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Folate Content in Tomato (*Lycopersicon esculentum*). Influence of Cultivar, Ripeness, Year of Harvest, and Pasteurization and Storage Temperatures

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The effects of cultivar, on-vine ripening, and year of harvest on the folate content of raw tomatoes were studied. Folate content in hot-break tomato puree (HTP) subjected to pasteurization at different temperatures and its evolution during the shelf life of tomato juice were also investigated. 5-Methyltetrahydrofolate (5-CH_3-H_4-folate) was the only folate compound identified in raw tomatoes and HTP, but tetrahydrofolate (H_4-folate) was 10% of the folate detected in tomato juice. The content of folates in raw tomatoes ranged from 4.1 to 35.3 μg/100 g of fresh weight and was highly influenced by all of the factors studied. No clear trend of folate content with ripening stage was observed. The extractability of 5-CH_3-H_4-folate from HTP increased significantly after pasteurization at 98 °C for 40 s, but higher temperatures decreased its content. Tomato juice showed folate losses during storage independent of the storage temperature. Folate losses were higher when tomato juice was packed in glass bottles than in Tetra Pak.

KEYWORDS: 5-Methyltetrahydrofolate; tetrahydrofolate; tomato; cultivar; ripeness; homogenization; pasteurization; storage

INTRODUCTION

Folates are essential vitamins that must be provided in the diet; humans cannot synthesize folates de novo. Plant foods, including vegetables, fruits, cereals, and potatoes, are the predominant contributors to folate intake in Europe (*1*). A deficiency of folates in the diet increases the risk of various pathologies such as cardiovascular diseases (CVD) (*2*), neural tube defects (*3*), colorectal cancer (*4*), and rheumatoid arthritis (*5*). Therefore, knowing the folate contents of food plants and selecting plant foods with high folate concentrations are important. However, the content of the different folate forms and their distribution and stability in vegetables, fruits, and cereals depend on a number of factors such as genetic background, growing conditions, harvest and postharvest handling, industrial processing, and storage during shelf life (*6*).

Folates are composed of a pterin, a *p*-aminobenzoic acid (*p*ABA), and a glutamate chain with a variable number of glutamate moieties. The cellular folate pool is a complex mixture of related molecules that differ in the oxidation state of the pterin ring, the one-carbon substitutions (formimino, formyl, methyl, methylene, and methenyl), and the number of glutamate moieties (*7*). Tetrahydrofolate (H_4-folate) and 5-methyltetrahydrofolate (5-CH_3-H_4-folate) (**Figure 1**) are the main forms present in plant foods, whereas 5-CH_3-H_4-folate is the major circulating form in humans.

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Tomato (*Lycopersicon esculentum*) is the plant food with the second-highest consumption in the world and has been considered an important functional food, due to its content of bioactive compounds (*8*). Folates are among the bioactive compounds in tomatoes that are considered to be beneficial for human health and, especially, for the prevention of cardiovascular diseases (*9*). Although tomatoes are not generally considered to be a rich source of folates in the human diet, the fact that they are widely consumed in practically all types of diets and cultures may contribute to their overall beneficial effects (*9*).

The content of bioactive antioxidant compounds, such as lycopene, β-carotene, phenolic compounds, flavonoids, and ascorbic acid, in tomatoes and the effects of agronomic and industrial factors on them have been widely studied in the past decade (*10*−*12*). Some data about the folate content of tomatoes have been reported (*13*). However, we could not find any investigations on the influence of on-vine ripening, environmental conditions, or industrial processing and storage on the content of folates in tomatoes.

This study was designed to assess the content of folates in both raw tomatoes and processed tomato products consumed in Spain. The main objectives of the present investigation were (1) to determine the content of folates in raw tomatoes harvested in Spain, considering the effect of the cultivar, on-vine ripeness stages, and year of harvest; (2) to study the content of folates in tomato puree, a raw material used in the tomato industry to obtain different processed products, subjected to different pasteurization temperatures; and (3) to assess the influence of

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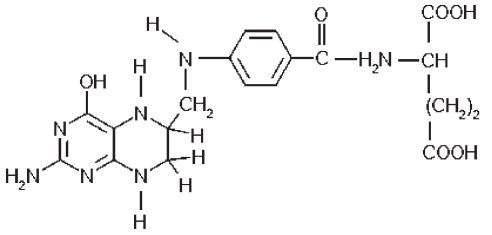
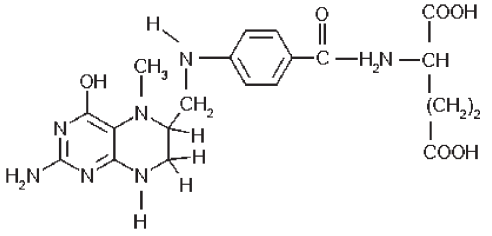


5,6,7,8-tetrahydrofolic acid



5-methyl-5,6,7,8-tetrahydrofolic acid

Figure 1. Chemical structures of folic acid's reduced forms tetrahydrofolate and 5-methyltetrahydrofolate.

temperature and packaging on the folate content of tomato juice during its shelf life of 12 months.

