Phenolic Content and Antioxidant and Antimutagenic Activities in Tomato Peel, Seeds, and Byproducts

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ABSTRACT: The phenolic content and antioxidant and antimutagenic activities from the peel and seeds of different tomato types (grape, cherry, bola and saladette type), and simulated tomato industrial byproducts, were studied. Methanolic extracts were used to quantify total phenolic content, groups of phenolic compounds, antioxidant activities, and the profile of phenolic compounds (by HPLC-DAD). Antimutagenic activity was determined by Salmonella typhimurium assay. The total phenolic content and antioxidant activity of tomato and tomato byproducts were comparable or superior to those previously reported for whole fruit and tomato pomace. Phenolic compounds with important biological activities, such as caffeic acid, ferulic acid, chlorogenic acids, quercetin-3-O-glycoside, and quercetin, were quantified. Differences in all phenolic determinations due to tomato type and part of the fruit analyzed were observed, peel from grape type showing the best results. Positive antimutagenic results were observed in all samples. All evaluated materials could be used as a source of potential nutraceutical compounds.

KEYWORDS: tomato byproducts, phenolic compounds, in vitro bioactivity, nutraceutical profiling

INTRODUCTION

In terms of cultivated area, tomato is the second most important crop in the world. Mexico has the ninth place in production (∼2 million metric tons/year), and Sinaloa state is the main producer with 37% of the national production. The main cultivated varieties in Sinaloa are saladette or Roma, bola, cherry type, and grape type. Tomatoes are used as food in fresh or processed (e.g., paste, juice, and sauce) forms, and worldwide consumption has increased in the past two decades. In our country, approximately 120,000 tons of tomato are industrialized annually, generating 6000 tons of byproducts; these residues are mainly composed of peel and seeds, which are usually used as feed and not for human consumption.

On the other hand, the whole tomato fruit has an important content of C and E vitamins and some minerals such as K, P, and Mg, carotenoids, mainly lycopene, and important phenolic compounds such as caffeic acid, ferulic acid, kaempferol, quercetin, and others have been identified in the fruit. In this way, tomato seed and peel generated as byproducts contain important quantities of the above-mentioned compounds, too, and even fiber, fatty acids, sitosterol, isorhamnetin, myricetin, and others. Although it has been found that the application of heating or storing processes decreases the content of these compounds, studies have shown that these compounds remain at acceptable levels and show nutraceutical activity. Furthermore, the consumption of fresh tomatoes, processed tomatoes, and dietary supplements based on tomato fruit has been associated with prevention of some diseases such as cancer, cardiovascular diseases, and Alzheimer’s. Most important biological activities demonstrated in tomato fruit, tomato food products, and byproducts are antimicrobial, antioxidant, antimutagenic, and anti-inflammatory. Traditionally, the bioactivity of tomato and its products has been attributed to carotenoids, although phenolic compounds have shown antioxidant and cytotoxic activities in vitro, in both processed products and fresh tomato. Phenolic compounds comprise a wide group of chemicals that contain one or more phenyl rings in their structure, with at least one hydroxyl bonded to the phenyl structure. The phenolic compounds mostly distributed in foods are phenolic acids and flavonoids (60 and 30% of the total phenolic consumed in the diet, respectively); several papers describe their attributes as natural and potent antioxidant, anticarcinogenic, and chemopreventive agents and other bioactivities. An important issue is that the bioactivity of the phenolic compounds from any natural source depends on the quality and quantity of compounds that are present, and the phenolic concentration could be affected by both cultivation system and genotype of the source. For that reason, it is necessary to strengthen the search for nutraceutical compounds as phenolics from several sources, including tomato byproducts from different varieties.

The purpose of this work was to determine the phenolic content and antioxidant and antimutagenic activities of the peel...
and seed of different tomato cultivars from Sinaloa, Mexico, to
take advantage of these byproducts to enrich animal or human
food and have ecological and economic benefits, reducing
tomato agricultural and industrial wastes.

MATERIALS AND METHODS

Chemicals and Reagents. HPLC grade acetonitrile and methanol
were purchased from Karal (Leon, Gto., Mexico). Minimal glucose
ag and Oxoid nutrient broth were bought from Oxoid Microbiology
Products (Thermo Fisher Scientific, New York, NY, USA). Solvents
and other reagents, including the phenolic standards (gallic acid,
cafeic acid, ferulic acid, p-coumaric acid, o-coumaric acid, (+)-catechin
hydrate, quercetin anhydrous, rutin hydrate, kaempferol, isorhamnetin,
and myricetin) were ACS grade solvents or reagents and pure grade
standards (Sigma Chemical Co., St. Louis MO, USA).

