Changes in Antioxidant Compounds during the Shelf Life of Commercial Tomato Juices in Different Packaging Materials

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Tomatoes provide an optimal mix of dietary antioxidants that may be responsible for the reported health benefits of tomato consumption. However, technological processing, packaging materials, and storage conditions have an impact on the nutritional quality of tomato products by affecting the stability of antioxidant nutrients to different extents. In this study, we evaluated the stability of the antioxidant compounds (lycopene, ascorbic acid, total phenols, and total flavonoids) present in commercially available tomato juices during storage extended for 12 months at three different temperatures (8, 22, and 37 °C). To further characterize the impact of storage conditions, two commonly used packaging materials (Tetra pack and glass bottles) were used to determine whether packaging materials affect antioxidant stability. Overall, the total lycopene, total phenolic compounds, and total flavonoids remained almost stable during storage for 12 months, regardless of the packaging material used, indicating that tomato juices maintain their nutritional value in terms of antioxidant composition during their shelf life. However, ascorbic acid was the most labile antioxidant and was markedly affected by storage conditions. The hydrophilic total antioxidant activity (TAA) paralleled the losses in ascorbic acid content, whereas the lipophilic TAA remained substantially stable throughout the storage trial.

KEYWORDS: Tomato juice; lycopene; antioxidant activity; ascorbic acid; total phenolic compounds; total flavonoids; packaging; shelf life

INTRODUCTION

It has been postulated that many chronic diseases such as cardiovascular diseases, cancer, inflammatory diseases, diabetes, eye diseases, and aging are a result of long-term oxidative stress (*1, 2*). Due to tomatoes' antioxidant content, the consumption of tomatoes and tomato products has been suggested to reduce the risk of such chronic diseases (*3−7*).

Tomatoes provide an optimal mix of dietary antioxidants such as phenolic compounds, ascorbic acid, vitamin E, and carotenoids, mainly lycopene, whose individual, additive, or synergistic actions may be responsible for the reported health benefits of consumption (*6−8*). Therefore, the assessment of the total antioxidant activity (TAA) provides useful information about the joint action of dietary antioxidants and may help to elucidate the potential protective effects of antioxidant-rich foods (*9*). The total hydrophilic antioxidant activity of the tomato mainly relies on the antioxidant capacity of ascorbic acid and hydrophilic phenolic compounds (e.g., chlorogenic acid), while the lipophilic activity can be explained by the combined action of tocopherols, carotenoids, and lipophilic phenolics (e.g., quercetin) (*9−12*).

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As reported in different studies, home cooking and industrial tomato processing have an impact on antioxidant stability. For instance, industrial and pilot-plant scale processing of tomatoes into different products has been shown to cause lycopene, ascorbic acid, and antioxidant activity losses, while phenolic compounds were shown to be more stable (*13−15*). In addition, commonly used cooking methods such as baking, frying, and microwaving have been reported to have a negative impact on lycopene stability, leading to 15−75% losses of lycopene, primarily due to oxidation reactions (*16*).

The nutritional quality of tomato products is affected by storage conditions in addition to thermal treatment during processing. Studies investigating the impact of storage conditions on the stability of tomato antioxidants have shown marked lycopene and ascorbic acid losses during the first months of storage (*17−20*). However, storage trials lasting longer than 6 months are scarce. As tomato juice typically has a commercial shelf life of 12 months, further studies are necessary to gather pertinent scientific information about the nutritional quality of tomato juice during storage.

It is also known that the packaging material influences the quality of liquid foods during storage. Traditional methods for juice packaging aim to reduce the exposure of the juice to oxygen through the use of high-barrier materials such as glass or foil laminates in brick packs (*21*). For instance, Tetra pack material has specially designed multilayered oxygen and light barriers to

protect ascorbic acid and flavor losses and to enhance shelf life. Packaging material has also been reported to have an impact on lycopene stability in tomato products (*22*).

The aim of this study was to evaluate the stability of antioxidant compounds present in commercially available tomato juices during extended storage for 12 months at three different temperatures (8, 22, and 37 °C). To further characterize the impact of storage conditions, two commonly used packaging materials (Tetra pack and glass bottles) were used to determine whether the packaging material affects antioxidant stability.

MATERIALS AND METHODS

Reagents and Chemicals. Gallic acid, (+)-catechin, butylated hydroxytoluene, Folin-Ciocalteu's phenol reagent, 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate), and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma (St. Louis, MO). Methanol, tetrahydrofuran, methyl *tert*-butyl ether, and L-(+)-ascorbic acid were purchased from Merck (Darmstadt, Germany). *trans*-β-Apo-8'-carotenal was purchased from Fluka (Buchs, Switzerland), and all-*E*-lycopene was purchased from CaroteNature (Lupsingen, Switzerland).

Study Design and Samples. A 1 year storage trial was designed involving the use of three different temperatures (8, 22, and 37 °C) to investigate the stability of the main bioactive antioxidant compounds of two commercially available tomato juices. The tomato juices were manufactured by Juver Alimentación SLU (Cabezo de Torres, Murcia, Spain). The tomato juice was prepared from hot-break tomato puree, which was subjected to a thermal treatment of 96 °C for 30 s and packaged in coated paperboard cartons (Tetra pack containers) or in glass bottles. It should be noted that the juices packaged in Tetra pack containers are commercialized in the format of vitamin C-enriched juices, a fact that is indicated on the label. In contrast, no vitamin C is added to the juices commercialized in glass bottles. Samples were taken immediately after manufacturing and every 2 months to analyze the physicochemical parameters, bioactive antioxidant compounds, and hydrophilic and lipophilic antioxidant activity.

