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Alterations of Vitamin C, Total Phenolics, and Antioxidant Capacity as Affected by Processing Tomatoes to Different Products

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This study was conducted to investigate the antioxidant vitamin C, the polyphenol content, and the hydrophilic antioxidant capacity of tomato juice, baked tomatoes, tomato sauce, and tomato soup. During the production of tomato juice and during the preparation of the other tomato products, samples were taken after different times, respectively, after each particular production step. High-performance liquid chromatography was used to determine the content of vitamin C. The total phenolics content was analyzed spectrophotometrically by using the Folin−Ciocalteu method. The hydrophilic antioxidant capacity was measured by using three different methods: the Trolox equivalent antioxidant capacity assay, the ferric reducing antioxidant power test, and the photochemiluminescence assay. The vitamin C contents of the tomato products decreased during the thermal processing of tomatoes. In contrast, the total phenolics concentration and the water soluble antioxidant capacity increased.

KEYWORDS: Vitamin C; total phenolics; antioxidant capacity; tomato products

INTRODUCTION

The cultivated tomato (*Lycopersicon esculentum*) originated in the New World from wild species that are native to the Andean region of South America. In the 16th century, the tomato was taken to Europe. Because of its relationship with poisonous members of the night-shade family, the fruit of the tomato plant was developed and was accepted as a food remarkably slowly at first but then became very popular (*1*). Processed tomatoes rank second to potatoes in dollar value among all vegetables produced (*2*). From 1996 to 2001, the quantity of processed tomatoes significantly increased from 7.88 million tons to 8.45 million tons in the European Union (*3*).

Tomatoes contain modest to high amounts of several nutrients. Regarding the vitamins, tomatoes have remarkable concentrations of folate, vitamin C, and vitamin E. In addition, they are the most important source of another constituent, the carotene lycopene, not having any provitamin A activity. They are also known for their content of the provitamin A active β-carotene as well as that of flavonoids and potassium (*1*). Beneficial effects of the Mediterranean diet have been stated manyfold (*4*, *5*). As compared to Northern Europe, in Italy, cancer rates have been lower. There, tomatoes are regarded as the second most important source of vitamin C after oranges. In a study of colorectal cancers, based on 1953 cases and 4154 controls, tomato intake was significantly protective on colorectal cancer risk (*6*). Among 72 epidemiological studies reviewed by Giovannucci (*7*), 57 of them reported inverse associations between tomato intake or blood lycopene level and the risk of cancer at a defined anatomic site. The evidence for a benefit was strongest for cancers of the prostate, lung, and stomach (*7*).

Investigations on the effects of food processing on the activity of naturally occurring antioxidants are scarce (*8*−*11*). Often, only the fate of lycopene and other carotenoids during processing of tomatoes was evaluated (*12*, *13*). Thermally processed tomato products showed an increased absorption of lycopene as compared to raw tomatoes (*14*).

The aim of the present study was to investigate how heat treatments affect the contents of vitamin C and polyphenols as well as the hydrophilic antioxidant capacity. For this, tomato juice was produced under industrial-like conditions, and baked tomatoes as well as tomato sauce and tomato soup were prepared under household conditions. With these results, it might be possible to optimize thermal processes or to get new insights in the quality of processed tomato products.

MATERIALS AND METHODS

Chemicals. All chemicals used were of analytical grade. Special reagents were ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) (Sigma no. A 1888, Sigma-Aldrich, Taufkirchen, Germany), myoglobin (Sigma no. M 1882), ACW-kit (ACW = integral antioxidant capacity of water soluble substances) (Analytik Jena AG no. 400.801, Analytik Jena AG, Jena, Germany), Trolox ((*S*)-(−)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) (Aldrich no. 39,192-1, Sigma-Aldrich), Folin−Ciocalteu's phenol reagent (Fluka no. 47641, Sigma-Aldrich), and TPTZ (2,4,6-tripyridyl-s-triazine) (Sigma no. T 1253).