Biological Material. Organically grown tomatoes, cultivated in
Sinaloa (a state in northwestern Mexico) during the autumn-winter
2010–2011 season, of four varieties (saladette, bala, grape, and cherry)
were collected (20 kg/cultivar) in the breaker stage (USDA, 1991)
from April 10 to May 10, 2011. Collected tomato materials were transported to the
breaking and slicing line of a processor in the tomato industry.

Preparation of the Methanolic Extract. Peel and seed byproducts
were manually separated, frozen at
−20 °C during 1 h in an aluminum pot with
constant stirring to get the higher yield of juice. The mixture was
filtered with a gauze swab, juice was discarded, and the remnants of
the peel and seeds were manually separated, frozen at −20 °C, and freeze-
dried. Dried byproducts were ground in a conventional blender until juice
suggested by an engineer from a local processor in the tomato industry
was collected (20 kg/cultivar) in the breaker stage (USDA, 1991)
were calculated from
ical measurement was performed to transport the
and stored at 20 °C until they reached the red stage (last
stage in the color scale). Commercial saladeetto tomatoes (20 kg) were
bought in a local market to get simulated industrial byproducts;
saladeetto tomatoes are the main material used for the regional tomato-
processing industry to produce tomato paste.

Color Analysis. The color of ripe tomatoes was measured with a
chroma meter CR-400 (Konica Minolta Sensing, Inc., Japan);
10 whole fruits of each type were evaluated in five points, three
equidistant equatorial points and two axial. Chroma and a* b*/b data
were calculated from L*a, a*, and b* CIELab parameters, using a white
standard CR-A43.

Sample Processing. For the chemical analysis, the peel and seeds of
all tomato types were manually separated from the pulp, frozen
(−20 °C) and freeze-dried (Labconco Co., Kansas City, MO, US).
The simulated commercial tomato byproducts were prepared as
suggested by an engineer from a local processor in the tomato industry
(personal communication); ripe saladeetto tomato (20 kg) was cut into
cubes of 2 cm3, ground in a conventional blender until juice
appearance, and heated at 80 °C during 1 h in a aluminum pot with
constant stirring to get the higher yield of juice. The mixture was
filtered with a gauze swab, juice was discarded, and the remnants of
peel and seeds were manually separated, frozen at −20 °C, and freeze-
dried. Dried byproducts were ground in a laboratory mill (Thomas
Wiley, Thomas Scientific, Swedesboro, NJ, USA) until we had a fine
powder (80 mesh size) and stored (4 ºC/protected from light) until
analyses. Three biological replicates of each variety of tomato
were processed.

Proximal Composition Analysis. Proximal composition was
Jeol JMS-700, Kyoto, Japan) with a mass range of 100 to 600
mass units at a resolution of 1000 (0.1% relative error).

Preparation of the Methanolic Extract. Peel and seed flour
were extracted as described by Ferreres et al.10 with some
modifications. Samples (0.5 g) were suspended in 5 mL of methanol
in 15 mL plastic tubes, sonicated during 1 h (mixed with a vortex
every 10 min), transferred into 125 mL Erlenmeyer flasks, and made up
to 20 mL with methanol. Then, samples were incubated for 22 h
(25 °C/darkness/220 rpm) and centrifuged at 1400g/3 min in a rotor
J6008R2004 (Solbat S.A. de C.V., Puebla de Zaragoza, Mexico),
the supernatant was decanted and stored at −20 °C, and the pellet
was re-extracted by adding 20 mL of methanol and following the
previously described steps. After supernatants were combined and concentrated to dryness at 37 °C in a rotary
evaporator (Yamato, Santa Clara, CA, USA). The extract
were reconstituted with 3 mL of methanol, filtered through 0.22 µm
Durapore syringe filters (Millipore, Carrigtwohill, Co. Cork, Ireland),

and kept stored at −20 °C until use (this was called the methanolic
crude extract). Each tomato sample was made in triplicate.

Total Polyphenol Content. The total polyphenol content of
 crude methanolic extract was quantified using the Folin–Ciocalteu
spectrophotometric method, according to Nurmi et al., with
modifications made by Ramirez-Mares et al.12. Briefly, methanolic
crude extract was dissolved in methanol (1:30 p/v), and 100 µL of
the solution was mixed with 1 mL of 0.5 N Folin–Ciocalteu’s phenol
reagent and 2 mL of a 20% (w/v) Na₂CO₃ solution; the mixture was
allowed to stand for 15 min at darkness, and the absorbance
was measured at 734 nm. Gallic acid (GA) and (+)-catechin hydrate
(CAT) were used to prepare calibration curves, and results were
reported as milligrams of gallic acid equivalents per gram of flour
(mg GAE/g) or catechin equivalents (mg CE/g). Groups of phenolic
compounds were quantified by spectrophotometry13 at 320 nm for
tartaric esters of phenolic acids and at 404 nm for flavonoids in a
Thermo Electron Genesys 2 spectrophotometer (Thermo Fisher
Scientific). Results of groups of phenolic compounds were expressed
as caffeic acid or quercetin equivalents per gram of peel or seed flour,
for phenolic tartaric esters or flavonoids, respectively.