MATERIALS AND METHODS

Materials and Study Design. Three batches of tomato samples were used in this study. First, commercial raw tomato samples belonging to the cultivars Ronaldo, Pera, Cherry Pera, Zoco, Tina, and Romario were harvested in July 2007 in Murcia (Spain) and provided to us by Tropicana-Alvalle S.L. (Puente Tocinos, Murcia). These samples were collected at four ripening stages, according to the color of the fruit, by the same person, following the criteria of the Californian Tomato Commission (2002) [unripe/green (stage 1), breaker/orange (stage 2), light red (stage 5), and fully ripe (stage 6)] to study the effect of tomato ripening on the folate content. In addition, raw tomatoes of Malva and Heinz (H-9776, H-9665, H-9661, and H-9997) cultivars subjected to similar agricultural conditions were collected at the fully ripe stage from the same area in Badajoz (Spain) during 2006, 2007, and 2008 and were provided to us by Conservas Vegetales de Extremadura S.A. (Villafranca del Guadiana, Badajoz) to evaluate the effect of the harvest year on the content of folates. Samples of tomatoes at the light red stage of maturity were also collected from Malva and Heinz cultivars harvested in 2006. After harvest, approximately 4000 g of each tomato sample was immediately brought to the laboratory, cleaned, and homogenized in an Omni-mixer (Omni, Waterbury, CT). Subsamples of 20 g were stored in screw-cap plastic containers at −80 °C until analysis.

Second, hot-break tomato puree (HTP) (scalded at 90 °C for 2 min) provided by Juver Alimentación, SLU (Cabezo de Torres, Murcia) and normally used in the industry to obtain tomato products, was subjected to different pasteurization temperatures (98, 108, or 128 °C for 40 s in a tubular pasteurizer) to assess the effect of thermal processing on folate content.

The third batch of samples consisted of industrial tomato juice produced from HTP and provided by Juver Alimentación, SLU. However, this tomato puree came from a batch different from the one mentioned above. To determine the variations in folate content during the shelf life of the tomato juice, different storage temperatures, times, and packagings were tested. The tomato juice was packed in Tetra Pak containers (1000 mL) or in glass bottles (200 mL) and stored at 8, 22, or 37 °C, in dark rooms, during 1, 3, 6, 9, or 12 months. Samples were exposed to light only when the door to the room was opened to pick the samples for analysis. Samples of 20 g were taken at the beginning of the shelf life study and every 3 months over a period of 12 months. Samples were placed in screw-cap plastic containers and stored at −80 °C until analysis.

The mean moisture contents of the samples were 94.1, 94.2, and 94.5% in raw tomatoes, HTP, and tomato juices, respectively.