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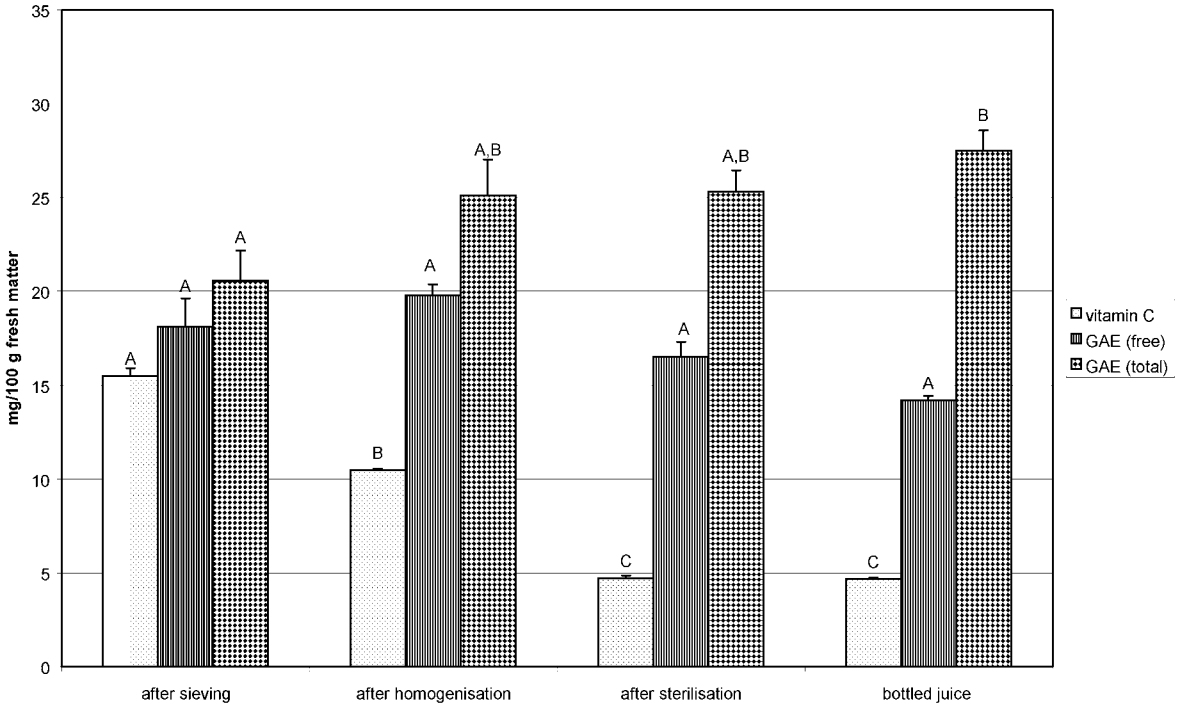

Figure 1. Alterations of vitamin C and free phenolics as well as sum of free and bound phenolics during the processing of tomato juice. Bars showing the same index are not significantly different ($p > 0.05$).

Sample Preparation. The tomato juice was produced in a pilot plant at the University of Applied Sciences Lippe and Höxter from canned tomatoes. Samples were taken after each processing step: (i) passing the tomatoes through a sieve, (ii) homogenization (70 bar, 62.5 kg/h), (iii) sterilization (121 °C, 2 min), and (iv) filling in bottles and pasteurization (80 °C, 20 min). The other tomato products were prepared from fresh tomatoes, which were purchased from a local supermarket. The tomatoes were cleaned and cut into equal pieces (cubes for sauce and soup and slices for baked tomatoes). The baked tomatoes were heated for up to 45 min in a preheated oven. Three different temperatures were used as follows: 180, 200, and 220 °C. Samples were taken after 15, 30, and 45 min. The sauces were cooked for 50 min. Samples were taken after 5, 25, and 50 min. For the soup, the peeled tomatoes were cooked for 50 min under stirring. After 25 min (after taking the first sample), cream was added to the soup as is mentioned in some recipes. Samples were taken after 25, 35, and 50 min. The distribution of vitamin C and total phenolic compounds within the tomato fruit was also determined. Three different fractions of the fruit were used for the determination: the outside layers (pericarp, peel), the inside layers (flesh, central axis), and the seeds. All samples were homogenized prior to analysis and stored at −18 °C until analysis.

High-Performance Liquid Chromatography (HPLC) Analysis of Vitamin C. Vitamin C was analyzed by using liquid chromatography on a RP-Phase with UV detection according to ref *15*. Briefly, 2 g of sample was mixed with 5 mL of *meta*-phosphoric acid (4.5 g/100 mL) and vigorously shaken for 1 min. After the mixture was centrifuged (5000 rpm), the liquid layer was transferred into a flask. This extraction was repeated twice. The combined extracts were membrane filtered and analyzed by using a Eurospher 100 colum (Knauer, Berlin, Germany) (250 mm × 4.0 mm, 5 μm) and UV detection at 245 nm with 1.0 mL/min water (pH 2.2) as the mobile phase.

Extraction of Soluble Free Phenolic Compounds. Two grams of the tomato sample was weighed into a tube and centrifuged (5000 rpm) for 5 min. The upper liquid layer was transferred into a flask. After 0.5 mL of water was added, the extraction was repeated twice. The water extracts were combined and frozen at −18 °C until analysis.