Physicochemical Determinations. The pH was measured using a pH-meter, a Crison Micro pH 2000 (Crison, Barcelona, Spain), and total titratable acidity (expressed as a percentage of citric acid) was analyzed by titrating the product with 0.1 N NaOH to pH 8.2. A Leica Abbe Mark II refractometer (Leica, Buffalo, NY) was used to quantify soluble solids.

Analysis of Lycopene and Its Isomers. Lycopene was analyzed using high-performance liquid chromatography (HPLC) with diode array detection according to Böhm (*23*) after three extractions with a methanol/tetrahydrofuran mixture (1:1, v/v) containing 0.1% butylated hydroxytoluene. Briefly, 400 mg of MgO, 200 μL of *trans*-β-apo-8'-carotenal (internal standard solution), and 35 mL of a methanol/tetrahydrofuran mixture were added to 0.6 g of the sample and homogenized for 5 min using a blender. The resulting solution was vacuum filtered through Whatman grade No. 5 filter paper. The extraction was repeated twice (until the residue was colorless), and the combined extracts were dried under vacuum at 30 °C in a rotary evaporator. The residue was redissolved in a methanol/methyl *tert*-butyl ether mixture (1:1, v/v) until the solution reached the defined volume of 10 mL. The solution was centrifuged at 11000 rpm for 10 min and then analyzed. The HPLC analysis was performed with methanol (solvent A) and methyl *tert*-butyl ether (solvent B) by using a gradient procedure on a C_{30} column (250 mm × 4.6 mm, 5 μm, Trentec, Gerlingen, Germany) at 17 °C and a rate of 1.3 mL/min. The injection volume was 90 μL, and the gradient elution started with 90% A and 10% B, reached 55% A at 35 min and 40% A at 40 min, was then isocratic for 10 min, and, finally, reached 90% A at 60 min. Lycopene and its *Z* isomers were quantified at 472 nm, and all-*E*-lycopene was identified by chromatographic comparison with the pure all-*E*-lycopene standard. However, since standards for lycopene *Z* isomers were not commercially available, these *Z* isomers were tentatively identified on the basis of the retention times and absorption spectrum characteristics described in the literature (*24*). Results are expressed as milligrams per kilogram of tomato juice, and lycopene *Z* isomers were quantified on the basis of the peak area of the all-*E*-lycopene standard. The percentages of *Z* isomerization were

calculated as follows:

$$Z \text{ isomerization } (\%) = [\text{total } Z \text{ isomers}/\text{total } (Z+E) \text{ lycopene}] \times 100$$

All extractions were conducted under subdued light and were performed in triplicate for each sample. The analytical method was validated for total lycopene using certified reference material BCR-485 (mixed vegetables). The indicative value for all-*E*-lycopene in the reference material is 14.2 mg/kg, and this study obtained a mean value of 15.0 ± 0.6 mg/kg after analysis of five samples.

Total Phenolic Content. Total phenolics in the juice were analyzed by a colorimetric assay using Folin-Ciocalteu's phenol reagent, as described by Singleton and Rossi (*25*). Before the colorimetric assay, the samples were subjected to a procedure of extraction and hydrolysis, as described by Gahler et al. (*14*). To hydrolyze the conjugated forms of polyphenols, 1 mL of 1 M HCl was added to 2 g of sample and the mixture was vortexed for 1 min and incubated at 37 °C for 30 min while being shaken constantly. Later, 1 mL of 2 M NaOH in 75% methanol was added, and the resulting mixture was vortexed for 1 min and incubated at 37 °C for 30 min while being shaken constantly. Then, 1 mL of 0.75 M metaphosphoric acid was added, and the resulting mixture was vortexed for 1 min and later centrifuged at 5000 rpm for 10 min. The supernatant was transferred into a 10 mL volumetric flask, and 1 mL of an acetone/water mixture (1:1, v/v) was added to the pellet; the mixture was vortexed for 1 min and centrifuged at 5000 rpm for 5 min. The two supernatants were combined, and the flask was filled to 10 mL with the acetone/water mixture. Finally, a 1 mL aliquot was taken, centrifuged at 11000 rpm for 10 min, and assayed for total phenolics. For the colorimetric assay, 500 μL of 0.2 N Folin-Ciocalteu's phenol reagent and 400 μL of a 2 M Na_2CO_3 solution were added to 100 μL of hydrolysate. After incubation 2 h in darkness at room temperature, absorbance at 750 nm was measured. Gallic acid was used as the standard, and the total phenolic content in the samples was expressed as milligrams of gallic acid equivalents (GAE) per kilogram of tomato juice.

