Antioxidant activity in different fractions of tomatoes

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Received 23 June 2004; accepted 15 October 2004

Abstract

The objective of this study was to determine the major antioxidants and antioxidant activity in different fractions (skin, seeds and pulp) of three tomato cultivars (Excell, Tradiro and Flavourine) grown under hydroponic conditions in a commercial greenhouse in New Zealand. It was found that the skin fraction of all cultivars had significantly ($p < 0.05$) higher levels of total phenolics, total flavonoids, lycopene, ascorbic acid and antioxidant activity (both in hydrophilic and lipophilic extracts as measured by the ABTS assay) compared to their pulp and seed fractions. The amount of antioxidants in each fraction was calculated on the basis of their actual fresh weights in whole tomato and it was found that the skin and seeds of the three cultivars on average contributed 53% to the total phenolics, 52% to the total flavonoids, 48% to the total lycopene, 43% to the total ascorbic acid and 52% to the total antioxidant activity present in tomatoes. These results show that removal of skin and seeds of tomato during home cooking and processing results in a significant loss of all the major antioxidants. Therefore, it is important to consume tomatoes along with their skin and seeds, in order to attain maximum health benefits.

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Keywords: Tomato; Fractions; Skin; Seeds; Pulp; Antioxidants; Antioxidant activity; Cooking; Processing

1. Introduction

Tomato is a versatile vegetable that is consumed fresh as well as in the form of processed products. More recently, there has been renewed attention given to the antioxidant content of tomatoes because many epidemiological studies suggested that regular consumption of fruits and vegetables, including tomatoes, can play an important role in preventing cancer and cardiovascular problems (Giovannucci, 1999; Heber, 2000; Rao & Agarwal, 2000). Tomato components like lycopene, phenolics, flavonoids and vitamins C and E are mainly responsible for the antioxidant capacity of raw tomatoes and processed tomato products (Beutner et al., 2001; Leonardi, Ambrosino, Esposito, & Fogliano, 2000; Stewart et al., 2000).

While most tomatoes produced worldwide are used in the production of tomato paste, an ingredient in different processed tomato products such as ketchup, sauces, and soups (Sanchez, Valencia, Ciruelos, Latorre, & Gallegos, 2003), a significant number of tomatoes are consumed fresh. For instance, in New Zealand, 40,000 tonnes of fresh tomatoes were supplied to the consumer market in the year 2002 (HortResearch, 2002). Most of these tomatoes are consumed raw, in salads, or after cooking at home. However, some consumers remove the skin and seeds of tomatoes before eating them raw, while some fresh tomatoes are cooked with or without the skin and seeds. Boiling and baking has been reported to have a relatively small effect on the antioxidant content of tomatoes, whereas, frying significantly ($p < 0.001$) reduced the antioxidant contents (Sahlin, Savage, & Lister, 2004). The skin of fruits and vegetables is commonly removed because they are thought to be indigestible and contain low levels of nutrients. Furthermore, approximately one-third of the
total weight of tomatoes in the form of skin and seeds is discarded during processing of tomatoes into paste (Al-Wandawi, Abdul-Rahman, & Al-Shaikhly, 1985). However, Wolfe, Wu, and Liu (2003) reported that the apple peels are a very rich source of antioxidants and had significantly higher amounts of phenolic compounds, antioxidant activity and antiproliferative activity than the flesh of apples. They suggested that regular consumption of apple peels may result in reduced risks of cardiovascular diseases and cancer.

