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Antioxidant Potential of Tomatoes Cultivated in Organic and Conventional Systems

Renata Galhardo Borguini[1], Deborah Helena Markowicz Bastos[2], José Machado Moita-Neto[3], Fernanda Sobral Capasso[2] and Elizabeth Aparecida Ferraz da Silva Torres[2*]

[1]Embrapa Agroindústria de Alimentos; Empresa Brasileira de Pesquisa Agropecuária; 23020-470; Rio de Janeiro - RJ - Brasil. [2]Departamento de Nutrição; Faculdade de Saúde Pública; Universidade de São Paulo; Av. Dr. Arnaldo 715; 01246-904; São Paulo - SP - Brasil. [3]Departamento de Química; Centro de Ciências da Natureza; Universidade Federal do Piauí; 64049-550; Teresina - PI - Brasil

ABSTRACT

The objective of the study was to compare the effect of organic and conventional cultivation on the antioxidant compound content and antioxidant activity of the Carmen tomato cultivar. Tomatoes were analyzed regarding ascorbic acid, phenolic compounds, lycopene content and antioxidant activity. Organic tomatoes presented higher content of ascorbic acid and total phenolics (641.39 and 4466.66 mg/100 g EAG on dry wt basis) than did the conventional tomatoes (510.16 and 3477.50 mg/100 g EAG on wt dry basis, respectively). There was no difference in lycopene concentrations between the organic and conventional. The ether, alcohol and aqueous extracts obtained from the tomatoes were subjected to the DPPH test and the β-carotene/linoleic acid system assay. The alcohol and aqueous extracts from organic tomatoes presented higher antioxidant activity in the DPPH test (25.43 and 14.28%, respectively) than the conventional tomatoes (19.52 and 11.33%, respectively). Organic tomatoes had higher antioxidant potential probably due to its higher ascorbic acid and total phenolic values.

Key words: Antioxidant activity, ascorbic acid, lycopene, phenolic compounds

INTRODUCTION

Organic foods are produced according to organic agriculture standards. The organic crops should grown based on a system of farming that maintains and replenishes soil fertility and crop health without the use of conventional pesticides, artificial fertilizers, human waste, or sewage sludge, and they should be processed without ionizing radiation, or food additives. The organic animals are those who are reared without the routine use of antibiotics and without the use of growth hormones. Organic produce must not be genetically modified. Products usually are certified by a third party certification body recognized at international, or national level, hence accountable in the case of fraud. Certification is made against the standards of the country where the product is sold. Certified organic food is recognized on the market by the organic label of the certification body (FAO 2009).

Tomatoes and tomato products are rich in food components that are antioxidant and considered to be a source of carotenoids, in particular lycopene, ascorbic acid and phenolic compounds (George et al. 2004; Sahlin et al. 2004; Ilahy et al. 2011; Pinela et al. 2012). Chemical composition of the fruit depends on factors such as genetics, fruit maturity and cultivation conditions (Abushita et al. 2000; Thompson et al. 2000; Giovanelli et al.

*Author for correspondence: eatorres@usp.br

2002; Martinez-Valverde et al. 2002). However, there is little information on the effect of different forms of cultivation on the antioxidant potential of tomatoes.

Regular consumption of tomatoes and their products has been correlated with reduction in the risk of several types of cancer and cardiovascular diseases (Giovannucci et al. 1995; Clinton 1998). This positive effect is attributed to the antioxidants, and particularly to the carotenoids (lycopene and β-carotene) and other phenolic compounds. Bourn and Prescott (2002) showed that only a small number of properly implemented studies capable of allowing valid comparison between the foods cultivated in organic and conventional systems exist. It is also observed that there is a lack of research aimed at comparing the levels of secondary metabolites (antioxidants) of vegetables cultivated in these two systems. Thus, the objective of the present study was to compare the effect of organic and conventional cultivation on the antioxidant compound content and antioxidant activity of the Carmen tomato cultivar. The main hypothesis put forward was that the different forms of cultivation would affect the plants' production of secondary metabolites.