Total Flavonoid Content. For total flavonoids, 200 μL of tomato extract was mixed with 1.25 mL of distilled water and then 75 μL of 5% $NaNO_2$ was added. After 6 min, 150 μL of a 10% $AlCl_3 \cdot 6H_2O$ solution was added and allowed to stand for an additional 5 min before 500 μL of 1 M NaOH was added. The mixture was brought to 2.5 mL with distilled water and mixed well. The absorbance was measured immediately against the blank at 510 nm using a Hitachi U-2000 spectrophotometer. (+)-Catechin was used as the standard using the following calibration solutions: 4, 8, 12, 16, and 20 μg/mL. The total flavonoid content was expressed as milligrams of catechin equivalents (CE) per kilogram of tomato juice (*26*).

Analysis of Ascorbic Acid. The ascorbic acid content was measured by reversed phase HPLC, as described by Esteve et al. (*27*). Ten grams of the sample was diluted to 100 mL with a 1% (w/v) meta-phosphoric acid solution and shaken for 10 min. The extracts were filtered first through Whatman No. 1 paper and then through a 0.45 μm Millipore filter before being analyzed via HPLC. The system conditions were an injection volume of 20 μL, a diode array detector wavelength set at 245 nm, isocratic elution at a flow rate of 1 mL/min, a Lichospher 100 RP-18 column (12.5 cm × 0.4 cm), and a 5 μm particle size (Merck, Darmstadt, Germany). The mobile phase was 0.1 M phosphate buffer (pH 3.5), and L-(+)-ascorbic acid was used as the standard. Results were expressed as milligrams of ascorbic acid per kilogram of tomato juice.

Total Antioxidant Activity (TAA). The TAA of the hydrophilic and lipophilic extracts was measured by using the Trolox equivalent antioxidant capacity (TEAC) test described by Miller et al. (*28*). The assay is based on the reduction of the radical cation of 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate) (ABTS), which is generated by filtering an ABTS solution through manganese dioxide powder. The antioxidant activity of the samples is calculated by determining the decrease in absorbance at 734 nm. Trolox was used as the standard, and results are expressed as millimoles of Trolox equivalents per kilogram of tomato juice. To obtain the hydrophilic extracts, 2 g of the sample was diluted in 10 mL of water, vortexed for 1 min, and centrifuged at 11000 rpm for 10 min. The supernatant was assayed for TAA. The lipophilic extracts were obtained by homogenizing 1 g of the sample in 20 mL of a hexane/acetone/methanol mixture (2:1:1, v/v/v) for 30 min protected from light. Then, 15 mL of

water was added, and the mixture was centrifuged at 5000 rpm for 5 min (*11*). The upper layer containing the lipophilic fraction was transferred into a 10 mL volumetric flask and filled to 10 mL with hexane. Finally, a 1 mL aliquot of the lipophilic fraction was dried under a nitrogen stream and then reconstituted into 0.5 mL of 2-propanol before being analyzed for TAA.

Statistical Analysis. Data were analyzed with Statistical Package SPSS version 15.0 for Windows. To study the variation in the analyzed compounds as a function of the time and temperature of storage, a two-factor analysis of variance (ANOVA) was performed using the general linear model (GLM). Tukey's test for pairwise comparison was used to determine significant differences in the different compounds analyzed for each juice. Relationships between variables were examined using Pearson correlation coefficients. *P* values of < 0.05 were considered statistically significant.

RESULTS

Table 1 shows the changes in pH, titratable acidity, and total soluble solids during the storage of tomato juices packaged in Tetra pack and glass containers. For both the Tetra pack- and glass-packaged samples, the evaluated parameters showed stability throughout the storage trial. For this reason, only data for the initial, 6 month, and 12 month samples are given.

Tables 2 and **3** show the evolution of the total lycopene content and the levels of *Z* isomerization in tomato juices packaged in Tetra pack and glass bottles at the three temperatures tested. As shown in **Table 2**, the total lycopene content remained substantially stable throughout the storage trial and varied from 99 to 120 mg/kg in tomato juice packaged in Tetra pack and from 96 to 115 mg/kg in samples stored in glass bottles. For both types of samples, no clear temperature dependency was revealed in the statistical analysis regarding the rate of total lycopene loss [$p > 0.05$ (**Table 4**)], which was affected by only storage time [$p < 0.05$ (**Table 4**)]. At the end of the storage trial, the final losses in the total lycopene content varied from 11 to 17% and from 7 to 15% in the samples stored in Tetra pack and glass containers, respectively.

Figure 1 shows the variations in the content of all-*E*-lycopene and total *Z*-lycopene isomers in the samples stored at 8, 22, and 37 °C. As can be seen, the all-*E*-lycopene content slightly decreased toward the end of the storage trial as a function of storage time [$p < 0.05$ (**Table 4**)]. The all-*E*-lycopene content varied from 88 to 115 mg/kg in the samples stored in Tetra pack and from 90 to 110 mg/kg in the samples stored in glass bottles, throughout the storage trial. The total *Z*-lycopene isomer content slightly increased in a time-dependent manner [$p < 0.05$ (**Table 4**)] in both the Tetra pack-packaged (5−11 mg/kg) and glass-packaged samples (4−7 mg/kg). As shown in **Table 3**, the percentage of *Z* isomerization slightly increased with storage time [$p < 0.05$ (**Table 4**)] in both types of samples, but the values always remained below 12%.