Antioxidant Activity. The antioxidant activity of crude meth-
anolic extracts was measured by the DPPH,²² ABTS,²² and ORAC²²
methods. DPPH and ABTS are free stable oxidative radicals, and we
measured spectrophotometrically their decrease in absorbance (as
the effect of the phenolic compounds presents in the samples). In the
ABTS method, the ABTS⁺ radical was chemically prepared (7.8 mM in
methanol); the ABTS reagent was mixed with potassium persulphate
(2.45 mM), incubated during 16 h at room temperature, and diluted
with methanol to get an absorbance value of 0.70 ± 0.02 at 734 nm.
To measure the antioxidant activity, 10 µL of the concentrated
methanol crude extract (controls or standard) was mixed with 1000 µL
of ABTS⁺ and vortexed gently during 20 s, and the absorbance at
734 nm was registered.

For the DPPH assay, the radical (125 µM in methanol) was daily
prepared and kept at 4 °C/darkness. Assay was done in a 96-well
microplate; 20 µL of the concentrated methanol crude extract
(controls or standard) was mixed with 200 µL of DPPH solution,
and absorbance (515 nm) was read at 0, 10, 20, 30, 40, 50, 60, 70, 80,
and 90 min with a DTX 880 multimode detector (Beckman Coulter,
Brea, CA, USA). Trolox standard curve was constructed at 30 min.

For the Oxygen radical absorbance capacity (ORAC) (performed
fluorometrically) assay,²² analysis was done at 37 °C in pH 7.4, 75 mM
phosphate buffer, peroxyl radical was generated using AAPH
(70 mM), and fluorescein (87 µM) was the substrate. Twenty-five
microliters of diluted (1:100) sample (control or standard) was mixed
with 200 µL of fluorescein and 50 µL of AAPH, and the increase
of fluorescence was calculated in a DTX 880 multimode detector, using
the following conditions: excitation wavelength of 485 nm and
emission wavelength of 530 nm; 76 cycles were read, every 2 min. In
all cases we report antioxidant activity as Trolox equivalents, a
standard curve was prepared with a correlation coefficient up to R =
0.99, and the equivalents were calculated as micromole equivalents
of Trolox per 100 g of flour. Three independent experiments
were conducted in each assay.

Profile of Phenolics by HPLC-DAD. The HPLC profile of
phenolics in the methanolic crude extract was established with a
Dionex UltiMate 3000 liquid chromatograph, equipped with a
titanium quaternary pump (LPG-3400AB), an autosampler (WPS-
3000TBP), a column oven, and a photodiode array detector DAD-
3000(RS) (scan range 190–800 nm and scan speed 2 nm/s) from
Thermo Scientific. Separation was carried out using an analytical
column Acclaim 120 Å (C18, 5 µm, 120 Å, 4.6 × 250 mm), from
Dionex (Thermo Fisher Scientific), at ambient temperature. Elution
was done with a gradient of two solvents: water acidified with acetic
acid (pH 2.8) (A) and acetonitrile (B). The gradient for phenolic acids
was 100% A, 6% phase B, from 0 to 8 min; from 8 to 14 min; from 12 to 20% B from 14 to 18 min; from 20 to 35% B from 18 to 24 min,
from 35 to 95% B from 24 to 27 min; from 95 to 100% B from 27 to 30 min; and from 100% B to 100% A in 10 min. The
fixed wavelengths were 260, 270, 275, 280, 285, 290, 295, and 300 nm.

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The proximal composition of peel and seed of all tomato types can be observed in Figure 1. All measured parameters were higher than the average values previously reported for whole tomato fruit, except total lipids in peel, and were closer than those reported for seed and peel from tomato pomace. Notable protein content was found in all seed samples, >24 g/100 g, closer than that reported by Fuentes et al. for tomato seed from pomace. Saladette type had the highest protein percentage (28.7 g/100 g of flour), and a high ash content was quantified in the majority of tomato types (6.7 g/100 g of flour, on average); the mentioned parameter was superior to that reported by different authors in flour.

## RESULTS AND DISCUSSION

### Color and Proximal Composition

Maturity stage was not significantly different among tomato varieties considered in this study. Tomato color was uniform inter- and intratomoato varieties, as indicated by the low variability of the color parameters ($L^*$, $a^*$, $b^*$, chroma, and $a^*/b^*$ values) and their statistical similarity (Table 1). The ripeness stage for these tomatoes corresponded with that accepted for marketing.