Al-Wandawi et al. (1985) reported that tomato skin contains high levels of lycopene compared to the pulp and seeds. In addition, tomato skin and seeds were reported to contain essential amino acids, and the tomato seeds had particularly high amounts of minerals (Fe, Mn, Zn, and Cu), and monounsaturated fatty acids (especially, oleic acid). However, they did not measure the other antioxidant compounds in their study. In most of the previous studies, antioxidants have been measured, mainly in, whole tomatoes or processed tomato products (Lavelli, Peri, & Rizzolo, 2000; Martinez-Valverde, Periago, Provan, & Chesson, 2002; Raffo et al., 2002). Stewart et al. (2000) reported that the majority of the flavonols in tomatoes are present in the skin. Similarly, Sharma and Le Maguer (1996) observed that most of the lycopene was associated with the skin and water insoluble fraction of the tomato pulp. Recently, George, Kaur, Khurdiya, and Kapoor (2004) studied antioxidant components in 12 field grown tomato genotypes, and reported that on an average, the tomato skin had 2.5 times higher lycopene levels than the pulp. They also reported that the tomato skin had significant amounts of phenolics and ascorbic acid. There is a lack of information on the levels of antioxidants in the seed fraction of tomatoes, and this could be an important contributor to the antioxidant activity of tomatoes. In general, limited data are available on the contribution of the different fractions (skin, pulp and seeds) towards the total amount of the antioxidant components and antioxidant activity of tomatoes. Therefore, it is difficult to assess the health benefits of including the skin and seeds of tomatoes during home consumption or the production of processed products. The main objective of this study was to compare the major antioxidants and total antioxidant activity in the skin, pulp, and seed fractions of three commercially grown New Zealand tomato cultivars.

### 2. Materials and methods

#### 2.1. Fruit sampling

Tomato fruits from three commercial tomato cultivars: Excell, Tradiro (De Ruiter Premier Seeds, Bergshoek, Holland) and Flavourine (Enza Zaden, Enkhuizen, Holland), which are grown for local fresh consumption, were grown using a hydroponic fertigation system in a commercial greenhouse located in Christchurch, New Zealand (43°40’S, 172°29’E) and tomatoes were harvested at the light red stage of ripeness [Maturity stage 5, (Californian Tomato Commission, 2002)]. The freshly harvested tomatoes were separated into three different fractions, skin, pulp and seeds. The skin (outer epidermis) of the tomatoes was carefully separated from the flesh using a sharp knife. The seed fraction of the tomatoes consisted of the seeds along with the jelly portion. Pulp was the portion of tomato remaining after removal of the skin and seed fractions. The weights of the whole tomatoes and their fractions were recorded (Table 1) and the dry matter in the tomato fractions was determined by the gravimetric method (AOAC, 2000).

#### 2.2. Sample storage

The tomato fractions were placed in air-tight plastic bags and frozen immediately. The samples were then freeze-dried and placed in oxygen barrier bags (Cryovac Barrier bags, 60 µm x 200 mm x 255 mm, W.R. Grace (NZ) Ltd.), vacuum packed (Multivac, D-8941, Germany) and stored frozen at −18 °C for a week until analysis of their antioxidant activity, total phenolics and total flavonoids.

#### 2.3. Analyses

##### 2.3.1. Extraction method for separation of hydrophilic and lipophilic extracts

A modified method of Prior et al. (2003) was used to separate the hydrophilic and lipophilic extracts of
different fractions of tomatoes. In brief, 0.5 g of finely ground freeze-dried tomato fraction was extracted twice with 10 ml hexane, in the dark, followed by centrifugation at 3400 rcf for 10 min. The lipophilic extract was prepared by drying a known volume (4 ml) of the hexane extract under nitrogen flow. This was then redissolved in 0.5 ml of 100% acetone and vortexed for 2 min followed by addition of 1.5 ml of 80% acetone. The samples were vortexed and sonicated for 5 min to completely dissolve the residue. After the hexane extraction, the sample was used for the extraction of hydrophilic antioxidants. The residual hexane in the samples was evaporated under nitrogen flow, followed by the extraction of the residual pellet with 10 ml of acetone:water:acetic acid (70:29.5:0.5). The samples were vortexed for 1 min, then sonicated for 5 min. The tubes were covered with aluminium foil and were placed on a rotary mixer for 15 min followed by centrifugation at 3400 rcf for 10 min. The supernatant (hydrophilic extract) was then transferred to a new tube.