Figure 2 shows the changes in the total phenolic and total flavonoid contents at different temperatures in the tomato juices tested. For both juices, the total phenolic and total flavonoid content remained virtually constant, although the statistical analysis indicates that the total phenolics were affected by both time and temperature [$p < 0.05$ (**Table 4**)], while the total flavonoids were affected by only storage time [$p < 0.05$ (**Table 4**)]. With regard to the juice packaged in Tetra pack, the total phenolic content varied from 260 to 300 mg of GAE/kg, whereas the total flavonoid content varied from 98 to 108 mg of CE/kg. Similar levels were observed in the tomato juices packaged in glass bottles, with the total phenolic content ranging from 240 to 285 mg of GAE/kg and the total flavonoid content ranging from 100 to 107 mg of CE/kg.

Table 1. Changes in the pH, Titratable Acidity (% of citric acid), and Soluble Solids (°Brix) of Juices during Storage at Three Different Temperatures

time	Tetra pack			glass bottle		
	8 °C	22 °C	37 °C	8 °C	22 °C	37 °C
pH						
0 months		3.93			3.93	
6 months	4.01	4.01	3.99	4.01	4.01	3.97
12 months	4.00	4.00	3.97	3.99	3.99	3.94
Titratable Acidity						
0 months		0.406			0.452	
6 months	0.407	0.417	0.421	0.443	0.442	0.439
12 months	0.427	0.423	0.418	0.418	0.454	0.425
Soluble Solids						
0 months		5.37			5.76	
6 months	5.40	5.39	5.36	5.4	5.69	5.86
12 months	5.27	5.28	5.26	5.27	5.80	5.73

Figure 3 shows the changes in the ascorbic acid content in tomato juice packaged in Tetra pack during storage at the different temperatures used in this study. The initial ascorbic acid concentration in the juice packaged in Tetra pack was 680 mg/kg, but no ascorbic acid was detected in the juices packaged in glass bottles (data not shown). It should be noted that in the case of the juices packaged in Tetra pack containers, ascorbic acid was added as an ingredient, a fact that is printed on the product label. As can be seen from the graph, the ascorbic acid content of the juice decreased in a time- and temperature-dependent manner [$p < 0.05$ (**Table 4**)], so the greatest losses of ascorbic acid were observed in the samples stored at 37 °C; their ascorbic acid content decreased by approximately 50% after storage for 2 months. However, at 22 and 8 °C, losses of approximately 50% of the initial content were observed after only 8 and 12 months, respectively.

Figure 4 illustrates the pattern of changes in the hydrophilic and lipophilic antioxidant activity of the juices tested. With regard tog the samples packaged in Tetra pack, the hydrophilic TAA dropped in a clear time- and temperature-dependent fashion [$p < 0.05$ (**Table 4**)], and approximately 30, 50, and 70% final losses occurred in the samples stored at 8, 22, and 37 °C, respectively. The lipophilic TAA, however, slightly decreased in the first 2 months from 0.4 to 0.2 mmol of Trolox/kg and then remained essentially unchanged for the duration of the study, regardless of the storage time or temperature [$p > 0.05$ (**Table 4**)]. In the samples stored in glass bottles, the hydrophilic TAA was affected by both time and temperature [$p < 0.05$ (**Table 4**)]. As can be seen, the hydrophilic TAA slightly decreased from 0.77 to 0.5 mmol of Trolox/kg in the first 2 months but then remained essentially constant until the end of the storage trial. In terms of the lipophilic TAA of the juices stored in glass bottles, the statistical analysis revealed that the lipophilic TAA was slightly affected by storage time [$p < 0.05$ (**Table 4**)], although the lipophilic TAA also remained virtually stable throughout the storage trial.

DISCUSSION

The lycopene content observed in the samples was similar to the values reported for tomato and tomato juices by other researchers (*19*, *20*, *22*, *29*, *30*). It should be noted that the slight differences observed in the lycopene content between juices at the beginning of the study are due to the variability of the hot-break tomato puree used as raw material for the elaboration of each type of juice. In the samples, all-*E*-lycopene was the major isomer

Table 2. Changes in the Total Lycopene Content (milligrams per kilogram)[a] of Juices Packaged in Tetra Pack Containers or Glass Bottles during Storage at Three Different Temperatures over 12 Months[b]

	Tetra pack			glass bottle		
time	8 °C	22 °C	37 °C	8 °C	22 °C	37 °C
0 months		118.97 ± 1.68 a			113.71 ± 2.53 a	
2 months	120.79 ± 2.20 a	116.22 ± 1.17 a	111.65 ± 8.31 a	113.37 ± 9.58 a	107.16 ± 3.82 a	113.98 ± 0.75 a
4 months	114.48 ± 3.36 a	112.7 ± 6.07 a	112.00 ± 4.61 a	114.76 ± 6.59 a	111.81 ± 5.81 a	114.54 ± 3.80 a
6 months	114.55 ± 6.38 a	114.02 ± 6.28 a	112.36 ± 10.27 a	115.32 ± 5.13 a	114.57 ± 0.88 a	110.20 ± 4.64 a
8 months	115.45 ± 6.20 a	111.56 ± 4.38 a	111.90 ± 5.37 a	104.70 ± 4.96 a	108.85 ± 4.09 a	111.54 ± 1.67 a
10 months	117.91 ± 2.09 a	111.36 ± 3.52 a	105.83 ± 7.32 a	101.00 ± 4.27 a	109.14 ± 3.16 a	112.85 ± 1.07 a
12 months	100.82 ± 0.55 a	105.68 ± 5.01 a	99.10 ± 4.53 b	103.82 ± 3.34 a	105.49 ± 1.79 a	96.04 ± 7.12 b

[a] Mean ± standard deviation. [b] Different letters within the same column mean statistical significance ($p < 0.05$).