Extraction of Bound and Free Phenolic Compounds. Two grams of the tomato sample were weighed into centrifuge tubes. At first, the samples were mixed with 1 mL of hydrochloric acid (1.0 mol/L) and incubated at 37 °C for 30 min. Then, 1 mL of sodium hydroxide solution (2.0 mol/L in 75% methanol) was used for the alkaline hydrolysis. A second incubation at 37 °C for 30 min followed. After that, the samples were mixed with 1 mL of *meta*-phosphoric acid (0.75 mol/L) and centrifuged (5000 rpm). One milliliter of acetone/water mixture (1 + 1) was added at the end of the extraction (*16*).

Determination of Total Phenolic Compounds. The content of total polyphenolics was analyzed photometrically using the Folin−Ciocalteu reagent. All tomato extracts were diluted with distilled water to obtain readings within the standard curve range (2−10.5 mg/100 mL gallic acid monohydrate). Afterward, 200 μL of sample solution was mixed with 800 μL of Na_2CO_3 solution and 1.0 mL of Folin reagent. The samples were allowed to stand for 120 min at room temperature before the absorbance at 750 nm was measured. Gallic acid monohydrate was used as the standard, and the total phenolic content is expressed as gallic acid equivalents (GAE) (*17*).

Trolox Equivalent Antioxidant Capacity (TEAC) Assay. Hydrophilic antioxidant activity was determined by using the TEAC assay. The principle of this assay is based on the oxidation of ABTS in the presence of H_2O_2 and metmyoglobin to the long-lived radical cation $ABTS^{\bullet+}$, which is measured photometrically at 734 nm (*18*).

Ferric Reducing Antioxidant Power (FRAP) Assay. The FRAP was analyzed by using a microtiter plate. Distilled water and sample solution were pipetted into this plate. Eight minutes after starting the reaction by adding the FRAP solution, the absorbance was measured at 595 nm (*18*).

Photochemiluminescence (PCL) Assay. In the PCL assay, the photochemical generation of free radicals is combined with the sensitive detection by using chemiluminescence. This reaction takes place in the Photochem. The antioxidant potential is analyzed by means of the lag phase (*18*).

Statistical Analysis. All results are presented as means ± standard deviation. Differences between variables were tested for significance by using a one way analysis of variance procedure, Tukey, using a level of siginificance of $p < 0.05$ (SPSS for Windows 10.0).

RESULTS

Vitamin C. Analyses of the different tomato fractions resulted in 230 ± 6 μg/100 g fresh matter inside of the tomato fruit, which is higher than the content in the outer layer (127 ± 10 μg/100 fresh matter). **Figure 1** and **Table 1** show the loss of

Table 1. Alterations of Vitamin C and Phenolic Contents (Free as Well as Total Contents) of Baked Tomatoes Heated at Different Temperatures[a]

sample	content of vitamin C (mg/100 FM)	content of free phenolics (mg GAE/100 g FM)	sum of free and bound phenolics (mg GAE/100 FM)
	baked tomatoes (180 °C)		
0 min	12.78 ± 0.08^{A}	9.86 ± 1.81^{A}	22.32 ± 2.06^{A}
15 min	13.42 ± 0.17^{A}	$11.55 \pm 0.89^{A,B}$	$23.49 \pm 1.63^{A,B}$
30 min	7.59 ± 0.17^{B}	$12.55 \pm 1.32^{A,B}$	$26.72 \pm 1.71^{A,B}$
45 min	7.11 ± 0.89^{B}	16.98 ± 1.20^{B}	32.23 ± 2.21^{B}
	baked tomatoes (200 °C)		
0 min	12.78 ± 0.08^{A}	9.86 ± 1.81^{A}	22.32 ± 2.06^{A}
15 min	12.99 ± 1.04^{A}	$13.21 \pm 0.25^{A,B}$	23.53 ± 2.17^{A}
30 min	11.81 ± 0.51^{A}	14.92 ± 0.20^{B}	$25.47 \pm 1.03^{A,B}$
45 min	7.35 ± 0.81^{B}	19.59 ± 0.51^{C}	31.33 ± 1.01^{B}
	baked tomatoes (220 °C)		
0 min	12.78 ± 0.08^{A}	9.86 ± 1.81^{A}	22.32 ± 2.06^{A}
15 min	12.53 ± 2.54^{A}	9.42 ± 0.94^{A}	26.66 ± 2.72^{A}
30 min	10.87 ± 0.02^{B}	$12.98 \pm 0.99^{A,B}$	$27.36 \pm 2.31^{A,B}$
45 min	4.88 ± 0.07^{C}	16.27 ± 1.69^{B}	30.68 ± 2.24^{B}