2.3.2. Total phenolics

Total phenolics in the hydrophilic and lipophilic extracts of the tomato fractions were measured by the method adapted from Spanos and Wrolstad (1990). In brief, the hydrophilic and lipophilic extracts were appropriately diluted and then oxidized with 2.5 ml of freshly diluted 0.2 M Folin–Ciocalteau reagent. This reaction was neutralized by adding 2.0 ml of 7.5% w/v sodium carbonate, and the samples were vortexed for 20 sec. The samples were then incubated at 45 °C for 15 min and the absorbance of the resulting blue colour was measured at 765 nm on an UV–Vis recording spectrophotometer (UV-2100, Shimadzu). Gallic acid was used as a standard, and results were expressed as gallic acid equivalents (GAE) per 100 g fresh weight (FW). The results were corrected for the contribution of ascorbic acid to the total phenolics as described by Toor (2004).

2.3.3. Total flavonoids

The flavonoid content of different fractions was measured using a colorimetric assay developed by Zhi-shen, Mengcheng, and Jianming (1999). A known volume (0.5 ml) of the hydrophilic extract or standard solutions of rutin (Sigma) was added to a 10 ml volumetric flask. Distilled water was added to make a volume of 5 ml. At zero time, 0.3 ml of 5% w/v NaNO₂ was added to the flask. After 5 min, 0.6 ml of 10% w/v AlCl₃ was added and after 6 min, 2 ml of 1 M NaOH was added to the mixture followed by the addition of 2.1 ml distilled water. Absorbance was read at 510 nm against the blank (water) and flavonoid content was expressed as mg rutin equivalents/100 g FW.

2.3.4. Lycopene

Lycopene from tomato skin, pulp and seeds was extracted using a mixture of hexane:acetone:ethanol (2:1:1) by the method of Sadler, Davis, and Dezman (1990). The lycopene content of samples was measured spectrophotometrically at 472 nm using an extinction coefficient (εₐ₅0) of 3450 (Davis, 1976; Sharma & Le Maguer, 1996) and the results were expressed as mg/100 g FW.

2.3.5. Ascorbic acid

The ascorbic acid of the fresh fractions was measured with a Metrohm 670 titroprocessor (Metrohm Herisau, Switzerland) using the AOAC method (AOAC, 1990). In brief, the samples were mixed with 40 ml of buffer (1 g/L oxalic acid plus 4 g/L anhydrous sodium acetate) and were titrated against the dye solution containing 295 mg/L DPIP (phenolindo-2,6-dichlorophenol) and 100 mg/L sodium bicarbonate. The standard curve was generated with concentrations of 0.2, 0.4, 0.6, 0.8 and 1 mg of standard L-ascorbic acid (AnalaR, BDH). The ascorbic acid content in the samples was determined from the standard curve and the results were expressed as mg/100 g FW.

2.3.6. Antioxidant activity

The radical scavenging capacity (antioxidant activity) of the acetone extracts was measured using the modified ABTS (2,2′ azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical decolorization assay (Miller & Rice-Evans, 1997). The ABTS+ radical was generated using manganese dioxide. Then 1 ml of the ABTS+ solution was added to 100 µl of standard or sample and vortexed for 10 s. The decolourisation caused by reduction of the cation by antioxidants from the sample was measured at 734 nm (Shimadzu UV21000, UV–Vis recording spectrophotometer with temperature controlled cell holder (Shimadzu TCC-260)) 1 min after addition of the sample. Assays were performed with three dilutions per extract, and duplicate assays per dilution. Trolox® (6 hydroxy-2,5,7,8-tri-methyl-chroman-2-carboxylic acid), a water-soluble vitamin E analogue, was used to prepare the standard curve and activity was reported as micromolar Trolox equivalent antioxidant capacity (µmole TEAC).

2.3.7. Statistical analysis

All data is reported as mean ± standard error of the mean for three replicates. One-way analysis of variance (ANOVA) was used and the least significant difference (LSD) at p < 0.05 was calculated using the GenStat 6th edition (Genstat, 2000) to determine significant differences between the different fractions of tomatoes. Two-way analysis of variance was also performed to study the interactions between cultivars and the different fractions of tomatoes.
3. Results and discussion

Tomatoes in this study were harvested at the same stage of development as they would be for domestic sale. The total weight of the tomatoes and the weight of the different fractions are given in Table 1. The data presented in Table 2 are the antioxidant compositions of the different fractions and are the mean values of three tomato cultivars and are based on a wet matter basis.