Table 3. Changes in the Percentages[a] of *Z* Isomerization in Tomato Juice Packaged in Tetra Pack Containers or in Glass Bottles during Storage over 12 Months[b]

	Tetra pack			glass bottle		
time	8 °C	22 °C	37 °C	8 °C	22 °C	37 °C
0 months		4.69 ± 0.07 b			3.80 ± 0.25 b	
2 months	5.03 ± 0.92 b	4.22 ± 0.37 b	5.57 ± 0.13 b	5.10 ± 0.66 b	4.09 ± 0.12 b	4.09 ± 0.12 b
4 months	6.30 ± 0.16 b	6.64 ± 0.94 b	6.36 ± 1.55 b	5.09 ± 0.88 ab	4.73 ± 1.21 b	4.52 ± 0.66 ab
6 months	6.25 ± 0.47 b	6.49 ± 0.24 b	6.35 ± 0.03 b	4.84 ± 0.34 ab	4.83 ± 0.57 b	4.03 ± 0.57 b
8 months	6.31 ± 0.42 b	6.10 ± 0.70 b	6.31 ± 0.32 b	5.17 ± 1.25 ab	4.49 ± 0.70 b	4.46 ± 0.94 b
10 months	5.76 ± 0.73 b	6.27 ± 0.65 b	6.83 ± 0.49 b	7.58 ± 0.72 ab	4.17 ± 0.36 b	5.39 ± 0.32 b
12 months	10.06 ± 1.28 a	11.98 ± 2.34 a	11.93 ± 0.92 a	6.23 ± 0.64 a	6.04 ± 1.36 b	6.70 ± 2.16 b

[a] Mean ± standard deviation. [b] Different letters within the same column mean statistical significance ($p < 0.05$).
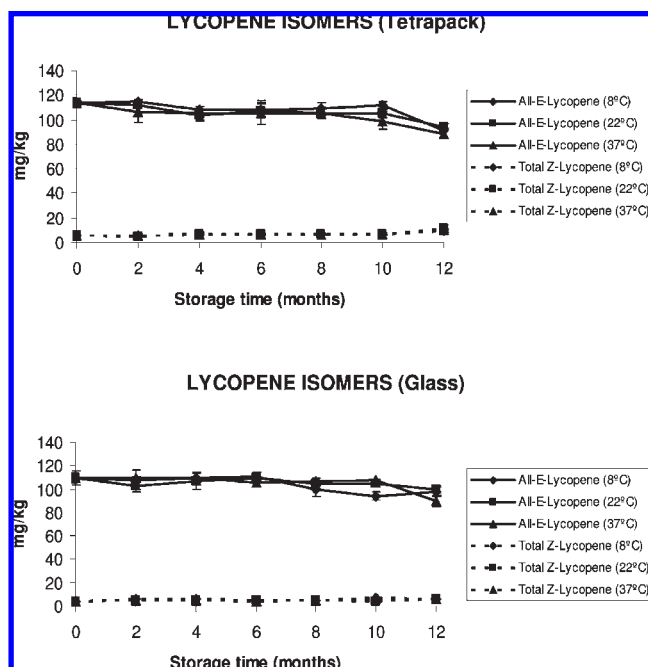
Table 4. Significance Values for Each Factor Obtained in the Two-Factor ANOVA by Using the General Linear Model

	time		temperature		time × temperature	
variable	Tetra pack	glass	Tetra pack	glass	Tetra pack	glass
total lycopene	0.000	0.009	0.094	0.912	0.782	0.400
E-lycopene	0.000	0.001	0.094	0.723	0.782	0.252
Z-lycopene	0.000	0.022	0.814	0.135	0.730	0.642
percent of *Z* isomerization	0.000	0.001	0.235	0.078	0.630	0.251
total phenolics	0.000	0.000	0.000	0.000	0.000	0.000
total flavonoids	0.000	0.008	0.329	0.260	0.398	0.131
hydrophilic TAA	0.000	0.000	0.000	0.000	0.000	0.000
lipophilic TAA	0.570	0.024	0.495	0.469	1.000	0.850
ascorbic acid[a]	0.000	—	0.000	—	0.000	—

[a] Ascorbic acid was not detected in glass-packaged samples.

Figure 1. Changes in all-*E*-lycopene and total *Z*-lycopene isomer contents during storage of tomato juice over 12 months in Tetra pack containers or glass bottles.

detected, accounting for approximately 90% of the total lycopene content. With regard to *Z*-lycopene isomers, we detected and tentatively identified three isomers in the samples: 13-*Z*-lycopene, 15-*Z*-lycopene, and 9-*Z*-lycopene. This lycopene isomer profile is consistent with that reported by other authors in different tomato products, in which all-*E*-lycopene generally accounts for more than 80% of the total lycopene content, with 13-, 15-, and 9-*Z*-lycopene being the main *Z* isomers detected (*19*, *24*, *31*).