Folate Analysis. Folates from tomato samples (10 g) were extracted following the procedure described by Pfeiffer et al. (*14*) and Konings (*15*). Samples were mixed with 25 mL of extraction buffer (50 mM CHES, 50 mM HEPES, containing 2% sodium ascorbate and 10 mM 2-mercaptoethanol, pH 7.85) under a nitrogen atmosphere. The extraction mixtures in screw-capped tubes were placed in a boiling water bath for 10 min, cooled on ice, and homogenized using an Omnimixer model 17106 (OMNI, Inc., Waterbury, CT). Then the pH was adjusted to 4.9 with 6 M HCl, and the samples were made up to a final volume of 50 mL with extraction buffer. Enzymatic deconjugation and purification of samples was carried out following the methodology described by Vahteristo et al. (*16*). Thus, an aliquot of 5 mL was incubated for 3 h at 37 °C under a nitrogen atmosphere with 1 mL of hog kidney conjugase prepared from fresh pig kidneys as described by Gregory et al. (*17*). To inactivate the enzyme, the samples were boiled for 5 min and then cooled on ice. The samples were then filtered through 0.45 μm pore size, 25 mm Ø nylon disposable filters (Whatman, Florham Park, NJ) and purified in strong anion-exchange (SAX) cartridges (3 mL/500 mg of quaternary amine N^+, counterion Cl^-, no. 52664-U, Bellefonte, PA) connected to a Supelco 12-port vacuum manifold (Supelco, Bellefonte, PA). First, the cartridges were conditioned with 3 mL of *n*-hexane (twice), methanol, and Milli-Q water and then equilibrated with 3 mL of purification buffer (0.01 M dipotassium hydrogen phosphate, 0.1% 2-mercaptoethanol, pH 7.0). Second, the sample was slowly loaded onto the cartridge at a rate of < 1 mL/min. Folate was eluted with 2 mL of elution buffer (10% sodium chloride, 0.1 M sodium acetate, 1% ascorbic acid) with a flow rate of < 0.5 mL/min. The eluted sample was weighed, and the purified extracts were kept under refrigeration for no longer than 2 h before they were placed in the autosampler and injected. The extraction, deconjugation, and purification procedures were carried out under subdued light to prevent photodegradation of folates. Folates were determined using a Merck-Hitachi 7000 (Merck, Darmstadt, Germany) HPLC equipped with a fluorescence detector (LaChrom, Merck-Hitachi, model 7485). A LiChrosphere 100 RP-18 (5 μm) column (Merck), protected with a guard column (LiChroCART 4-4, Merck), was used to separate the folate compounds. The column was eluted with a gradient of acetonitrile and 30 mM phosphate buffer (potassium phosphate and orthophosphoric acid 85%, pH 2.2) at a flow rate of 0.9 mL/min. The gradient started at 6% acetonitrile, which was maintained isocratically for the first 6 min, and then the acetonitrile concentration was increased to 25% over 24 min and decreased to 6% after 5 min. The injection volume was 40 μL. The running time was 30 min, and the time between injections was 40 min. Fluorescence absorbance, at excitation and emission wavelengths of 280 and 350 nm, respectively, was used to detect and quantify the naturally occurring folate forms present in the tomato samples, namely, H_4-folate, 5-CH_3-H_4-folate, and 5-formyltetrahydrofolate (5-HCO-H_4-folate). Peak identification was based on the retention time compared with standards, and spiking (addition of standard compounds into the purified sample extract) to confirm peaks for any sample in which identification using the retention time was inaccurate. (6*R*,*S*)-5,6,7,8-Tetrahydrofolic acid calcium salt (H_4-folate), (6*R*,*S*)-5-methyl-5,6,7,8-tetrahydrofolic acid sodium salt (5-CH_3-H_4-folate), and (6*R*,*S*)-5-formyl-5,6,7,8-tetrahydrofolic acid sodium salt (5-HCO-H_4-folate) were obtained from Dr. Schirck's Laboratories (Jona, Switzerland). The folate content was expressed as micrograms per 100 g of fresh weight in all samples.

The certified reference material (BCR 485 freeze-dried mixed vegetables) used for the quality control of analytical measurement of total folate content was obtained from the European Commission, Institute for Reference Materials and Measurement (Brussels, Belgium). The indicative 5-CH_3-H_4-folate content in BCR 485 was 2.14 mg/kg (*18*). The certified material was analyzed in each set of analyses, and an average value of 2.10 ± 0.09 mg of 5-CH_3-H_4-folate/kg of reference material was obtained. The precision of the HPLC analysis including sample extraction, deconjugation, and purification showed recoveries of spiked folates on the three types of samples studied (n = 3) at the level of 50 ng/mL that ranged from 75 to 100% for H_4-folate, from 70 to 99% for 5-CH_3-H_4-folate, and from 80 to 100% for 5-HCO-H_4-folate. The coefficient of inter- and intra-assay variation for folate analysis was below 10%. The limits of quantification were 2.34 ng/mL for H_4-folate, 2.67 for 5-CH_3-H_4-folate, and 34.20 ng/mL for 5-HCO-H_4-folate.

