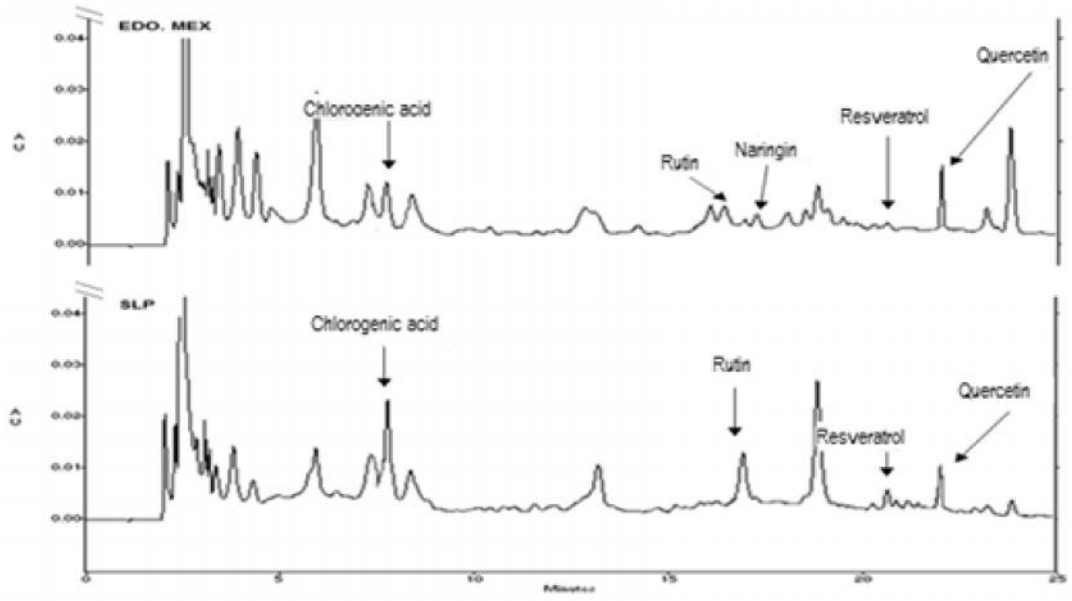


and glycosylated *trans* and *cis* resveratrol in addition to the phenols mentioned above.

Quantification of Phenolic Compounds in Skin and Seeds

Table 2 shows the quantity of each phenolic compound by cultivar and geographical origin. It can be observed that chlorogenic acid is present in both the skin and seeds of all the samples, with the highest values in seeds regardless of geographical origin. Some studies demonstrated that the concentration of this phenolic acid is related to ripening stage, being highly concentrated in the green stage and decreasing as color ripens, as shown by Nicoletti *et al*. (2007). These authors identified chlorogenic acid in green fruits (35.39 mg/kg fresh weight) but not in red fruits. Other authors note that the highest concentration of chlorogenic

Figure 5: Chromatogram of Cherry Variety Tomato Seed



acid in tomato is found in the seeds (Manach *et al.*, 2004), which agrees with the present work. The lowest value of chlorogenic acid in seeds was observed in *Saladet* from San Luis Potosí, while the highest value was found in *Cherry* from San Luis Potosí.

Unlike chlorogenic acid, contents of flavonoids such as rutin, quercetin and naringin were highest in the skin of the fruits. Navarro *et al*. (2011) also reported the presence of these flavonoids in tomato skin. Rutin was highest in the skin of *Saladet*, followed by *Cherry* from San Luis Potosí and Estado de México. Nicoletti *et al*. (2007) reported rutin concentrations of 80.3 mg/kg of fresh weight in tomato skin from *Saladet*, which is higher than that of the San Luis Potosí samples.

Naringin was mostly detected in the skin fraction, and *Cherry* samples showed the highest concentration of this flavonoid. Nicoletti *et al*. (2007) reported naringenin (obtained by hydrolysis of naringin) at a concentration of 31.68 mg/kg of fresh weight in the skin of *Saladet*, which is higher than the value observed in the present study.