In this storage trial, the stability of lycopene was higher than that previously reported, as the observed final losses were always < 20% in the total lycopene content. For example, Odriozola-Serrano et al. (*20*) reported final losses of approximately 70% in tomato juice stored at 4 °C for 3 months in polypropylene bottles. Similarly, in tomato juices either thermally stabilized or processed by pulsed electric fields, Min et al. (*18*) reported 60−65% losses in lycopene content after storage for 3.7 months at 4 °C in polypropylene containers. In addition, Lin and Chen (*19*) reported lycopene losses of approximately 65% in canned tomato juice stored at 4, 25, and 37 °C for 3 months, and losses of approximately 75% when the juices were stored in glass containers (regardless of exposure to light). Moreover, similar lycopene

degradation patterns (35−75%) have been reported during the storage of tomato paste (*17*). In contrast, in line with our data, Ordoñez-Santos et al. (*29*) reported lycopene stability in tomato pulp stored at 20 °C, but their storage trial lasted only 3 months so does not allow a proper comparison of results. The highest stability of lycopene observed in our study may have been aided by the presence and stability of other antioxidants such as polyphenolic substances and/or by the thermal inactivation of oxidizing enzymes during tomato processing, as has been suggested by other researchers (*13*, *22*, *29*).

Article

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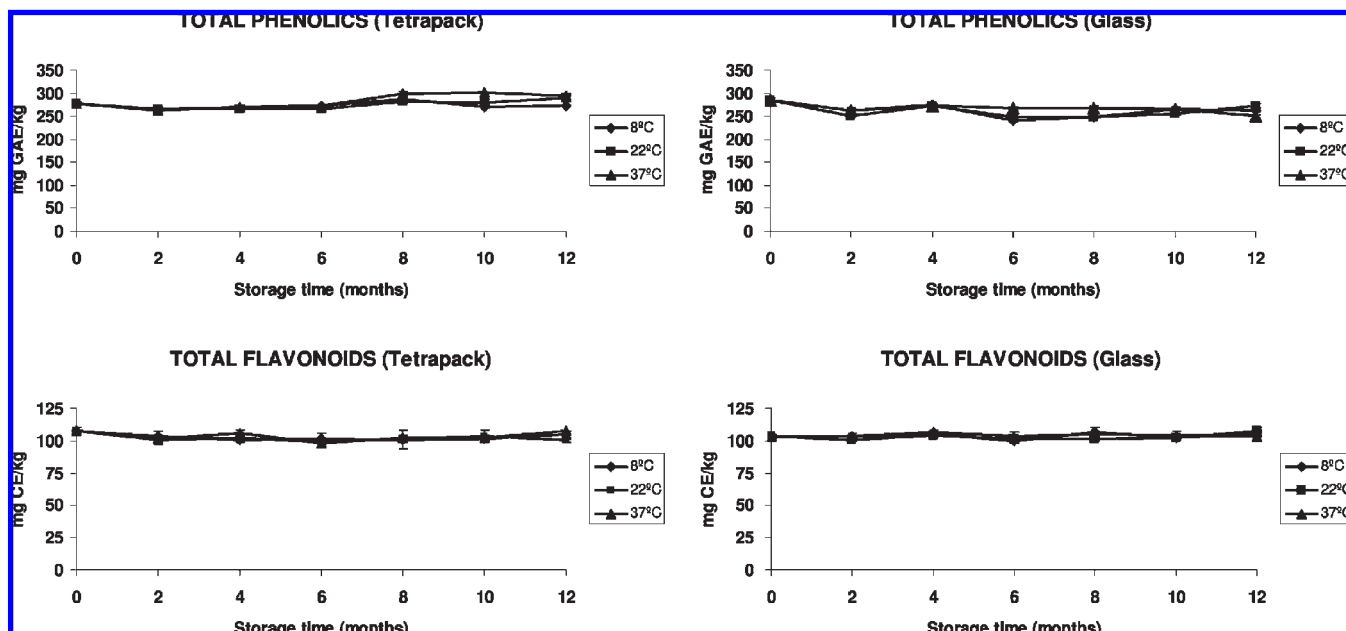

Figure 2. Changes in total phenolics and flavonoids during storage of tomato juice over 12 months in Tetra pack containers or glass bottles.
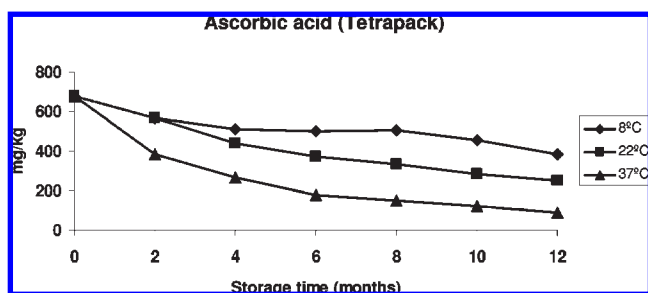

Figure 3. Changes in ascorbic acid content during storage of tomato juice over 12 months packaged in Tetra pack containers.