![Figure 1. Proximal composition of flour from peel (A) and seeds (B) of different tomato types and tomato byproducts. Data are the average of three replicates ± standard deviation. Statistical analysis of proximal composition is not shown in this figure, but significant differences between treatments were observed.](image)

### Antimutagenic Activity Test

The antimutagenicity of the methanolic crude extracts was determined, the extracts were concentrated to dryness under a nitrogen flow, and the solid extract was resuspended in sterilized DMSO. The antimutagenic activity was done by the microsuspension assay using YG1024 *Salmonella typhimurium* as tester strain and 1-nitropyrene as mutagen, according to the method of Santos-Cervantes et al. A dose–response curve of all flavonoids and antioxidant or antimutagenic activity was done through the response curve of positive control plate.

\[
\text{% antimutagenic activity} = \left( \frac{\text{His}^+ \text{ revertants of positive control plate}}{\text{His}^+ \text{ revertants of sample test plate}} \right) \times 100
\]

### Statistical Analysis

A totally random experimental design was used to evaluate the color parameters and a factorial design $5 \times 2$ for the chemical and antimutagenic analysis; the first factor was tomato type, and the second one was the anatomic part of the tomato fruit. ANOVA analysis and a multiple-ranges test (Tukey, $p \leq 0.05$) were done with the SAS 7.0 statistic program (SAS Institute Inc., Cary, NC, USA). A correlation test between total phenolics/individual phenolic content and antioxidant or antimutagenic activity was done through the Pearson’s correlation coefficient option. Three biological replicates and at least two independent experiments were performed.

![Table 1. Parameters of Color of Whole Tomato Fruits from Several Varieties Cultivated in Northwestern Mexico (X ± SD, n = 10)\(^a\)](image)

<table>
<thead>
<tr>
<th>tomato type</th>
<th>$L^*$ ± SD</th>
<th>$a^*$ ± SD</th>
<th>$b^*$ ± SD</th>
<th>chroma ± SD</th>
<th>$a^<em>/b^</em>$ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>cherry</td>
<td>31.4 ± 1.45 b</td>
<td>13.8 ± 1.64 ab</td>
<td>11.2 ± 1.29 b</td>
<td>17.5 ± 3.72 a</td>
<td>1.24 ± 0.17 a</td>
</tr>
<tr>
<td>grape</td>
<td>34.2 ± 1.58 a</td>
<td>17.2 ± 1.50 a</td>
<td>13.0 ± 0.96 ab</td>
<td>21.6 ± 1.36 a</td>
<td>1.32 ± 0.15 a</td>
</tr>
<tr>
<td>sola</td>
<td>36.7 ± 1.30 a</td>
<td>15.9 ± 1.74 a</td>
<td>15.1 ± 0.96 a</td>
<td>20.1 ± 3.14 a</td>
<td>1.05 ± 0.13 ab</td>
</tr>
<tr>
<td>saladette</td>
<td>35.6 ± 1.36 a</td>
<td>15.9 ± 1.35 a</td>
<td>15.0 ± 0.92 a</td>
<td>19.4 ± 4.03 a</td>
<td>1.06 ± 0.09 ab</td>
</tr>
<tr>
<td>byproducts</td>
<td>34.8 ± 1.80 a</td>
<td>17.5 ± 2.60 a</td>
<td>12.9 ± 2.10 ab</td>
<td>18.2 ± 4.40 a</td>
<td>1.37 ± 0.11 a</td>
</tr>
</tbody>
</table>

\(^a\)Values marked by the same letter within the same column are not statistically significantly different (multiple-range test; Tukey, $p \leq 0.05$).

The gradient for the elution of flavonoids was 100% A, 10% B, from 0 to 2.5 min; from 10 to 12% B from 2.5 to 6 min; from 12 to 23% B from 6 to 18 min; from 23 to 35% B from 18 to 24 min; from 35 to 95% B from 24 to 27 min; from 95 to 100% B from 27 to 30 min; and from 100% B to 100% A in 10 min; 260 and 342 nm were the fixed wavelengths for flavonoids. The flow rate was 0.5 mL/min. The injection volume was 10 μL. Chromatographic peaks were identified by comparing the retention times and UV–visible absorption of pure standards (i.e., gallic, caffeic, ferulic, p-coumaric, and o-coumaric acids; and flavonoids (+)-catechin hydrate, quercetin, rutin hydrate, kaempferol, isorhamnetin, and myricetin). A standard curve was constructed with the mix of all phenolic acids and another with the mix of all flavonoids. Each sample was injected three times. The data were processed using Chromelope 7.0 software (Dionex, Thermo Fisher Scientific).
Crude fiber of seed plus peel of each tomato type was superior that previously reported for tomato pomace. The byproducts contained the highest fiber content (38.5 g/100 g of flour) ($p \leq 0.05$). This effect could be due to the result of the heat process, as it has been previously reported in other vegetables that insoluble dietary fiber increased as a result of a cooking process. Total lipids in peel correspond to 3% of the proximal composition and are represented by waxes, mainly. In seeds, total lipids represented up to 15 g/100 g of flour, and in the case of grape tomato seeds, these compounds reached 20 g/100 g of flour; it could be important to analyze the fatty acid profile and other hydrophobic compounds as phytosterols to determine a potential commercial use. Analysis of variance (ANOVA) showed that all proximal composition parameters were affected by tomato genotype, the part of the fruit analyzed (peel or seed), and the interaction of both factors.