### 3.1. Antioxidant composition in skin, seed and pulp of different cultivars

Phenolic compounds tend to accumulate in the dermal tissues of plant body because of their potential role in protection against ultraviolet radiations, to act as attractants in fruit dispersal, and as defence chemicals against pathogens and predators (Strack, 1997). In this study, significantly \((p < 0.05)\) higher levels of total phenolics (both hydrophilic as well as lipophilic) and total flavonoids were detected in the skin of tomatoes (Table 2). The hydrophilic phenolics in the skin of three cultivars ranged from 26.9–30.3 mg GAE/100 g. Flavourine had significantly \((p < 0.05)\) lower hydrophilic phenolics in its skin than Excell and Tradiro (Fig. 1). The pulp of Tradiro had significantly \((p < 0.05)\) lower hydrophilic phenolics (2.8 mg GAE/100 g) than Excell and Flavourine (3.9 and 3.6 mg GAE/100 g, respectively) (Fig. 2). In addition, this study has also identified the seed fraction of tomatoes as an important reservoir of phenolics. The mean total phenolics (hydrophilic + lipophilic) and total flavonoids in the seeds of three cultivars were significantly \((p < 0.05)\) higher than the mean phenolic content of their pulps (Table 2).

In a recent study on red grapes by Negro, Tommasi, and Miceli (2003), it was reported that the total phenolic

![Fig. 1. Total phenolics in the hydrophilic extract of tomatoes.](image)

### Table 2

Major antioxidants and antioxidant activities in the skin, pulp and seed fractions (means ± standard error of means of the three cultivars)

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Total phenolics (mg GAE/100 g)</th>
<th>Total flavonoids (mg rutin eq/100 g)</th>
<th>Lycopene (mg/100 g)</th>
<th>Ascorbic acid (mg/100 g)</th>
<th>Antioxidant activity (μM TEAC/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophilic</td>
<td>Lipophilic</td>
<td>Hydrophilic</td>
<td>Lipophilic</td>
<td>Hydrophilic</td>
<td>Lipophilic</td>
</tr>
<tr>
<td>Skin</td>
<td>29.1 ± 1.12^a</td>
<td>5.6 ± 0.21^a</td>
<td>20.4 ± 0.61^a</td>
<td>8.7 ± 1.12^a</td>
<td>16.9 ± 0.89^a</td>
</tr>
<tr>
<td>Pulp</td>
<td>12.7 ± 2.03^c</td>
<td>2.3 ± 0.12^c</td>
<td>8.2 ± 0.37^c</td>
<td>2.8 ± 0.14^b</td>
<td>8.9 ± 0.59^b</td>
</tr>
<tr>
<td>Seeds</td>
<td>22.0 ± 3.76^b</td>
<td>3.5 ± 0.33^b</td>
<td>12.1 ± 1.18^b</td>
<td>1.6 ± 0.10^c</td>
<td>8.4 ± 0.78^b</td>
</tr>
</tbody>
</table>

Data followed by different letters in the same column are significantly different at 0.05 probability level.
substances, total flavonoids and antioxidant activity were significantly \((p < 0.01)\) higher in the seed extract of grapes compared to the peel and they suggested that by-products of grape processing could be used as sources of natural antioxidants.

Flavonoids, which are the major components of the total phenolic content of tomatoes, were also quantified in different fractions of tomato (Fig. 3). No significant difference was observed in the total flavonoids of the skin and pulp of the three cultivars. However, the flavonoid content in the seed fraction of Tradiro \((9.8 \text{ mg rutin equivalents/100 g})\) was significantly \((p < 0.05)\) lower than Excell and Flavourine \((12.8 \text{ and 13.7 mg rutin equivalents/100 g})\).