[a] FM, fresh matter; A–C, values within columns with different letters are significantly different ($p < 0.05$).

the water soluble vitamin C during the production of tomato juice and preparation of baked tomatoes. With increasing time or processing steps, the vitamin C contents decreased. Vitamin C is a heat instable vitamin; thus, high temperatures led to a loss of vitamin C. Differences in temperature had a minor influence on the losses of vitamin C. Only baking tomatoes for 45 min at 220 °C led to a higher decrease of vitamin C as compared to the lower temperatures investigated. In contrast, the preparation of tomato sauce and tomato soup did not affect the contents of vitamin C (**Figure 2** and **Table 2**) when using the results based on fresh matter. Calculations using dry matter as a basis (data not shown) resulted in a decrease in vitamin C content as shown for the other products.

Table 2. Alterations of Vitamin C and Phenolic Contents (Free as Well as Total Contents) of Tomato Soup[a]

sample	content of vitamin C (mg/100 FM)	content of free phenolics (mg GAE/100 g FM)	sum of free and bound phenolics (mg GAE/100 FM)
0 min	12.40 ± 1.14^{A}	10.30 ± 0.32^{A}	18.39 ± 0.97^{A}
25 min	11.56 ± 0.20^{A}	11.70 ± 1.06^{A}	23.98 ± 2.06^{A}
35 min	10.39 ± 0.65^{A}	12.36 ± 0.20^{A}	37.14 ± 2.26^{B}
50 min	11.54 ± 0.07^{A}	18.46 ± 1.27^{B}	44.40 ± 3.34^{B}

[a] FM, fresh matter; A–B, values within columns with different letters are significantly different ($p < 0.05$).

Phenolic Compounds. The distribution of the phenolic compounds within the tomatoes was examined as well and led to surprising results. Inside the tomato fruit, 27.0 ± 0.6 mg/100 fresh matter was determined. The content in the outer layer was lower (18.9 ± 0.3 mg/100 g fresh matter). A 31.0 ± 0.6 mg/100 g fresh matter amount was comprised in the seeds. Fruits and vegetables normally have higher contents of phenolic compounds in the outer parts. Regarding the mass partition within the tomato, the pericarp comprised 68.5% of the tomato, the inner part (without seeds) comprised 28.5%, and the seeds comprised 3.0%. Thus, the outer part of the tomato comprised the highest absolute amount of polyphenolics as compared to the inside of the fruit. Hydrolysis led to higher contents of total phenolics in all fractions of the tomato. The highest increase was observed for the seeds leading to 91.9 ± 1.8 mg/100 g fresh matter.

No significant changes of the free phenolics were determined during the processing of tomato juice, whereas the sum of free and bound phenolics significantly increased from 20.56 ± 1.62 to 27.49 ± 1.09 mg GAE/100 g fresh matter after four production steps (**Figure 1**).

The contents of the free phenolic compounds and the total phenolic compounds of baked tomatoes are listed in **Table 1**. After 15, 30, and 45 min of heating at 180, 200, or 220 °C,