High protein and total fiber contents found in our samples suggest that the peel or seed of tomato dry flour can be used as a source of those substances to enrich animal or human food.

**Total Phenolic Content, Groups of Phenolic Compounds, and Antioxidant Capacity.** Crude methanolic extracts showed levels of phenolic content (Table 2) comparable to those of other fruits recognized for their phenolic content, such as kiwi and grapes. The ranges were from 71.6 to 351.6 mg equiv gallic acid (GA)/100 g flour samples (208.2 mg equiv GA/100 g flour, on average) and from 67.3 to 121.8 mg equiv GA/100 g seed flour (95.2 mg equiv GA/100 g flour, on average); these values were superior to those reported for peel and seed from several tomato varieties and tomato byproducts. In the case of the total phenolic content expressed as milligram equivalents of (+) catechin (CAT), data ranged between 464.3 and 1824.1 mg equiv CAT/100 g peel (1136.8 mg equiv CAT/100 g peel, on average) and between 438.3 and 682.8 mg equiv CAT/100 g seed flour (569.4 mg equiv CAT/100 g, on average) in seed samples and were superior to those previously reported by George et al. in tomato peel. There were found statistically significant differences ($p \leq 0.05$) due to the interaction of tomato type and part of the fruit and the notable presence of a higher amount of phenolic compounds in peel flour than in seed flour, as has been reported by Chandra et al. Grape peel flour was the material with the best total phenolic content in both ways of expressing phenolic concentration (351.6 mg equiv GA/100 g flour and 1824.1 mg equiv CAT/100 g flour), whereas byproduct seed flour showed the lowest phenolic content (67.3 mg equiv GA/100 g flour and 464.3 mg equiv CAT/100 g flour) than saladeet (byproducts were produced from a saladette tomato type); perhaps this decrease in phenolic content could be due to the heat processing. In addition, phenolics oxidation could have occurred during homogenization of the fresh fruit due to polyphenol oxidase, but the total phenolic concentration in byproducts remains attractive for their use as a source of phenolic compounds. Sagdic et al. in 2011 reported the content of total phenolic compounds in byproducts of grape fruit; our data obtained in cherry and grape tomato peel flours were comparable to those reported by these authors. Differences in the phenolic content for effect of tomato genotype have been reported; the highest amount of phenolic compounds found in cherry and grape tomato types could be due to these genotypes being less genetically manipulated than saladeet and bola, and it is known that

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Table 2. Total Phenolic Content, Groups of Phenolics, and Antioxidant Capacity of Methanolic Extracts from Peel or Seed Flour of Different Tomato Varieties ($X \pm SD/100$ g).

<table>
<thead>
<tr>
<th>Sample/Flour Type</th>
<th>Total Phenols* (mg equiv GA/100 g)</th>
<th>Catechins* (mg equiv CAT/100 g)</th>
<th>Tartaric Esters* (mg equiv TART/100 g)</th>
<th>Other Phenolic Esters* (mg equiv ESR/100 g)</th>
<th>Antioxidant Capacity (mg equiv Trolox/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peel</td>
<td>208.2 ± 31.6</td>
<td>1136.8 ± 31.6</td>
<td>304.0 ± 15.6</td>
<td>209.2 ± 15.6</td>
<td>1136.8 ± 31.6</td>
</tr>
<tr>
<td>Bola</td>
<td>83.7 ± 15.6</td>
<td>50.0 ± 15.6</td>
<td>20.4 ± 15.6</td>
<td>10.2 ± 15.6</td>
<td>50.0 ± 15.6</td>
</tr>
<tr>
<td>Saladette</td>
<td>109.6 ± 15.6</td>
<td>417.9 ± 15.6</td>
<td>27.8 ± 15.6</td>
<td>10.4 ± 15.6</td>
<td>417.9 ± 15.6</td>
</tr>
<tr>
<td>Byproducts</td>
<td>67.3 ± 15.6</td>
<td>59.0 ± 15.6</td>
<td>15.8 ± 15.6</td>
<td>4.2 ± 15.6</td>
<td>59.0 ± 15.6</td>
</tr>
</tbody>
</table>