Among the three fractions of tomato, the skin was found to contain the highest levels of lycopene (Fig. 4). The lycopene content of pulp was found to be significantly \((p < 0.05)\) higher than the seed fraction. This finding is consistent with results of Sharma and Le Maguer (1996) who showed that lycopene is mostly attached to the fibre fraction of the pulp. The skin of Excell had significantly \((p < 0.05)\) lower lycopene \((6.5 \text{ mg/100 g})\) than Tradiro and Flavourine \((9.4 \text{ and 10.2 mg/100 g, respectively})\). Al-Wandawi et al. (1985) reported a lycopene content of 12 mg/100 g in the skin of tomatoes used for processing in Northern Iraq, whereas, Sharma and Le Maguer (1996) reported a value of 54 mg/100 g in skin of tomatoes obtained from Canada. George et al. (2004) reported that the lycopene content ranged from 5–14 mg/100 g in the skin of tomatoes grown outdoors in India. The variation in the lycopene content of tomatoes obtained from different parts of the world is probably due to the differences in their growing conditions. Generally, the field grown tomatoes have been reported to contain higher levels of lycopene, ranging from 5.2 to 23.6 mg/100 g (Abushita, Daood, & Biacs, 2000; Gomez et al., 2001; Takeoka et al., 2001), whereas, the greenhouse grown tomatoes are reported to contain lycopene between 0.1 and 10.8 mg/100 g (Leonardi et al., 2000). The cultivar and ripening stage of tomatoes can also affect their levels of lycopene and other antioxidants (Abushita et al., 2000; Davies & Hobson, 1981; Gomez et al., 2001; Thompson et al., 2000). These factors could account for the variation in the lycopene levels reported in different studies. The red colour of tomatoes is mainly due to presence of lycopene (Sharma & Le Maguer, 1996). Excell had a less bright red colour compared to Tradiro and Flavourine, and Excell was also found to have a significantly \((p < 0.05)\) lower amount of lycopene in its skin \((6.5 \text{ mg/100 g})\) compared with Tradiro and Flavourine, whereas, no significant differences were observed in the lycopene content of the seed and pulp fractions in the three cultivars. The results of the present study suggest that the variation in the redness of different cultivars is mainly due to a difference in the levels of lycopene accumulated in their skins.

The mean ascorbic acid content in the skin of the three cultivars was significantly \((p < 0.05)\) higher compared to the pulp and seeds (Table 2). The skin of Excell had significantly \((p < 0.05)\) higher ascorbic acid \((18.6 \text{ mg/100 g})\), whereas, the pulp and seeds had significantly \((p < 0.05)\) lower ascorbic acid content \((2.3 \text{ and 0.5 mg/100 g})\) respectively. The mean ascorbic acid content in the skin of Tradiro and Flavourine was significantly \((p < 0.05)\) lower than that of Excell. The results of the present study suggest that the variation in the levels of ascorbic acid is mainly due to a difference in the levels of ascorbic acid accumulated in their skins.
mg/100 g) than Tradiro and Flavourine (15.7 and 16.2 mg/100 g FW, respectively) (Fig. 5). The pulp of Flavourine had significantly ($p < 0.05$) lower ascorbic acid (7.8 mg/100 g) than Excell and Tradiro (9.8 and 9.1 mg/100 g, respectively), whereas, the seed fraction of Flavourine had significantly ($p < 0.05$) higher ascorbic acid (9.9 mg/100 g) than Excell and Tradiro (7.6 and 7.5 mg/100 g, respectively). These results of this study are in accordance with George et al. (2004), who reported that the ascorbic acid content ranged from 8–56 mg/100 g in the skin, and 8–32 mg/100 g in the pulp of Indian tomatoes.