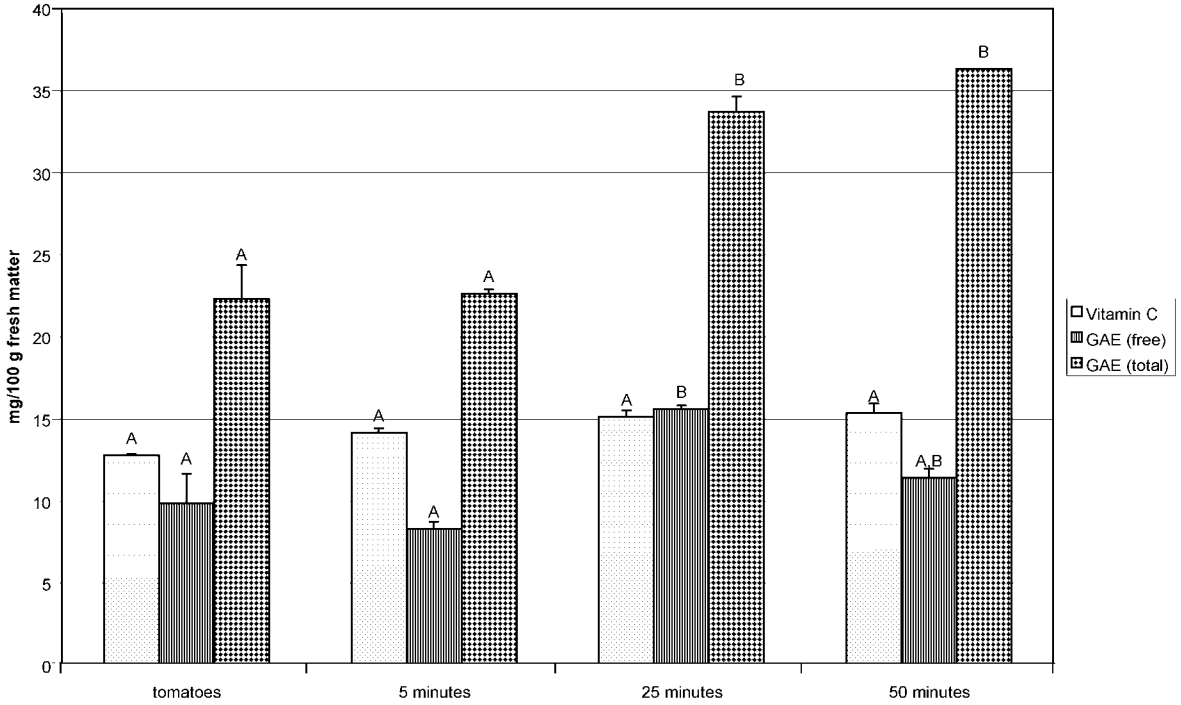

Figure 2. Alterations of vitamin C and free phenolics as well as sum of free and bound phenolics during the processing of tomato sauce. Bars showing the same index are not significantly different ($p > 0.05$).

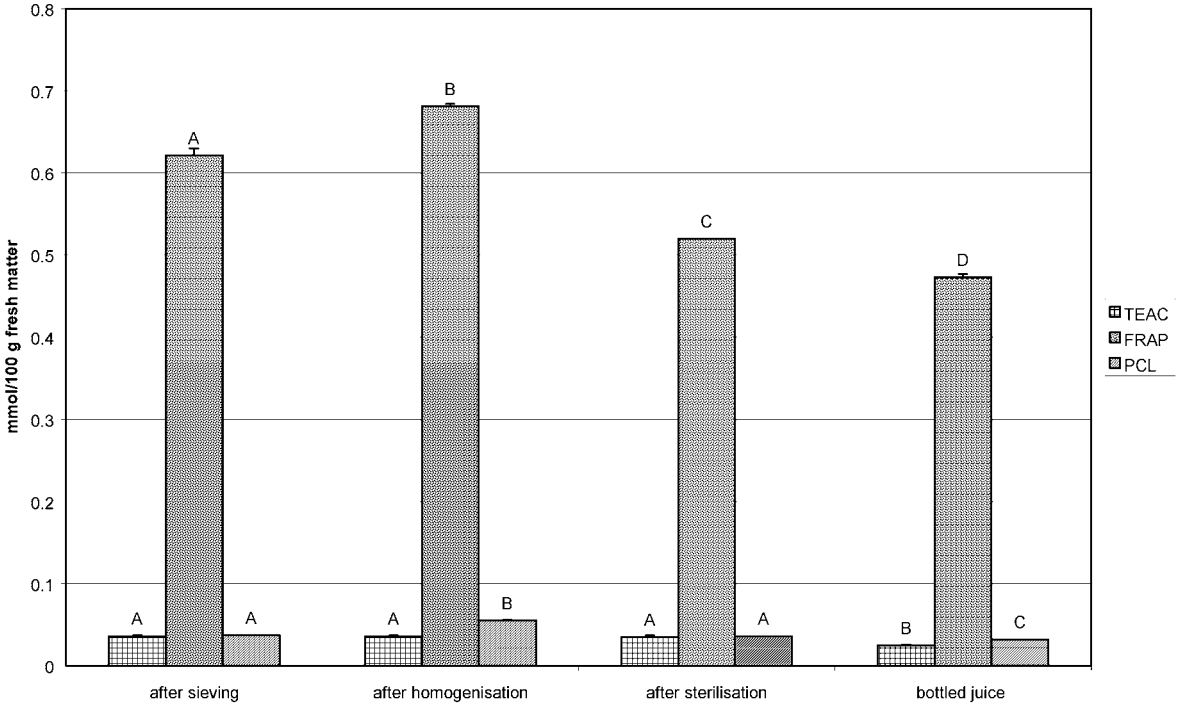

Figure 3. Hydrophilic antioxidant capacity, determined with the TEAC assay, the FRAP assay, and the PCL assay, during the processing of tomato juice. Bars showing the same index are not significantly different ($p > 0.05$).

both free and total phenolic contents of baked tomatoes increased. Significant differences between the respective temperatures for the free phenolic contents existed between the baked tomatoes at 180 and 200 °C and between 200 and 220 °C. Like the free phenolics, the contents of the total phenolics of baked tomatoes at 200 and 220 °C were significantly different (these differences are not marked in **Table 1**). The other tomato products, which were analyzed, followed a similar course (**Figure 2** and **Table 2**).