In the study presented here, we paid special attention to the isomerization of lycopene because human studies point to the putative nutritional benefits of consuming tomato products with an increased percentage of *Z*-lycopene isomers, as they seem to be better absorbed in the intestine (*32–34*). Additionally, these *Z*-isomers have been previously shown to have higher TAA than all-*E*-lycopene (*35*). In our study, a slight increase in *Z*-lycopene isomer content, which was accompanied by a slight decrease in all-*E*-lycopene content, was observed at the end of the storage trial. This agrees with data reported by Lin and Chen (*19*), who observed a favored formation of lycopene *Z* isomers, particularly 9-*Z* and 13-*Z*, during storage of tomato juice for 3 months. Interestingly, the formation of these *Z* isomers was accompanied by a decrease in the all-*E*-lycopene content. The explanation the researchers gave for this phenomenon was that isomerization is an important factor leading to lycopene loss during storage. This is because all-*E*-lycopene can be converted to 13-*Z*-lycopene, which later can undergo further isomerization to other *Z* isomers. This could explain the changes observed in our study regarding lycopene *Z* isomerization and all-*E*-lycopene losses at the end of the storage trial. Nevertheless, *Z* isomerization is an extremely complicated phenomenon and can vary depending on the storage conditions (time and temperature), moisture, and acidity of the product (*19*).

Although in our study storage conditions increased the degree of lycopene isomerization, the percentage of *Z* isomers remained below 12% for the duration of the study. This amount of *Z*-lycopene isomers is within the range reported as being normal for tomato and tomato products, generally 5–20% (*19, 36, 37*). Hence, no additional nutritional advantages would be expected upon consumption of the juices, as improved absorption in human subjects has been reported after the intake of tomato products containing more than 40% *Z* isomers (*33, 38*).

The total phenolic content observed in the samples is in the range of those reported by other researchers in tomato and tomato products (100–500 mg/kg) (*10, 14, 20, 26, 30*). Similarly, the total flavonoid content in the samples is consistent with values previously reported for tomatoes (2–200 mg/kg) (*10, 26*). Overall, the total phenolic and flavonoid content in the samples remained essentially unchanged during storage, although a slight increase in total phenol content was observed in the Tetra pack samples stored at 37 °C after 8 months. Phenolic compounds have been shown to be very stable during storage of tomato juice at 4 °C for 3 months (*20*) and storage of tomato pulp at 4 and 20 °C for 3–5 months (*17*). In addition, Giovanelli and Paradiso (*17*) observed that total phenolic compound levels increased in tomato pulp stored at 37 °C, and the researchers assumed that such an effect was likely due to the formation of Maillard reaction products, which are capable of reacting with Folin-Ciocalteu's phenol reagent. Hypothetical formation of these Maillard products via nonenzymatic browning could have contributed to the slight increase in total phenolic content observed in the samples of this study stored at 37 °C. Nevertheless, the possibility of phenolics being released from the juice matrix as a consequence of time and storage temperature should not be ruled out as a contributor to this slight increase. In addition to Maillard products, ascorbic acid can also react with Folin-Ciocalteu's phenol reagent, giving an overestimation of phenolic compound content. However, previous analysis has demonstrated that natural ascorbic acid is fully degraded during the hydrolysis of the tomato sample before total phenolic determination (data not shown).

Ascorbic acid is a reactive compound, and it is particularly vulnerable to processing and storage conditions. Although ascorbic acid is a natural antioxidant found in tomatoes (*10, 12*), the thermal processing during juice processing leads to total degradation, since no ascorbic acid was detected in the glass-bottled
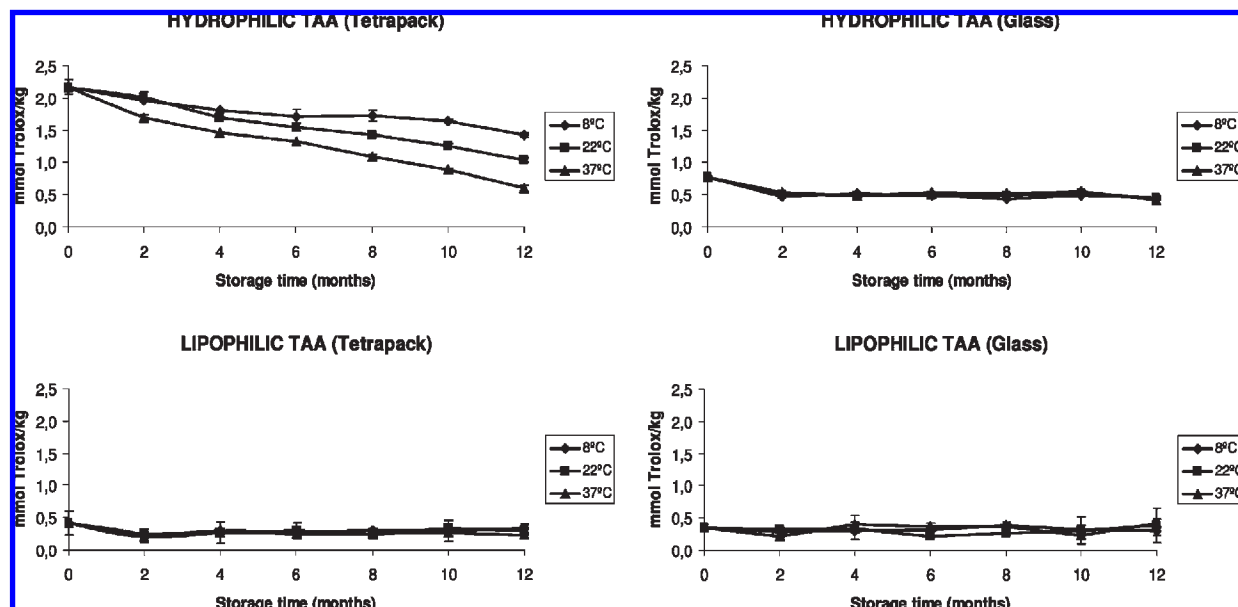
Figure 4. Changes in hydrophilic and lipophilic total antioxidant activity (TAA) during storage of tomato juice over 12 months in Tetra pack containers or glass bottles.