MATERIALS AND METHODS

Samples

Fruit from the Carmen tomato cultivar destined for table consumption, which had been produced by means of organic and conventional cultivation, were selected. While selecting the food samples, care was taken to control for various factors, such as: tomato cultivar (Carmen), production region (southeastern Brazil), time of planting and harvesting, degree of ripeness (salad), transportation conditions (highway), packaging used (wooden boxes) and exposure to humidity, heat and light. The organically produced tomatoes came from the municipality of Indaiatuba, State of São Paulo, and presented certification from the Biodynamic Institute, which was authorized by the International Federation of Organic Agricultural Movements (IFOAM), and from the certifier of the Organic Agriculture Association for commercialization as organic product. These tomatoes were destined for sale as organic product. The conventionally produced tomatoes came from the municipality of São José de Ubá, State of Rio de Janeiro, and were acquired at the central wholesale market of the city of São Paulo (Companhia de Entrepostos e Armazéns Gerais de São Paulo, CEAGESP).

The fruits were harvested upon reaching the commercial state, known as "salad", based on their color classification as determined by CEAGESP and used by producers. The samples were prepared 24h after receiving them. The tomatoes were washed with running water and distilled water, and were then dried using paper towels. The fruits were cut into piece and then mashed and homogenized in a domestic blender at maximum speed.

Analysis

Ascorbic acid content

Was determined by titration with 2,6-dichlorophenolindophenol, until a slightly pinkish coloration that was stable for 15 seconds was obtained and the content in the samples was calculated based on an ascorbic acid standard that had previously been determined. The results were expressed as mg of ascorbic acid per 100 g of sample in dry basis (Pregnolatto and Pregnolatto 1985).

Total phenolic

Compounds were extracted by the method of Genovese et al. (2003) using the Folin-Ciocalteu reagent. The samples were extracted in proportions of 1:20 (m/v) with methanol, using a homogenizer (Ultra Turrax® model T18 basic, IKA, Staufen, Germany) for 1 minute at speed 5. The residue was re-extracted in the same proportions. The extracts that were obtained were filtered using filter paper and the volume was made up to 50 mL. The total phenolics determination was carried out in accordance with Zieliski and Kozowska (2000), modified by Genovese et al. (2003). The blue color that was produced through the reducing action of the phenolics on the Folin-Ciocalteu reagent was measured in a spectrophotometer at 750 nm (model CE 1020, Cecil, England). The results were expressed as mg of gallic acid equivalent per 100 g of sample in dry basis.

Lycopene

Five grams of the homogenized samples were weighed in a 125 mL Erlenmeyer flask, which was wrapped in aluminum foil for protection against the light. Fifty milliliter of a mixture of hexane/acetone/ethanol (2:1:1, v/v/v) was added to solubilize the carotenoids (Sadler et al. 1990). The

samples were stirred for 30 minutes and transferred to a separation funnel, and then 10 mL of distilled water was added. The solution was separated into a polar fraction (35 mL) and an apolar fraction (25 mL), the latter containing lycopene. The extraction residue did not present coloration. The lycopene content was determined by reading the hexane solution absorbance at 472 nm. The conversion of the absorbance into lycopene concentration was based on the specific extinction coefficient for the pigment in hexane (3.450) (Gross 1987). The results were expressed as mg of lycopene per 100 g of sample in dry basis.

Moisture

Was determined gravimetrically according to the AOAC (1995). Five grams of each sample were weighed in a porcelain capsule, which was subsequently placed in an oven at 105ºC ± 1ºC to constant weight. The results were expressed as percentage of water in the sample.

Extraction

The samples were subjected to a freeze-drying, ground and placed in amber flasks under a nitrogen atmosphere, and then stored in a freezer at - 18ºC. The ether, alcohol and aqueous extracts were obtained from the freeze-dried samples, by means of a sequential extraction process (Sotero 2002). The extractions were carried out in the proportions of 1:20 (sample:solvent) and the solvents used were ethyl ether, ethyl alcohol and distilled water, following the order of polarity for the extraction. The samples were homogenized for one hour at room temperature and were protected from the light. Following this, they were filtered using a Büchner funnel with the aid of a vacuum pump. The residue from the filtration was dried, weighed and subjected to extraction with the subsequent solvent. The extracts obtained were collected in amber glass flasks and stored in a freezer at - 18ºC.