Antioxidant Capacity. Homogenization and thermal treatment increased the hydrophilic antioxidant capacity of different tomato products. **Figure 3** shows the alterations of the hydrophilic antioxidant capacity (TEAC, FRAP, and PCL) while producing the tomato juice. A significant increase (FRAP, PCL) resulted only after the homogenization step. Sterilization and bottling led to decreases of the antioxidant capacity. During the course of thermal processing at 180, 200, and 220 °C, the hydrophilic antioxidant capacity of baked tomatoes increased (TEAC, FRAP, and PCL). The height of temperature did not influence significantly the sequence of the FRAP and PCL values (**Table 3**) while the TEAC values significantly increased only at 200 and 220 °C. For the tomato sauce only, the TEAC assay resulted in significantly increased values after 50 min of heating (**Figure 4**). Producing the tomato soup showed significantly enhanced activities within the results of the TEAC assay and the PCL test (**Table 4**).

Table 3. Hydrophilic Antioxidant Capacity, Determined with the TEAC Assay, the FRAP Assay, and the PCL Assay, of Baked Tomatoes Heated at Different Temperatures[a]

sample	TEAC value (mmol TE/ 100 g FM)	FRAP value (mmol Fe^{2+}/ 100 g FM)	PCL value (mmol TE/ 100 g FM)
	baked tomatoes (180 °C)		
0 min	0.045 ± 0.002^{A}	0.425 ± 0.000^{A}	0.067 ± 0.000^{A}
15 min	0.052 ± 0.003^{A}	$0.521 \pm 0.034^{A,B}$	0.065 ± 0.001^{A}
30 min	0.052 ± 0.001^{A}	$0.554 \pm 0.027^{A,B}$	0.054 ± 0.000^{B}
45 min	0.053 ± 0.004^{A}	0.649 ± 0.002^{B}	0.065 ± 0.000^{A}
	baked tomatoes (200 °C)		
0 min	0.045 ± 0.002^{A}	0.425 ± 0.000^{A}	0.067 ± 0.000^{A}
15 min	0.063 ± 0.001^{B}	$0.600 \pm 0.017^{A,B}$	0.062 ± 0.000^{B}
30 min	$0.069 \pm 0.001^{B,C}$	0.656 ± 0.011^{B}	0.054 ± 0.000^{C}
45 min	0.083 ± 0.000^{C}	0.640 ± 0.010^{B}	0.061 ± 0.001^{B}
	baked tomatoes (220 °C)		
0 min	0.045 ± 0.002^{A}	0.425 ± 0.000^{A}	0.067 ± 0.000^{A}
15 min	0.038 ± 0.002^{A}	0.474 ± 0.036^{A}	0.044 ± 0.000^{B}
30 min	0.051 ± 0.000^{A}	0.554 ± 0.027^{A}	0.061 ± 0.000^{A}
45 min	0.078 ± 0.000^{B}	0.624 ± 0.050^{A}	0.041 ± 0.001^{B}

[a] TE, Trolox equivalents; FM, fresh matter; A–C, values within columns with different letters are significantly different ($p < 0.05$).

DISCUSSION

Results on the behavior of vitamin C were comparable to existing data. The vitamin C content of tomatoes depended on the species and the cultivation conditions (*19*). For our investigations, Dutch tomatoes were used with a vitamin C content of 12.78 ± 0.08 mg/100 g fresh matter. The vitamin C content of greenhouse tomatoes has been analyzed as 12−16 mg/100 g fresh matter. In contrast, sun-ripened tomatoes contained much more vitamin C: 14.7−44.6 mg/100 g fresh matter (*20*). Many studies showed the decline in vitamin C during the production and cooking (*10*, *21*). With increasing heating time and processing steps of different tomato products (tomato juice, baked tomatoes, tomato sauce, and tomato soup), a continuous loss of the water soluble vitamin C was observed in our investigations. For the sauce and the soup, the loss was masked by an increase in dry matter. Thus, the loss was only visible when dry matter was used as the calculation basis (data not shown).