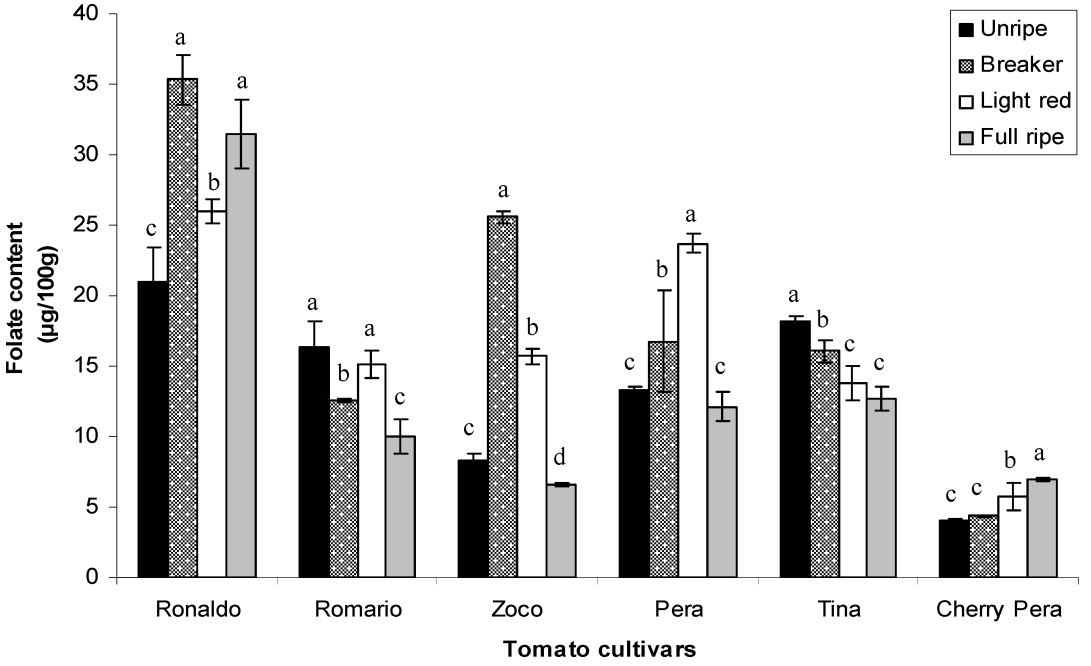


Figure 2. Folate content (5-CH_3-H_4-folate in μg/100 g of fresh weight) in tomato cultivars depending on the ripeness stage. All results are means of quadruplicates, and the error bars show standard deviations. Different letters within each tomato cultivar show significant differences ($p < 0.05$) in the folate contents among the four ripeness stages evaluated.

Table 1. Content of 5-CH_3-H_4-folate (Micrograms per 100 g of Fresh Weight) in the Maturity Stages Mostly Consumed (Light Red and Full Ripe) from Tomato Cultivars Harvested in 2006 and Their Contribution to the RDA

	ripening stage			
tomato cultivar	light red	full ripe	av[a]	contribution to the RDA (%)[b]
Ronaldo	25.7 ± 0.8	31.5 ± 2.4	28.6 ± 1.6a	10.7
Romario	15.1 ± 0.8	9.9 ± 1.2	12.5 ± 0.7c	4.7
Zoco	15.8 ± 0.5	6.6 ± 0.1	11.2 ± 0.3cde	4.2
Pera	23.9 ± 0.8	12.1 ± 1.0	18.0 ± 0.2b	6.7
Tina	13.4 ± 1.2	12.6 ± 0.9	13.0 ± 1.0c	4.9
Cherry Pera	6.0 ± 1.0	7.0 ± 0.1	6.5 ± 0.5g	2.4
Malva	14.9 ± 0.8	9.8 ± 0.2	12.4 ± 0.3cd	4.6
H-9776	6.5 ± 0.2	5.5 ± 0.1	6.0 ± 0.1g	2.3
H-9661	8.7 ± 0.7	9.3 ± 0.5	8.9 ± 0.6f	3.3
H-9997	13.9 ± 1.5	6.3 ± 0.6	10.2 ± 1.0ef	3.8
H-9665	12.2 ± 0.5	8.7 ± 0.1	10.5 ± 0.2def	3.9

[a] Different letters show significant differences ($p < 0.05$) in the folate content among the tomato cultivars studied. All results are means of quadruplicates ± standard deviation. Content of folates as the average content of folate from light red and full red stages for each tomato cultivar. [b] The contribution to the RDA for folate (400 μg/day) (*32*) has been calculated on the basis of the consumption of one tomato per day (serving size = 150 g).

Statistical Analysis. Results are expressed as mean value ± SD from four replicates based on fresh weight (FW). An analysis of variance was used to test the variation in the content of folates among the different cultivars of tomato and ripening stages, as well as the variation in the content of folates due to year of harvest and the temperature and time of storage. Tukey's pairwise comparison was used to determine significant differences between means. Differences were considered to be significant for $p < 0.05$. Statistical analysis of the data was performed using the SPSS 15.0 software package (Chicago, IL).