Antioxidant activity

DPPH test

The capacity of the sample extracts to capture free radicals was measured using the method described by Brand-Williams et al. (1995), with modifications. This method is based on the removal of the stable DPPH radical (2,2-diphenyl-1-picrylhydrazyl) from the reaction medium by the action of the antioxidants that are present in the sample. The degree of discoloration of the DPPH radical at 517 nm, after adding the extract, was measured in a methanol solution using a spectrophotometer, until the absorbance remained constant. This indicated the efficiency of the added sample for removing the radical.

The ether, alcohol and aqueous extracts were diluted in methanol at a concentration of 0.2 mg/mL.

Butylhydroxytoluene (BHT) was used as the standard, at a concentration of 0.1 mg/mL.

Assay in β-carotene/linoleic acid system

The antioxidant activity was evaluated by means of a spectrophotometric assay based on the discoloration (oxidation) of β-carotene that was induced by the products from oxidative degradation of a fatty acid (linoleic acid), in accordance with a procedure described by Melo and Mancini-Filho (1989). The substrate used was a β-carotene and linoleic acid emulsion. To prepare the emulsion, β-carotene diluted in chloroform (20 mg/mL) was used, to which 20 mg of linoleic acid and 200 mg of Tween 40 were added. After the chloroform had evaporated under a nitrogen atmosphere, approximately 100 mL of water enriched with oxygen (distilled water treated with O_2 for 30 minutes) was added. In the test tube, 5.0 mL of this emulsion was added to 1.0 mL of the appropriate dilution of the extracts (extract concentrations: ether extract 2.5 x 10^{-2} mg/mL; alcohol and aqueous extracts 1.0 mg/mL). After homogenization, the reading was done in a spectrophotometer at 470 nm, taking this reading at time zero (starting time). The tubes were then placed in a water bath at 50°C and the other sequential readings were done every 15 minutes for a total period of 2 h. BHT was used as the standard. The results were expressed as the percentage (%) of oxidation inhibition.

Statistical analysis

All the results were presented as means ± standard deviations. The statistical analyses were carried out using the SPSS for Windows software, version 15.0. The differences between treatments were determined by applying the Student's t-test.

RESULTS AND DISCUSSION

Antioxidant compounds

Table 1 presents the ascorbic acid, total phenolics and lycopene content of the samples.

The organic tomatoes presented mean ascorbic acid content that was higher than that of the conventional tomatoes (Table 1). The variation in vitamin C content in the tomatoes depends mainly on the environment conditions. According to Martinez-Valverde et al. (2002), many experiments have shown that variation in light intensity prior to the harvest has a great influence on vitamin C content. Therefore, it is important to compare the organic and conventional foods that are planted and harvested during the same season of the year and that originate from the regions with similar incidence of solar radiation. It is important to emphasize that this control was adopted regarding the fruits used in the present study.

Table 1 - Ascorbic acid, total phenolics and lycopene content, expressed as mg/100 g (dry basis), of tomato fruits obtained through organic and conventional cultivation.

Cultivation	Ascorbic acid*	Total phenolics*	Lycopene*
Organic	641.39 ± 39.58[a]	4466.66 ± 395.51[a]	37.43 ± 4.91[a]
Conventional	510.16 ± 57.16[b]	3477.50 ± 419.32[b]	40.80 ± 6.69[a]

*The values represent means ± standard deviations, obtained from six determinations of ascorbic acid, total phenolics and lycopene. **Values with the same letters in the same column indicate that there was no statistically significant difference between the means at the 5% level. Sample moisture content: organic 95.44 % and conventional 95.57 %.

In the study of Toor et al. (2006), the mean ascorbic acid content in organically fertilized tomatoes was 29% higher than the results obtained from tomatoes that were fertilized with mineral solutions. Caris-Veyrat et al. (2004) found that ascorbic acid content was 31% higher in organic tomatoes. They also found that fertilizer that was rich in soluble nitrogen (N) could cause a decrease in the ascorbic acid content, probably for indirect reasons, since the nitrogen supply increased the plants' leaf density, which promoted shading over the fruits. According to Dumas et al. (2003), exposure to light is a favorable factor for ascorbic acid accumulation. Premuzic et al. (1999) compared the ascorbic acid content in the tomatoes cultivated with an organic substrate with hydroponically cultivated tomatoes. They found higher ascorbic acid content in the fruits produced using organic compost.