Phenolic compounds belong to the secondary plant substances. Together with other antioxidants such as vitamin C, vitamin E, and carotenoids, they protect the human body tissue against oxidative attacks. The daily intake of phenolic com-

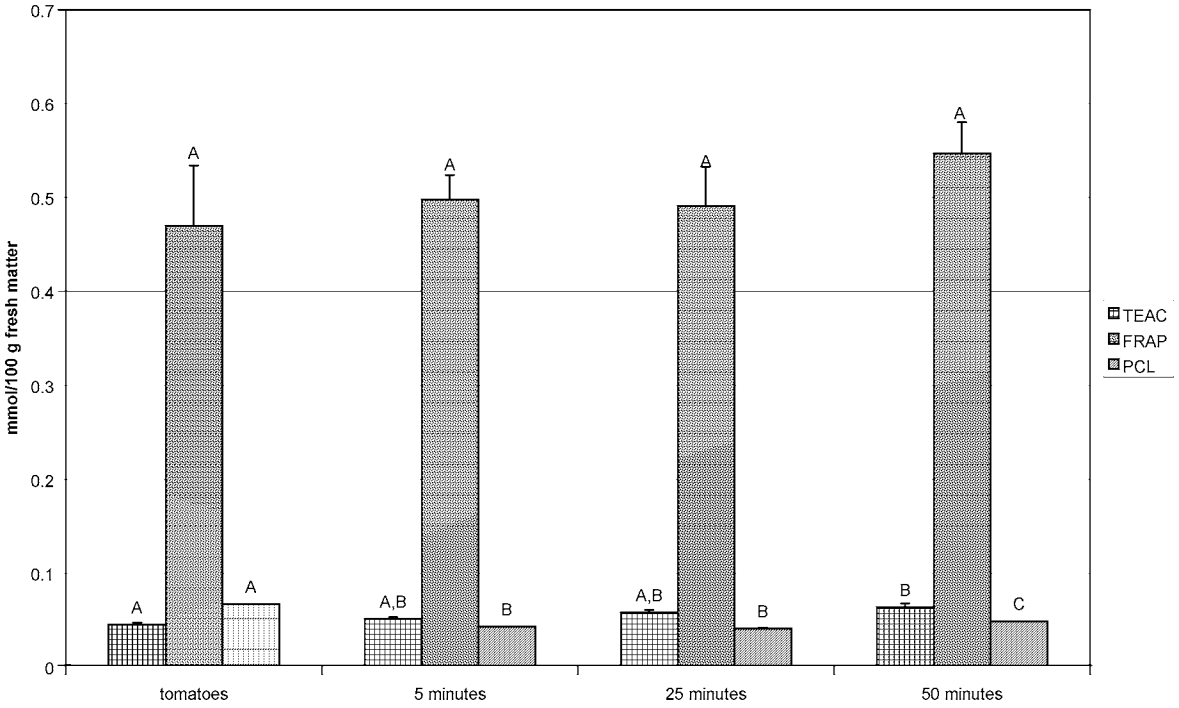

Figure 4. Hydrophilic antioxidant capacity, determined with the TEAC assay, the FRAP assay, and the PCL assay, during the processing of tomato sauce. Bars showing the same index are not significantly different ($p > 0.05$).

Table 4. Hydrophilic Antioxidant Capacity, Determined with the TEAC Assay, the FRAP Assay, and the PCL Assay, of Tomato Soup[a]

sample	TEAC value (mmol TE/ 100 g FM)	FRAP value (mmol Fe^{2+}/ 100 g FM)	PCL value (mmol TE/ 100 g FM)
0 min	0.017 ± 0.001^{A}	0.451 ± 0.008^{A}	0.042 ± 0.001^{A}
25 min	0.056 ± 0.003^{B}	0.561 ± 0.031^{A}	0.035 ± 0.001^{B}
35 min	0.049 ± 0.001^{B}	0.575 ± 0.011^{A}	0.043 ± 0.002^{A}
50 min	$0.041 \pm 0.012^{A,B}$	0.582 ± 0.000^{A}	0.056 ± 0.006^{C}

[a] TE, Trolox equivalents; FM, fresh matter; A–C, values within columns with different letters are significantly different ($p < 0.05$).

pounds is about 1 g (*22*). Results in the literature are contrary to our own investigations. Total phenolics did not change significantly during processing of tomatoes (*10*, *23*). In contrast, our own results showed an increase in total phenolics within thermal processing of tomatoes, possibly due to liberation of phenolics from the matrix. The addition of cream to the tomato soup is a common practice in some recipes. It could be assumed that the proteins from the cream bind phenolic compounds and affect the antioxidant capacity. Our results showed only a slight, nonsignificant increase of the free phenolics after the addition of cream. In parallel, the sum of free and bound phenolics significantly increased. The TEAC and FRAP results did not show any alteration after the addition of cream. Only the PCL results significantly increased. Different actions and reactions with the Folin reagent have been examined. The interaction of carbohydrates (glucose, fructose) with the Folin reagent was excluded (*24*, *25*). Tertiary aliphatic amines, tryptophan, hydroxylamine, and other reducing agents reacted with the Folin reagent (*26*).