Regarding resveratrol, the results reveal small amounts in both seeds and skin of most samples. Higher amounts of this stilbene were found in the skin of the fruits from San Luis Potosí, particularly in *Saladet* (7.01 ± 0.94 µg/100 g of fresh sample), followed by *Cherry* (3.14 ± 0.10 µg/100 g) and *Bola* (2.09 ± 0.08 µg/100 g). It is noteworthy that the *trans* isomer of resveratrol is usually detected, even though in nature, higher amounts exist in the glycosylated form. Nicoletti *et al*. (2007) have studied the development of tomatoes enriched with polyphenolic functional compounds such as stilbenes by experimenting with genetically modified fruits. It has been possible to increase the amount of resveratrol in this type of transgenic tomato up to 48.48 ± 0.99 mg/kg of fresh sample. However, in traditional non-modified tomatoes, resveratrol was not found (isomer or glycosylated). Much of the epidemiological and clinical evidence for the prevention of diseases associated with resveratrol comes from the consumption of red wine, grapes and grape juice (Signorelli and Ghidoni, 2005; Lin *et al.*, 2010; and Gresele *et al.*, 2011). Further studies on this antioxidant are needed in other plant sources such as tomato, due to its wide availability and potential to provide significant amounts to a larger population.

Results for quercetin are shown in Table 2. The highest concentrations of this metabolite were found in both skin and seeds of all the samples, with the highest concentrations in the skin, regardless of cultivar or region. Among these, the concentration was highest in *Saladet* skin samples from Estado de México (14.43 ± 0.10 µg/100 g of fresh simple), followed by *Cherry* and *Bola*.

Regarding seeds, the lowest quercetin values were observed in *Bola* samples from San Luis Potosí (2.75 ± 0.24

µg/100 g of fresh sample), while the highest value was found in *Cherry* (8.31 ± 0.23 µg/100 g) from Estado de México. Nicoletti *et al*. (2007) did not identify this flavonoid in ripe tomato samples. It has been reported that quercetin has antioxidant, anti-cancer, anti-inflammatory, anti-aggregatory and vasodilation effects (Erlund, 2004).

CONCLUSION

Mexican tomatoes are an important source of antioxidant compounds, and it is imperative to consume the whole fruit, since the loss of the skin and seed fractions during processing – at home or at the industrial level – undermine health benefits. The highest concentrations of phenolic compounds and antioxidant activities are found in the skin and seeds of the fruit, regardless of variety or geographic origin. The highest phenolic concentration was found in *Cherry*, followed by *Saladet* and *Bola*. Single phenolic compounds such as chlorogenic acid, naringin, resveratrol, rutin and quercetin were identified in samples of the three cultivars selected in the present study, which indicates the presence of antioxidant compounds other than lycopene in Mexican crops. The concentrations and types of antioxidant compounds in tomatoes depend on several factors, such as environmental and agronomic conditions. The study of phytonutrients from tomato may be the basis for future projects involving technological development for the formulation of new products with functional characteristics.

ACKNOWLEDGMENT

The authors wish to thank the Secretaría de Investigación y Posgrado of Instituto Politécnico Nacional (SIP-IPN) for the financial support of this study.