The contribution of vitamin C towards the total antioxidant capacity of extracts of vegetal origin food generally varies according to the type of food. Significant losses of nutrients, especially vitamin C, may occur during the storage, and this also contributes towards variations in the fruit composition (Chitarra 1994). It is worth emphasizing that, in the present study, the fruits were stored under identical conditions and that the analyses were carried out after the same length of time had elapsed following the harvesting and sample acquisition for both types of tomatoes. This procedure ensured control over the conditions relating to the preservation of ascorbic acid content.

Regarding the total phenolics, the organic tomatoes presented higher content than did the conventional tomatoes (Table 1). This result was concordant with the data obtained by Toor et al. (2006), who found a higher level of total phenolics in organically fertilized tomatoes than in those that received conventional fertilization. Caris-Veyrat et al. (2004) found rutin and naringenin content that was respectively 170 and 12.5 % higher in organic tomatoes then in the conventional ones. Phenolic compounds are originated from plants' secondary metabolism. Many secondary metabolites act as fungicides and antibiotics to protect the plants from fungi and bacteria (Vickery and Vickery 1981). Thus, it can be inferred that the higher content of phenolic compounds in organic food occurs because of the possible incidence of pests and pathogens in the organic cultivation method, in which pesticides are not used. This higher incidence could cause some sort of stress and, therefore, caused an increase in phenolic compounds production, with the purpose of increasing the natural defenses.

Ren et al. (2001) evaluated the polyphenol content of five widely consumed vegetables in Japan (kale, Chinese cabbage, spinach, garlic and green pepper) produced in organic and conventional cultivations. The flavonoid quercetin and caffeic

acid content was respectively, 1.3 to 10.4 times higher in the organic products, therefore suggesting that different cultivation practices had an influence. Tomatoes originating from hydroponic cultivation in greenhouses in New Zealand (Giovanelli et al. 2002) presented total phenolics content of between 21.3 and 36.40 mg/100 g (moist basis), expressed as gallic acid equivalent.

Light incidence is one of the main environmental factors that influence the phenolics production of the plants. Higher levels are recorded in the plants that receive abundant light. In the present study, all the fruits (organic and conventional) were cultivated in the fields in the southeastern region of the country. Therefore, it could be inferred that the difference that was found in the phenolics content occurred due to handling differences.

Regarding the lycopene content of the tomatoes, there was no significant difference between the organic and conventional types (Table 1). The carotenoid content in fruits and vegetables, and particularly in tomatoes, depends on the factors such as exposure to light, temperature and degree of fruit ripeness (Abushita et al. 2000). Thus, the absence of any difference between the organic and conventional tomatoes could be due to the control over the ripening, transportation and storage conditions. Furthermore, both types of tomatoes were of the Carmen cultivar and both of them originated from the southeastern region of Brazil.

The ascorbic acid, phenolic compounds and lycopene concentrations in the tomatoes are determined by agricultural, geographic and seasonal factors, as well as the variety cultivated (Martinez-Valverde et al. 2002). Explaining the relationship between these factors and the content of components with antioxidant potential is necessary when it is desired to explore the potential benefits to human health from consuming tomatoes and their products. Comparisons relating to the antioxidant compounds content are not easy to establish, considering the inherent variability of food samples *in natura* and also because quantitative results are expressed in different forms (relative percentages, moist sample weight, or dry sample weight) by different authors. According to Tavares and Rodriguez-Amaya (1994), different cultivars, climatic effects, the exact stage of ripeness (even though all samples may be described as ripe), different processing and storage conditions for the produce and different analytical procedures may contribute towards the differences identified through various studies.