To our knowledge, this is the first time that the antioxidant activity in the skin, pulp, and seeds of tomatoes has been reported. Due to higher levels of all major antioxidant compounds, the skin of tomatoes also had a significantly ($p < 0.05$) higher antioxidant activity compared to pulp and seeds. Antioxidant activity in both the hydrophilic and lipophilic extracts of tomatoes was measured and it was found that the antioxidant activity in the hydrophilic extracts of skin, seeds and pulp was the major contributor (91–93%) to the total antioxidant activity (hydrophilic + lipophilic) of the fractions. Excell skin was found to have a significantly ($p < 0.05$) higher antioxidant activity in its hydrophilic extract (242 μM TEAC/100 g) than Tradiro and Flavourine (198 and 197 μM TEAC/100 g, respectively) (Fig. 6). This is due to the higher levels of all the major contributors of the hydrophilic antioxidant activity (ascorbic acid, total flavonoids and hydrophilic phenolics) in the skin of Excell compared to the skins of other cultivars. Tradiro had a significantly ($p < 0.05$) lower antioxidant activity in its pulp (63 μM TEAC/100 g) compared to Excell and Flavourine (94 and 88 μM TEAC/100 g, respectively). This is possibly due to the lower levels of hydrophilic phenolics detected in the pulp of Tradiro.

As shown in Fig. 7, the antioxidant activity in the lipophilic extract of skin of Flavourine was significantly ($p < 0.05$) higher than Excell and Tradiro and it is probably due to the higher amount of lycopene present in the skin of Flavourine. There was no significant difference in the antioxidant activity of the lipophilic extracts in the pulps of the three cultivars. However, the seed fraction of Tradiro had significantly ($p < 0.05$) lower antioxidant activity (7.1 μM TEAC/100 g) in its lipophilic extract compared to Excell and Flavourine (10.9 and 10.1 μM TEAC/100 g, respectively), and this is probably due to the lower levels of lipophilic phenolics detected in the seed fraction of Tradiro.

3.2. Percent contribution of skin, seed, and pulp fractions to the total antioxidant content in whole tomatoes

The results (based on 100 g FW basis) show that the amount of antioxidants and antioxidant activity is higher in the skin of tomatoes compared to the pulp and
seeds (Table 2). But in fact, the amount of skin and seed fraction present in whole tomato is very low as compared to the pulp (Table 1). In order to determine the contribution of the skin and seed fractions to the total antioxidant composition of tomato, it is important to measure the antioxidants in each fraction on the basis of their actual weight in the tomato. Therefore, the amount of antioxidants in each fraction was calculated on the basis of their actual weights in whole tomatoes. Percent contribution of each fraction to the total amount of antioxidants present in tomato (skin + seeds + pulp) was calculated and the results are given in Table 3.

The skin and seeds of the three cultivars on average contributed 53% to the total phenolics, 52% to the total flavonoids, 48% to the total lycopene, and 52% to the total antioxidant activity, present in tomatoes. These results show that the skin and seed fractions are important contributor to the major antioxidants and overall antioxidant activity of tomatoes. Therefore, removal of tomato skin and seeds during their fresh consumption or home cooking means a significant loss of the antioxidants. Similarly, the discard of tomato slurry (consisting of skin and seeds of tomatoes) during the preparation of processed tomato products means a loss of antioxidants from the tomato processing wastes. The skin and seed fractions of tomatoes could also be used as a value added ingredient in other food products. Along with a diet rich in other plant produce, tomatoes, and their skins and seeds, could play an important role in improving antioxidant intake in the human diet.

### 4. Conclusions

This study suggests that the skin and seed fractions of tomato are a very rich source of antioxidant compounds, and the incorporation of the skin and seeds fraction during home consumption or processing could lead to about a 40–53% increase in the amount of all the major antioxidants in the final product. Therefore, removal of these fractions during home cooking or processing results in a loss of their potential health benefits. Consumer demand for healthy food products provides an opportunity to develop foods rich in antioxidants as new functional foods. By adopting slight changes during processing, the antioxidant and nutrient composition of the final products can be increased, and a valuable reserve of antioxidants would be optimally utilized. The results of this study will be also of value in designing a unit on-site for the extraction of lycopene and other antioxidants from the tomato processing wastes. The skin and seed fractions of tomatoes could also be used as a value added ingredient in other food products. Along with a diet rich in other plant produce, tomatoes, and their skins and seeds, could play an important role in improving antioxidant intake in the human diet.

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