REFERENCES

- Acuña-Avila P E, Vásquez-Murrieta M S, Hernández M O F and del Socorro López-Cortéz M (2016), "Relationship Between the Elemental Composition of Grapeyards and Bioactive Compounds in the Cabernet Sauvignon Grapes Vitis Vinífera Harvested in Mexico", *Food Chem*., Vol. 203, pp. 79-85.
- Al-Wandawi H, Abdul-Rahman M and Al-Shaikhly K (1985), "Tomato Processing Wastes as Essential Raw Material Sources", *J. Agric. Food Chem*., Vol. 33, No. 5, pp. 804-807.
- AOAC (1975), *Oficial Methods of Analysis of the Association of Oficial Analytical Chemists*,12th Edition.
- Bastida C O A (2012), "Métodos de cultivo hidropónico de jitomate (*Solanum lycopersicum* L.) bajo invernadero basados en doseles escaleriformes", Universidad Autónoma Chapingo Departamento de Fitotecnia, Instituto de Horticultura, Tesis, México.
- Boeing H, Dietrich T, Hoffmann K, Pischon T, Ferrari P, Lahmann P H and Skeie G (2006), "Intake of Fruits and Vegetables and Risk of Cancer of the Upper Aero-Digestive Tract: The Prospective EPIC-Study", *Cancer Causes & Control*, Vol. 17, No. 7, pp. 957-969.
- Brand-Williams W, Cuvelier M E and Berset C (1995), "Use of a Free Radical Method to Evaluate Antioxidant Activity", *LWT Food Science Technol*., Vol. 28, No. 1, pp. 25-30.
- Bravo L (1998), "Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance", *Nutr Rev*., Vol. 56, No. 11, pp. 317-333.
- Elbadrawy E and Sello A (2016), "Evaluation of Nutritional Value and Antioxidant Activity of Tomato Peel Extracts", *Arab J Chem*., Vol. 9, No. 2, pp. S1010-S1018.
- Erlund I (2004), "Review of the Flavonoids Quercetin, Hesperetin, and Naringenin, Dietary Sources, Bioactivities, Bioavailability, and Epidemiology", *Nutr Res.*, Vol. 24, No. 10, pp. 851-874.
- FAO (1985), "II Mesa redonda de la red latinoamericana de agroindustria en frutas tropicales", Oficina Regional FAO para América Latina y el Caribe.
- George B, Kaur C, Khurdiya D S and Kapoor H C (2004), "Antioxidants in Tomato (*Lycopersicon esculentum*) as a Function of Genotype", *Food Chem.*, Vol. 84, No. 1, pp. 45-51.
- Giuntini D, Graziani G, Lercari B, Fogliano V, Soldatini G F and Ranieri A (2005), "Changes in Carotenoid and Ascorbic Acid Contents in Fruits of Different Tomato Genotypes Related to the Depletion of UV-B Radiation", *J. Agric. Food Chem.*, Vol. 53, No. 8, pp. 3174-3181.
- Gresele P, Cerletti C, Guglielmini G, Pignatelli P, Gaetano G and Violi F (2011), "Effects of Resveratrol and Other Wine Polyphenols on Vascular Fuction: An Update", *J Nutr Biochem.*, Vol. 22, No. 3, pp. 201-211.
- Guil-Guerrero J L and Rebolloso-Fuentes M M (2009), "Nutrient Composition and Antioxidant Activity of Eight

Tomato (*Lycopersicon esculentum*) Varieties", *J Food Comp Anal.*, Vol. 22, No. 2, pp. 123-129.