Antioxidant activity

The presence of different antioxidant components in the plant tissue, and especially in the fruits and vegetables, makes it relatively difficult to measure the antioxidant activity of each component separately. Thus, various methods have been developed to calculate the total antioxidant activity of the samples. Researchers test different solvents and extraction methods to certify the maximum solubility of the antioxidants present in the sample. With the objective of obtaining reliable results regarding the mechanisms for the action of antioxidant compounds and their relative contributions, the antioxidant activity of the tomato samples was evaluated by obtaining three different extracts (ether, alcohol and aqueous). Two tests (DPPH and β-carotene/linoleic acid) were used to evaluate the *in vitro* antioxidant activity of the sample extracts, so as to estimate the potential health benefits provided by tomato consumption. These tests are widely recognized for evaluating plant extracts *in vitro*. The antioxidant activity is a measurement of the capability of substances that are extracted from the food matrix to capture free radicals (DPPH test), or to delay the lipid oxidation process in a controlled system (β-carotene / linoleic acid system). Therefore, the term “antioxidant” will be used here to refer to both activities: the anti-radical effect (activity of capturing free radicals) and the antioxidant action (activity of preventing or delaying lipid oxidation).

Table 2 brings together results relating to the capture of free radicals by the sample extracts, through the DPPH test.

The antioxidant activity of the ether extract by the DPPH test did not present any difference between the two types of cultivation (Table 2). This was probably due to the absence of any difference in the lycopene content between the organic and conventional tomatoes. Since lycopene is a liposoluble micronutrient, its levels are relatively higher in the lipophilic fraction for most foods (Djuric and Powell 2001). Raffo et al. (2002) observed that the antioxidant activity of the liposoluble fraction of the *in natura* tomato samples resulted essentially from the carotenoids, particularly lycopene. This was confirmed by the high correlation coefficient value between its

content and the antioxidant activity of this fraction.

Lycopene antioxidant activity has been exhaustively evaluated, based on its ability to capture free radicals, using *in vitro* methods; or its capability to protect cellular components from oxidative damage, using cell, or animal culturing methods (Yaping et al. 2002). According to Stahl and Sies (2003), among the various radicals that are formed in the human body under oxidative conditions, the carotenoids react most efficiently with the peroxyl radical. These radicals are generated from the lipid peroxidation process and their capture interrupts the reaction sequence that would ultimately lead to damaging of the lipophilic compartments, such as cell membranes and lipoproteins.

Table 2 - Antioxidant activity (% of DPPH discoloration) of the ether, alcohol and aqueous extracts (concentration 0.2 mg/mL) obtained from tomato fruits originating from organic and conventional cultivation.

Cultivation	Ether extract*	Alcohol extract*	Aqueous extract*
Organic	72.34 ± 15.28^{a}	25.43 ± 1.08^{a}	14.28 ± 1.08^{a}
Conventional	65.31 ± 8.26^{a}	19.52 ± 2.07^{b}	11.33 ± 1.04^{b}

*The values represent means ± standard deviations from four determinations of antioxidant activity by means of the DPPH method. **Values with the same letters in the same column indicate that there was no statistically significant difference between the means at the 5% level. Percentage of DPPH discoloration for the BHT assay at 0.1 mg/mL = 91.32%.

Regarding the alcohol extract, the organic tomatoes presented higher antioxidant activity than did the conventional ones (Table 2). This could be explained based on the results shown in Table 1, relating to the total phenolics content of the samples studied, in which it was observed that the organic tomatoes presented higher total phenolics content.

In relation to the aqueous extract, the organic tomatoes presented higher antioxidant activity than did the conventional ones (Table 2). In this, ascorbic acid, which was water-soluble, was present in higher quantity in the organic tomatoes (Table 1), which could have contributed significantly towards the higher antioxidant activity of the aqueous extract. It was possible that the total antioxidant activity also depended on the synergistic effect among all the water-soluble antioxidants and their interactions with other components of this fraction.

The fractions that were soluble in ethyl ether, ethanol and water behaved differently in the DPPH test. As a general tendency, it was observed that there was increased percentage DPPH discoloration with increasing extract concentration (data not presented). Thus, it was observed that the antioxidant activity of the sample depended on the type of solvent used for the antioxidant compound extraction and on the concentration of the extract applied in the test. The BHT standard presented elevated activity in relation to the samples, although it had been used at a lower concentration (0.1 mg/mL).

To determine the extract concentrations to be applied in the test of β-carotene/linoleic acid, various assays were carried out. It was observed that the most suitable concentration ones for the ether extract would be 2.5×10^{-2} mg/mL, since at higher concentrations, the extracts would act as pro-oxidants. For the alcohol and aqueous extracts, the concentration determined was 1.0 mg/mL. For the BHT, the concentration used was 0.2 mg/mL. This latter value is the maximum limit for the use of this additive in foods in Brazil, according to Resolution No. 04/88 of the National Council for Sanitary Surveillance (*Conselho Nacional de Vigilância Sanitária*, CNS), of November 24, 1988, published in the Federal Official Gazette of December 19, 1988.