The hydrophilic antioxidant capacity was analyzed by using three test systems. Recent investigations (*18*) showed differences between the test systems for the determination of antioxidant capacity. Thus, it was recommended to use at least two methods. We decided to use three assays showing different sensitivity and using different reactions. The hydrophilic antioxidant capacity of tomatoes was already the object of different investigations. One study described an increase in total antioxidant activity of tomatoes after thermal processing at 88 °C (*10*). Our own results confirmed these analyses. The hydrophilic antioxidant activity increased during the preparation of baked tomatoes, tomato sauce, and tomato soup, although the vitamin C content of the tomato products decreased. The increase of the hydrophilic antioxidant capacity is primarily based on the increase of the phenolics (*27*, *28*). Our own investigations resulted in correlations between contents of free phenolics and FRAP ($R^2 = 0.64-0.93$) as well as TEAC ($R^2 = 0.52-0.97$) results for the baked tomatoes while the PCL results of the these tomato products were not correlated to the free phenolics concentrations. Contents of free phenolics and antioxidant capacity of the tomato juice processing step samples were correlated for all test systems used ($R^2 = 0.73-0.96$). Contents of free phenolics and the TEAC as well as the PCL results of the tomato soup samples were correlated ($R^2 = 0.73-0.92$). All other results showed correlations below $R^2 = 0.5$. Another reason for the increase of the hydrophilic antioxidant capacity is the formation of Maillard products. Maillard products are antioxidant active substances that are formed at high temperatures. They can balance the loss of vitamin C (*11*) or even lead to an increase in the hydrophilic antioxidant capacity (*29*). The tomato juice production has to be discussed separately. After an increase in antioxidant activity by homogenization, sterilization and filling resulted in reduced activity. Further model experiments (heating of juice without or with oxygen saturation under pressure) were done to get an idea why the antioxidant potential decreased in contrast to the observations with the other tomato products. The results (data not shown) did not show any influence during sterilization (heating under pressure) and heating alone (temperature). In another study, high-pressure treatment (500/800 MPa/5 min) of tomato homogenate did not affect the hydrophilic antioxidant capacity when using the TEAC

assay (*8*). The decrease after the third as well as the fourth processing step in our investigation might be based on the interaction of the juice with oxygen during the processing.

Comparing the results of the three test systems, differences can be seen. The increase of the antioxidant capacity after homogenization within the juice production was determined only with the FRAP and the PCL assay. The same two test systems showed the decrease after sterilization and bottling. The TEAC values were only significantly decreased after bottling the tomato juice. All three test systems showed significantly increased values with increasing heating time of the baked tomatoes (exceptions: TEAC, 180 °C; FRAP, 220 °C). The tomato sauce preparation resulted in significantly increased TEAC values after 50 min and significantly decreased values for the PCL test after 5 min. During the preparation of the tomato soup, significantly increased antioxidant capacity was observed with increasing heating time for the TEAC and the PCL assay. For the tomato soup samples, these two test systems showed a correlation to the contents of free phenolics. Recently, five tomato juices, four canned tomatoes, and four tomato pastes were analyzed on their antioxidant capacity by using the TEAC assay and a linoleic acid emulsion (formation of thiobarbituric acid reactive substances). In the TEAC assay, the tomato pastes had the highest antioxidant capacities, followed by the juices and the canned tomatoes. Using the linoleic acid emulsion resulted in comparable antioxidant capacities for all groups of tomato products (*30*). Nine commercial varieties of tomatoes produced in Spain were investigated on their antioxidant capacity by using the TEAC and the DPPH assay. Total antioxidant status (TEAC) and antiradical efficiency (DPPH) showed different rankings for the tomatoes investigated (*31*). Thus, as shown for reference materials and beverages (*18*), antioxidant capacities are not comparable between different test systems.

Concluding the results, the experiments led to new results in the field of the hydrophilic antioxidant capacity and the total phenolic contents of tomatoes. For the judgment of the alterations in total phenolics, it might be very important to determine the phenolic compounds (free and bound phenolics). For a complete statement on the antioxidant activity of tomatoes, the lipophilic part of this parameter has to be determined as well. These experiments are under investigation.

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