- Guzmán J (2004), *El cultivo del tomate*, 5th Edition, Espasande S R L (Ed.), pp. 4-30, Caracas, Venezuela.
- Ilahy R, Hdider C, Lenucci S M, Tlili I and Dalessandro G (2011), "Antioxidant Activity and Bioactive Compound Changes During Fruit Ripening of High-Lycopene Tomato Cultivars", *J Food Comp Anal*., Vol. 24, Nos. 4-5, pp. 588-595.
- Lenucci M S, Cadinu D, Taurino M, Piro G and Dalessandro G (2006), "Antioxidant Composition in Cherry and High-Pigment Tomato Cultivars", *J. Agric. Food Chem.*, Vol. 54, No. 7, pp. 2606-2613.
- Leonardi C, Ambrosino P and Fogliano V (2000), "Antioxidative Activity and Carotenoid and Tomatine Contents in Different Typologies of Fresh Consumption Tomatoes", *Agric. Food Chem*., Vol. 48, No. 10, pp. 4723-4727.
- Lin Y L, Chang H C, Chen T L, Chang J H, Chiu W T and Chen R M (2010), "Resveratrol Protects Against Oxidized LDL-Induced Breakage of the Blood-Brain Barrier by Lessening Disruption of Tight Junctions and Apoptotic Insults to Mouse Cerebrovascular Endothelial Cells", *The Journal of Nutrition*, Vol. 140, No. 12, pp. 2187-2192.
- Liu Q, Cai W and Shao X (2008), "Determination of Seven Polyphenols in Water by Hig Performance Liquid Chromatography Combined Whit Preconcentration", *Talanta.*, Vol. 77, No. 2, pp. 679-683.
- Luthria D L, Mukhopadhyay S and Krizek D T (2006), "Content of Total Phenolics and Phenolic Acids in Tomato (*Lycopersicon esculentum Mill.*) Fruits as Influenced by Cultivar and Solar UV Radiation", *J Food Comp Anal.*, Vol. 19, No. 8, pp. 771-777.
- Manach C, Scalbert A, Morand C, Remesy C and Jimenez L (2004), "Polyphenols: Food Sources and Bioavailability", *Am J Clin Nutr*., Vol. 79, No. 5, pp. 727-747.
- Martínez C J P, Antúnez B A, Pertuzé C R and Araya L H (2010), "Tomate: el manejo de la salinidad cambia la productividad y calidad en especial frutales y hortalizas", *INIA Tierra adentro.*, pp. 12-14.
- Martínez-Valverde I, Periago M J, Provan G and Chesson A (2002), "Phenolic Compounds, Lycopene and Antioxidant Activity in Commercial Varieties of Tomato (*Lycopersicum esculentum*)", *J Sci Food Agric*., Vol. 82, No. 3, pp. 323-330.
- McCollum J P (1954), "Effects of Light on the Formation of Carotenoids in Tomato Fruits", *Food Res Int*., Vol. 19, Nos. 1-6, pp. 182-189.
- Mondragón L (2007), "Producción de jitomate en invernadero, Gobierno del Estado de México", *Secretaria de Desarrollo Agropecuario*, Instituto de investigación y capacitación agropecuaria, acuícola y forestal del Estado de México, 1st Edición.
- Muñoz M, Ledesma S J A, Chávez V A, Pérez-Gil R F, Mendoza M E and Calvo C C (2010), "Composición de alimentos Miriam Muñoz de Chávez", *Valor nutritivo de los alimentos de mayor consumo*, 2nd Edition, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ). Ed. McGraw Hill, México.
- Navarro G I, Valverde G V, Alonso G J and Periago M J (2011), "Chemical Profile, Functional and Antioxidant Properties of Tomato Peel Fiber", *Food Res Int.*, Vol. 44, No. 5, pp. 1528-1535.
- Nicoletti I, De Rossi A, Giovinazzo G and Corradini D (2007), "Identification of Stilbenes in Fruits of Transgenic Tomato Plants (*Lycopersicon esculentum Mill*) by Reversed Phase HPLC with Photodiode Array and Mass Spectrometry Detection", *J Agric Food Chem*., Vol. 55, No. 9, pp. 3304-3311.
- NMX-F-102-S-1978 (1978), "Determinación de la acidez titulable en productos elaborados a partir de frutas y hortalizas", *Dirección general de normas*, p. 4, México DF.
- NMX-F-103-1982 (1982), "Frutas y derivados Determinación de grados brix", *Dirección general de normas*, p. 2, México DF.
- NMX-F-317-S-1978 (1978), "Determinación de pH en alimentos", *Dirección general de normas*, p. 3, México DF.
- NMX-FF-031-1997-SCFI (1997), "Productos alimenticios no industrializados para consumo humano - hortalizas frescas - tomate - (*lycopersicun esculentum mill.*) – Especificaciones", *Normas mexicanas*, p. 29, Dirección general de normas, México DF.