*Values coded by the same letter within the same column are not statistically significantly different (multiple-range test; Tukey, $p \leq 0.05$). Total phenolic compounds were quantified with the +catechins (CAT) per 100 g of sample, mg equivalents of gallic acid (GA) per 100 g of sample, mg equivalents of quercetin (QUER) per 100 g of sample, and mg equivalents of Trolox per 100 g of sample.
crops that have been largely genetically improved show reduced concentration of some metabolites, even phenolic compounds. The amount of the different phenolic groups can be observed in Table 1. The major group was flavonoids, ranging from 83.7 mg/100 g flour in byproducts to 572.2 mg/100 g of grape tomato peel flour; in the case of seed samples, flavonoids were quantified from 50.0 to 69.2 mg/100 g in byproducts and grape tomato flour, respectively. On the other hand, phenolic tartaric esters ranged from 3.1 to 16.9 mg/100 g for byproducts and cherry tomato peel flours, respectively; meanwhile in seeds a decrease in content until 2.6 times ranged from 3.2 to 6.3 mg/100 g for byproducts and grape tomato seed, respectively. In the same way as happened with total phenolic content, flavonoid and ester phenolics concentrations were higher in the peel than in seed flour, in most of cases. Statistically significant differences (p ≤ 0.05) were observed between treatments. We observed that approximately 60% of total phenolic content corresponded to flavonoids and 30% to phenolic acids, in the same way that has been found in several samples. Oomah et al. reported levels of flavonoids similar to those that we found in tomato peel in hull fractions and residues from red and green lentils, but this previous study was done with acetone and hot water extracts, and the proportion of phenolic compounds extracted varies widely according to the extraction solvent, so our results and those reported previously are not completely comparable.

Antioxidant activity was determined with three methodologies, ABTS, DPPH, and ORAC, and results were expressed as micromole equivalents of Trolox per 100 g of flour (peel or seed) in all cases. Peel samples showed the best results in all cases. ABTS and DPPH methodologies have a similar chemical makeup, but the data obtained with these two methods were different between the same samples. ABTS measured a more major antioxidant activity than DPPH in the peel samples of all evaluated genotypes except in byproducts, in which case there was no significant difference (p ≤ 0.05) in the antioxidant activity due to the radical used (ABTS or DPPH). In contrast, in seed samples, the DPPH methodology showed the best results in all genotypes. Due to the previously described behavior, it can be inferred that the qualities of the extracted compounds are different between peel and seed samples because both extracts reacted differently on the same free radical, as observed before. The best results were obtained in grape tomato genotype, again. This sample showed 405.7 μmol equiv Trolox/100 g flour by ABTS and 181.4 μmol equiv Trolox/100 g flour by DPPH in peel and 139.6 μmol equiv Trolox/100 g flour by ABTS and 156.2 μmol equiv Trolox/100 g flour with DPPH methodology in seed sample. Antioxidant activity determined by ABTS was superior to that previously reported in aqueous extracts from tomato plants and yellow cherry whole tomato fruits.

Antioxidant capacity calculated with ORAC methodology was significantly higher than those quantified with ABTS and DPPH methodologies (p ≤ 0.05); values were in the range between 705.3 and 4131.7 μmol equiv Trolox/100 g of flour in peel samples and from 1028.3 to 1825.0 μmol equiv Trolox/100 g flour in the case of seed samples. In both cases the lowest activity corresponded to byproducts. The highest activity was measured in grape tomato type (p ≤ 0.05). Despite this, the antioxidant activity of the byproducts remained at high levels. Several studies have shown that ORAC methodology is more adequate to quantify antioxidant activity, due to its resemblance to a biological process in vivo, regarding the peroxyl free radicals. It is, however, desirable that our results were validated through an in vivo antioxidant activity methodology, reducing confounding factors that affect the action of phenolic compounds over free radicals. The ORAC methodology does not take into account those factors; thus, further studies are required. There was observed an influence of the tomato type, as has been reported before by Chandra et al., the part of the fruit analyzed, and the interaction of these two factors on the antioxidant activity measured by the three methodologies used.

Correlations analysis (Pearson, SAS 7.0) between total phenolic and antioxidant activity and between groups of phenolic compounds and antioxidant activity were done (data not shown). We calculated R values >0.8 in all cases except when we correlated flavonoids with antioxidant activity determined by the DPPH method. R values >0.8 are considered higher. It has been reported that not all extractable compounds that can be measured as total phenols or belonging to a phenolic group may have the capacity to act against a free radical used in a particular methodology to determine antioxidant activity (scavenging activity, mainly); this may be due to structural aspects or steric impediments of the different compounds that are present in the extract.