Considering the antioxidant activity of the three extracts evaluated, there were no differences between the fruits originating from the organic and conventional cultivations (Table 3). In this assay, better performance was observed in the alcohol and aqueous extracts, possibly due to the actions of phenolic compounds and ascorbic acid, which acted efficiently as lipid oxidation inhibitors. The BHT standard (positive control for the assay) presented high activity in relation to the samples.

Taking the antioxidant efficiency of the ether extract into consideration, it was interesting to note that its capacity to capture free radicals was higher than its power to inhibit lipid oxidation. Therefore, the antioxidant capacity of this extract related strongly to its ability to capture the free radicals, probably due to the presence of lycopene,

rather than its effectiveness as a lipid oxidation inhibitor.

Although the sample extracts showed different performances, when using two methods as indicators for the antioxidant activity, this type of measurement allowed higher precision in indicating the antioxidant potential of the food as well as on the many antioxidant compounds present in the extracts tested. The antioxidant activity also depends on the synergistic effect between these compounds. Interactions between the antioxidants may present effects that would not be observed when the elements are tested separately.

Table 3 - Antioxidant activity (% inhibition of lipid oxidation) of the ether extract (concentration 2.5 x 10^{-2} mg/mL) and the alcohol and aqueous extracts (concentration 1.0 mg/mL) obtained from tomato fruits originating from organic and conventional cultivation.

Cultivation	Ether extract*	Alcohol extract*	Aqueous extract*
Organic	12.02 ± 5.65[a]	39.35 ± 1.74 [a]	40.24 ± 4.97 [a]
Conventional	11.93 ± 1.56 [a]	42.00 ± 3.16 [a]	36.08 ± 2.07 [a]

*The values represent means ± standard deviations obtained from four determinations of antioxidant activity through the β-carotene / linoleic acid system. **Values with the same letters in the same column indicate that there was no statistically significant difference between the means at the 5% level. Percentage of lipid oxidation inhibition for the BHT assay at 0.2 mg/mL = 58.31%.

Considering the results obtained, the antioxidant capacity of the samples was shown to be dependant both on the solvent used for the extraction and the method (DPPH and β-carotene/linoleic acid), as was observed in previous studies (Martinez-Valverde et al. 2002; Larrosa et al. 2002). Thus, it was observed that the ether extracts, which were rich in lycopene, showed higher antioxidant activity when analyzed using the DPPH test. According to Stahl and Sies (1996), lycopene is effective in capturing free radicals. However, the phenolic compounds of the alcohol and aqueous extracts, which have been shown to have high antioxidant activity in lipid emulsions (Cheung et al. 2003), attained higher antioxidant activity in the β-carotene and linoleic acid system, in contrast with its lower effectiveness against free radicals, as was observed in the DPPH test.

Antioxidant levels in contrast with other antioxidant defenses of the organism are influenced by dietary intake. Fruit and vegetables are the main sources of antioxidants, which makes these foods essential to human health. Considering that tomatoes are one of the main components of daily meals in many nations it becomes increasingly important to invest in evaluating their nutritional value regarding antioxidant content. Many studies have shown that environmental, genetic and agronomic factors may change the chemical composition of plant foods. Thus, studies aiming to compare the nutritive value of plant foods from organic and conventional cultivation are difficult to implement as well as to interpret the results (Bourn and Prescott 2002). Nevertheless, because of the growing interest in this topic and the increasing production and consumption of organic foods further research should be conducted to fill the information gaps that still persist.

CONCLUSION

In the present study, the organic tomatoes of the Carmen cultivar showed a relatively higher antioxidant potential than shown by the conventional tomatoes, since they had significantly higher content of ascorbic acid and total phenolics. This confirmed the hypothesis that the type of production would affect the production of secondary metabolites by the plants. Furthermore, the alcohol and aqueous extracts obtained from the organic tomatoes revealed higher antioxidant activity, when evaluated using the DPPH test.

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