- NMX-V-032-S-1980 (1980), "Bebidas alcohólicas. Determinación de densidad relativa", *Dirección general de normas*, p. 2, México DF.
- Nuez F, Rodríguez A and Tello J (2001), *El cultivo del tomate*, Primera Edición, pp, 16-18, 32, 45, 80-81, 96-99, Ediciones Mundi - Prensa, España.
- Odriozola S I R, Soliva F O and Martín B (2008), "Effect of Minimal Processing on Bioactive Compounds and Color Attributes of Fresh-Cut Tomatoes", *LWT Food Science Technol*., Vol. 41, No. 2, pp. 217- 226.
- Paradiso A, D'Amico L, Nicoletti I, Santino A, De Gara L and Giovinazzo G (2013), "Resveratrol Biosynthesis Up-Regulates the Ascorbate/Glutathione Pathway in Transgenic Tomato Fruit", *J Plant Biochem Physiol*., Vol. 1, No. 2, pp. 1-7.
- Raffo A, La Malfa G, Fogliano V, Maiani G and Quaglia G (2006), "Seasonal Variations in Antioxidant Components of Cherry Tomatoes (*Lycopersicon esculentum* cv. *Naomi F1*)", *J Food Comp Anal*., Vol. 19, No. 1, pp. 11-19.
- Ragab S A, Van F J, Jankowski B, Park J-H and Bobzin S C (2006), "Detection and Quantitation of Resveratrol in Tomato Fruit (*Lycopersicon esculentum* Mill.)", *J. Agric. Food Chem*., Vol. 54, No. 19, pp. 7175-7179.
- Ríos O O, Chávez S J L and Carrillo R J C (2014), "Producción tradicional y diversidad de tomate (*Solanum lycopersicum* L.) nativo: un estudio de caso en Tehuantepec-Juchitán, México", *Rev. ASyD*., Vol. 11, No. 1, pp. 35-51.
- Rosales-Martínez P, Arellano-Cárdenas S, Dorantes-Álvarez L, García-Ochoa F and López-Cortez M D S (2014), "Comparison Between Antioxidant Activities of Phenolic Extracts from Mexican Peanuts, Peanuts Skins, Nuts and Pistachios", *J Mex Chem Soc*., Vol. 58, No. 2, pp. 185-193.
- SAGARPA Jitomate (2010), "Monografía de cultivos, Subsecretaría de Fomento a los agronegocios", México DF.
- Saleem M, Kim H J, Ali M S and Lee Y S (2005), "An Update on Bioactive Plant Lignans", *Nat Prod Rep*., Vol. 22, No. 6, pp. 696-716.
- Sánchez R E, Moreno D A, Ferreres F, Rubio W M and Ruiz J M (2011), "Differential Responses of Five Cherry Tomato Varieties to Water Stress: Changes on Phenolic Metabolites and Related Enzymes", *Phytochemistry*, Vol. 72, No. 8, pp. 723-729.
- Signorelli P l and Ghidoni R (2005), "Resveratrol as an Anticancer Nutrient: Molecular Basis, Open Questions and Promises", *J Nutr Biochem*., Vol. 16, No. 8, pp. 449-466.
- Singleton V L and Rossi J A (1965), "Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents", *Am J Enol Vitic*., Vol. 16, pp. 144-158.
- Slimestad R and Verheul M (2009), "Review of Flavonoids and Other Phenolics from Fruits of Different Tomato (*Lycopersicon esculentum* Mill.) Cultivars", *J Sci Food Agric*., Vol. 89, No. 8, pp. 1255-1270.
- Stewart A J, Bozonnet S, Mullen W, Jenkins G I, Lean M E J and Crozier A (2000), "Occurrence of Flavonols in Tomatoes and Tomato Based Products", *J Agric Food Chem.*, Vol. 48, No. 7, pp. 2663-2669.
- Strack D (1997), "Phenolic Metabolism", in P M Dey and J B Harborne (Eds.), *Plant Biochemistry*, Academic Press, London.
- Toor R K and Savage G P (2005), "Antioxidant Activity in Different Fractions of Tomatoes", *Food Res Int.*, Vol. 38, No. 5, pp. 487-494.
- Toor R K, Savage G P and Lister C E (2006), "Seasonal Variations in the A1ntioxidant Composition of Greenhouse Grown Tomatoes", *J Food Comp Anal.*, Vol. 19, No. 1, pp. 1-10.
- Vitale A, Bernatene E and Pomilio A (2010), "Carotenoids in Chemoprevention: Lycopene", *Acta Bioquim Clin Latinoam*, Vol. 44, No. 2, pp. 195-238.
- Wang H, Cao G and Prior R L (1996), "Total Antioxidant Capacity of Fruits", *J Agric Food Chem.*, Vol. 44, No. 3, pp. 701-705.
- Wittwer S H and Castilla N (1995), "Protected Cultivation of Horticultural Crops Worldwide", *Horttechnology*, Vol. 5, No. 1, pp. 6-23.
- World Health Organization (2009), "Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks", Consultado 15/08/2012, Disponible en: http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_full

- Wu C, Yang J Y, Wang F and Wang X X (2013), "Resveratrol: Botanical Origin, Pharmacological Activity and Applications", *Chin J Nat Med*., Vol. 11, No. 1, pp. 1-15.
- Zambrano J, Moyeja J and Pacheco L (1996), "Efecto del estado de madurez en la composición y calidad de frutos de tomate", *Agron Trop*., Vol. 46, No. 1, pp. 61-72.


IJFANS
International Journal of Food
And Nutritional Sciences