Phenolic Compounds Profile by HPLC-DAD. Results of phenolic compounds profile by HPLC-DAD of each tomato sample (peel and seed flour) are summarized in Table 3, and Figure 2 shows the chromatograms obtained in the peel of grape tomato type. We have identified and quantified 16 compounds in total, 8 phenolic acids and 8 flavonoids. It is interesting that byproducts did not always present the lowest concentration of individual phenolic compounds, but the trend was to diminish the content of the evaluated compounds in byproducts, perhaps by the heat-processing effect, as has been mentioned above. Flavonoids were found in higher concentration than phenolic acids in all tomato types (as happened in phenolic groups evaluation). The most abundant phenolic acids were caffeic acid, in concordance with that reported before in whole red tomato fruit, followed by vanillic, ferulic, sinapic, and chlorogenic acids; in the case of flavonoids, the most concentrated were quercetin-3-β-D-glucoside, rutin, isorhamnetin, and kaempferol. Rutin (and its diverse chemical derivatives) is localized in the last steps of the phenolic biosynthesis route and is related to a commercially mature stage of fruits. The content of all phenolic acids and flavonoids determined by HPLC-DAD, in our samples, was 3–5 times greater than that evaluated with the total phenolic methodology (Folin–Ciocalteu). There are diverse reports about the individual content of phenolic compounds in tomato fruits and seeds generated as byproduct, including an effort to characterize tomato metabolome, from a mix of 96 cultivars from the Iberian peninsula; these works were done by HPLC and HPLC coupled to mass spectrometry. The main phenolic compounds found in tomato in previous studies (caffeic, ferulic, p-coumaric, and sinapic acids, rutin, quercetin, and kaempferol) are almost the same as those found in our work and were present in comparable levels to those reported by other authors. There have been reported attractive phenolic compounds such as caffeic, ferulic, and chlorogenic acids and rutin in tomato industrialized products (tomato juices and ketchup) in important levels, and we observed the presence of these phenolics in our byproduct samples. Therefore, we can mention that these compounds remain after a heat or cooking process.
Table 3. Profile of Phenolic Compounds, Phenolics Acids, and Flavonoids in the Peel and Seed of Different Tomato Types Cultivated in Northwestern Mexico (X ± SD, n = 3)*

<table>
<thead>
<tr>
<th>Phenolic acid</th>
<th>mg/100 g DWa,b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>caffeic acid</td>
</tr>
<tr>
<td>peel</td>
<td></td>
</tr>
<tr>
<td>cherry</td>
<td>5.11 ± 1.24</td>
</tr>
<tr>
<td>grape</td>
<td>22.06 ± 3.24</td>
</tr>
<tr>
<td>bolo</td>
<td>7.85 ± 0.00</td>
</tr>
<tr>
<td>saladette</td>
<td>10.51 ± 0.38</td>
</tr>
<tr>
<td>byproducts</td>
<td>1.05 ± 0.17</td>
</tr>
<tr>
<td>seeds</td>
<td></td>
</tr>
<tr>
<td>cherry</td>
<td>2.19 ± 0.17</td>
</tr>
<tr>
<td>grape</td>
<td>1.67 ± 0.16</td>
</tr>
<tr>
<td>bolo</td>
<td>2.13 ± 0.03</td>
</tr>
<tr>
<td>saladette</td>
<td>0.95 ± 0.04</td>
</tr>
<tr>
<td>byproducts</td>
<td>1.95 ± 0.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>mg/100 g DWa,b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>quercetin-3-O-β-glucoside</td>
</tr>
<tr>
<td>peel</td>
<td></td>
</tr>
<tr>
<td>cherry</td>
<td>15.12 ± 2.04</td>
</tr>
<tr>
<td>grape</td>
<td>47.99 ± 2.71</td>
</tr>
<tr>
<td>bolo</td>
<td>18.30 ± 0.05</td>
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<tr>
<td>saladette</td>
<td>25.24 ± 0.23</td>
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<tr>
<td>byproducts</td>
<td>4.47 ± 0.10</td>
</tr>
<tr>
<td>seeds</td>
<td></td>
</tr>
<tr>
<td>cherry</td>
<td>3.94 ± 0.07</td>
</tr>
<tr>
<td>grape</td>
<td>2.61 ± 0.14</td>
</tr>
<tr>
<td>bolo</td>
<td>3.23 ± 0.01</td>
</tr>
<tr>
<td>saladette</td>
<td>3.37 ± 0.03</td>
</tr>
<tr>
<td>byproducts</td>
<td>2.68 ± 0.16</td>
</tr>
</tbody>
</table>

*Values marked by the same letter within the same column are statistically significantly different between treatments (multiple-range test; Tukey, p ≤ 0.05). a,b Dry weight.
Attractive biological activities have been reported in the phenolic compounds found in the higher concentrations in this work; that is, ferulic and sinapic acids have been correlated with antioxidant and anti-inflammatory activities and act as chemoprotector agents, if they are frequently consumed in the diet.\cite{43}

Mathematical correlation analysis (data not shown) revealed that the compounds that showed the highest level of correlations ($R = 0.8$) with antioxidant activity were those that were detected.