juices. With regard to the samples stored in Tetra pack, which were enriched with ascorbic acid, the data confirm the detrimental effect of storage conditions on such a labile antioxidant. Similar loss rates have been reported during storage of tomato juice at 4 °C for 70 days (*18*) or 3 months (*20*), and in the case of tomato pulp stored at 20 °C for 3 months (*29*). It has been suggested that ascorbic acid and other antioxidants, such as phenolics, might aid in preventing the degradative oxidation of lycopene during storage (*13, 22*). However, on the basis of these results, it seems that ascorbic acid plays a minor role in this preventive effect. This is because lycopene stability was similar in the samples stored in Tetra pack and those stored in glass bottles, irrespective of the addition of ascorbic acid in the formulation of the juices. Therefore, as the polyphenolic compounds (total phenols and flavonoids) remained essentially unchanged during storage, it is reasonable to assume that these compounds play a key role in preventing lycopene losses.

It is generally well accepted that ascorbic acid, together with phenolics, is a major contributor to the hydrophilic TAA (*9, 10, 12, 26*), and a clear time and temperature dependency was observed in the losses of hydrophilic TAA in juices packaged in Tetra pack containers, which closely reflected the changes observed in the ascorbic acid content. This is supported by the significant positive correlation observed between the ascorbic acid content and the hydrophilic TAA of the Tetra pack samples, with correlation coefficients higher than 0.900 ($p < 0.05$). A similar trend was observed by Odriozola-Serrano et al. (*20*) during cold storage of tomato juice extended for 3 months; they reported that the ascorbic acid losses were paralleled by the decrease in hydrophilic TAA.

In the case of the samples stored in glass containers, the initial hydrophilic TAA values were lower than those of the Tetra pack samples, clearly demonstrating the contribution of ascorbic acid to hydrophilic TAA, since the total phenolic content and total flavonoid content in the Tetra pack- and glass-packaged juices were very similar. Thus, it is also clear that, in glass-packaged samples, hydrophilic TAA relied on the total phenolic and flavonoid content, as changes in hydrophilic TAA during the storage trial showed a trend similar to that of total phenolics and flavonoids, with correlation coefficients of > 0.600 ($p < 0.05$).

Unlike for hydrophilic TAA, relationships between the changes in lycopene content and lipophilic TAA were not as clear as expected, and no significant correlation was observed, although these parameters were very stable during the storage trial. Therefore, it is reasonable to assume that other antioxidants, most likely lipophilic phenolic compounds, may have contributed to maintaining the lipophilic TAA of the samples during storage.

Lipophilic compounds such as lycopene are responsible for lipophilic antioxidant activity in tomatoes, but the contribution of such compounds to TAA is considered to be small. In general, hydrophilic antioxidant activity accounts for $> 90\%$ of the TAA of fruits and vegetables (*39*). In tomatoes, the hydrophilic fraction displays greater antioxidant activity than the lipophilic fraction; the difference depends on the analytical method used. For example, hydrophilic activity accounted for ~92% of the TAA determined by the oxygen radical absorbance capacity (ORAC) (*39*) and TEAC assays (*40*), approximately 87% as measured by the xanthine oxidase/xhantine system, and practically 100% when evaluated by the myeloperoxidase/$NaCl/H_2O_2$ system (*41*). Accordingly, we observed that the hydrophilic TAA values were always higher than those of the lipophilic fractions, with values accounting for 70−84% of the TAA in the samples stored in glass and in Tetra pack, respectively. In this regard, Toor and Savage (*40*) have suggested that the low antioxidant activity in the lipophilic tomato extracts, as measured by the TEAC assay, is probably due to the low levels of lipophilic phenolics.

As a final remark regarding the effect of packaging material, we did not observe relevant differences in antioxidant stability when using Tetra pack and glass. As shown for lycopene, total phenols, and total flavonoids, the time trend of changes was similar regardless of the packaging material used, and similar behavior was also observed for lipophilic TAA.

In summary, the bioactive compounds lycopene and phenolic compounds (total phenols and flavonoids) remained essentially stable during storage for 12 months, indicating that tomato juices maintain their nutritional value in terms of antioxidant composition during their shelf life. However, as expected, ascorbic acid was shown to be a more labile antioxidant that is markedly affected by storage conditions. Storage led to a slight increase in the content of *Z*-lycopene isomers, but the levels achieved would

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not confer a nutritional advantage in terms of improving bioavailability. No advantage is achieved by using Tetra pack or glass in terms of improving antioxidant stability. Storage at room temperature (22 °C) is appropriate for the maintenance of the antioxidant compounds during shelf life, except for the ascorbic acid content. This information is of interest for consumers and nutritionists; they can expect a beneficial effect from the consumption of tomato juice during the entire shelf life. It is also useful for the industry, since room-temperature storage reduces product distribution and storage costs.

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