Figure 2. HPLC-DAD chromatograms of phenolic compound standards and phenolics and flavonoids determined in a methanolic extract from the peel of grape tomato. (A1) Phenolic acids standard mix, absorbance at 260 nm, labeled peaks in order from left to right are gallic, protocatechuic, chlorogenic, caffeic, vanillic, ellagic, $p$-coumaric, ferulic, sinapic, and trans-cinnamic. (A2) Phenolic acids detected in grape tomato peel methanolic extract at 260 nm (retention times): 23.7 min = chlorogenic acid, 27.1 min = caffeic acid, 27.9 min = vanillic acid, 31.3 min = $p$-coumaric acid, 31.8 min = ferulic acid, 32.3 min = sinapic acid, and 38.9 min = trans-cinnamic acid; other signals unknown. (B1) Flavonoids standard mix, absorbance at 360 nm, labeled peaks from left to right are catechin, epicatechin, rutin, quercetin 3-$\beta$-glucoside, epicatechin gallate, myricetin, quercetin, apigenin, naringenin, kaempferol, and isorhamnetin. (B2) Flavonoids found in grape tomato peel methanolic extract, main signals detected at 360 nm, retention times of the identified compounds are 27.7 min = rutin, 29.1 min = quercetin 3-$\beta$-glucoside, 33.3 min = myricetin, 35.1 min = quercetin, 37.9 min = apigenin, 38.4 min = naringenin, 39.0 min = kaempferol, and 40.1 min = isorhamnetin; other signals unknown.

Attractive biological activities have been reported in the phenolic compounds found in the higher concentrations in this work; that is, ferulic and sinapic acids have been correlated with antioxidant and anti-inflammatory activities and act as chemoprotector agents, if they are frequently consumed in the diet.\cite{43} Rutin and quercetin are able to suppress mutagenesis and have antitumoral capacity.\cite{43}

Mathematical correlation analysis (data not shown) revealed that the compounds that showed the highest level of correlations ($R = 0.8$) with antioxidant activity were those that were detected.
in high levels, but only quercetin-3-β-O-glycoside, quercetin, and naringenin showed correlation with antioxidant activity evaluated with at least two methodologies. The high correlations found between individual phenolic concentrations and antioxidant activity, in many cases, suggest that the antioxidant activity determined in this work could be given by the action of the quantified phenolic compounds or by the interaction between the different compounds present in the extract, although further studies are necessary to prove this.

**Antimutagenic Activity Test.** Antimutagenic activity, expressed as percentage of mutagenic inhibition, as a response to methanolic tomato peel and seed extracts was determined (Figure 3). A dose–response curve using 0.0, 5.0, 10.0, 20.0, 30.0, and 40.0 mg/mL of solid extract (using seed saladette tomato type extract) was constructed; no differences in the percentage of mutagenic inhibition were obtained at concentrations ≥20 mg/mL; for that reason, the following experiments were done at 20 mg/mL extract concentration. There were no statistically significant differences (p ≤ 0.05) between peel and seed flours, except for byproduct flours, where peel samples had a higher antimutagenic activity than seed samples. Percentages of mutagenic inhibition were between 40 and 60%; peel extracts from byproducts showed an antimutagenic activity greater than those of bola and saladette tomato types and very similar to the activities determined in cherry and grape tomato genotypes. We suggested that the heat process did not affect compounds, which confirmed the antimutagenic activity. Bunkov a et al.44 reported in antimutagenic studies made with green tea (using TA98 S. typhimurium strain) that the percentage of mutagenic inhibition between 40 and 60% represents an antimutagenic activity from moderate to strong.

Correlation analyses between total phenolic content and antimutagenic activity and between groups of phenolic compounds and antioxidant activity and also profile of individual phenolic compounds (by HPLC-DAD) and antimutagenicity were done. We were not able to obtain a strong linear, second- or third-grade correlation between all evaluated parameters and the percentage of antimutagenicity. In relation to this negative correlation, it could be that the antimutagenic activity observed results from the action of several compounds (or their interactions), some of them not identified with the used methodology. More work is needed to determine which compounds impart the antimutagenic activity observed in our extracts and their mechanisms of action.

Finally, we can affirm that methanolic extracts from peel and seed of several tomato types cultivated in northwestern Mexico, including industrial byproducts, can be a potential source of compounds with attractive antioxidant and antimutagenic activities. These extracts could be utilized as nutraceutical ingredients in animal or human food or as nutritional supplements. For this reason, further studies are needed to complete the metabolite characterization of these materials and to realize in vivo assays.

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**Notes**

The authors declare no competing financial interest.

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