RESULTS

Effects of Cultivar, Ripeness Stage, and Year of Harvest on Folate Content in Raw Tomatoes. The contents of folates in six different tomato cultivars classified by their ripeness stages are shown in **Figure 2**. 5-CH_3-H_4-folate was the only folate form detected in the different tomato cultivars; its concentration showed a great variation ranging from 4.1 to 35.3 μg/100 g of FW depending on the cultivar and ripeness stage. Ronaldo tomatoes had the highest 5-CH_3-H_4-folate content in all four ripeness stages, compared to the other cultivars. The content of folates in Ronaldo samples varied from 21.0 to 35.3 μg/100 g of FW for green and fully ripe fruits. The tomato cultivar with the lowest content in 5-CH_3-H_4-folate was Cherry Pera (4.1−7.0 μg/100 g of FW). The data in **Figure 2** show the great variability in the content of 5-CH_3-H_4-folate from the unripe to the fully ripe stage; no common trend could be established for all tomato cultivars. For instance, the content of 5-CH_3-H_4-folate significantly increased during ripening of cultivar Cherry Pera (from 4.1 to 7.0 μg/100 g of FW, $p < 0.0001$), whereas it steadily decreased in cultivar Tina (from 18.2 to 12.7 μg/100 g of FW, $p < 0.0001$). On the contrary, the content of 5-CH_3-H_4-folate in Ronaldo, Romario, Zoco, and Pera cultivars showed a random distribution among the four ripening stages. However, in Romario, Zoco, and Pera cultivars the content of 5-CH_3-H_4-folate significantly ($p < 0.0001$) decreased in the last ripening stage compared to the previous stage.

The contents of 5-CH_3-H_4-folate in tomato cultivars Malva and Heinz (four cultivars) fully ripened on the vines and harvested in 2006, 2007, and 2008 are presented in **Figure 3**. The content of 5-CH_3-H_4-folate was significantly higher ($p < 0.0001$), at least 4.3-fold, in tomatoes harvested in 2008 compared to 2006 and 2007. On the contrary, the lowest content of folates was measured in tomatoes harvested in year 2006, except for cultivar Malva. Additionally, in three of the Heinz cultivars (H-9776, H-9661, and H-9997) a steady and significant ($p < 0.0001$) increase of the 5-CH_3-H_4-folate content from 2006 through 2008 was observed.

Light red and fully ripe are the tomato ripeness stages most commonly consumed by the general population; the mean value of their respective 5-CH_3-H_4-folate contents as well as the estimated average 5-CH_3-H_4-folate intake after the consumption of these two tomato stages is presented in **Table 1**. The Ronaldo cultivar showed the highest content followed by the Pera cultivar,

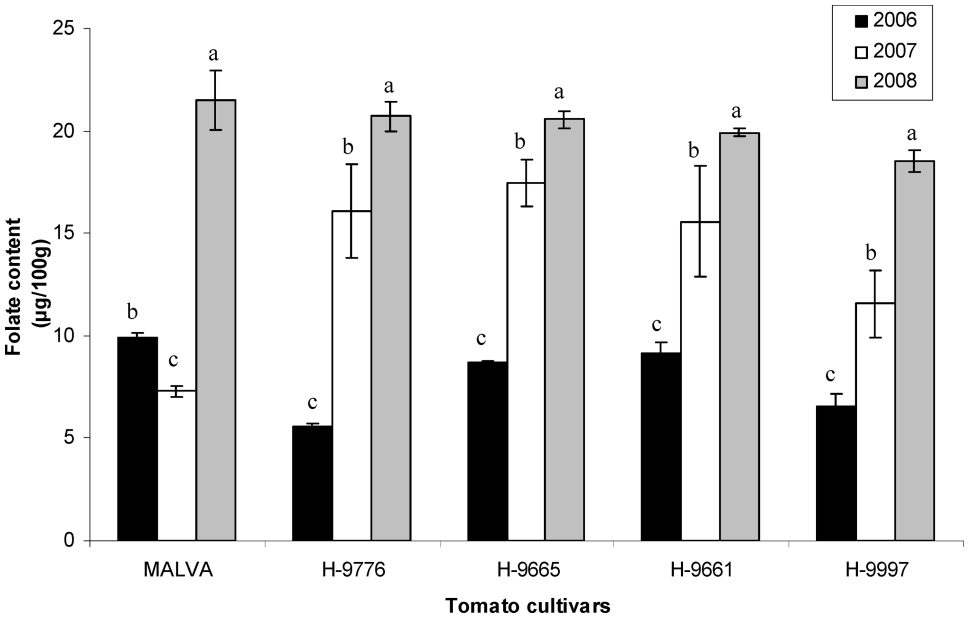


Figure 3. Folate content (5-CH_3-H_4-folate in μg/100 g of fresh weight) in tomato cultivars harvested in 2006, 2007, and 2008. All results are means of quadruplicates, and the error bars show standard deviations. Different letters within each tomato cultivar show significant differences ($p < 0.05$) in the folate contents among the three years of harvest.

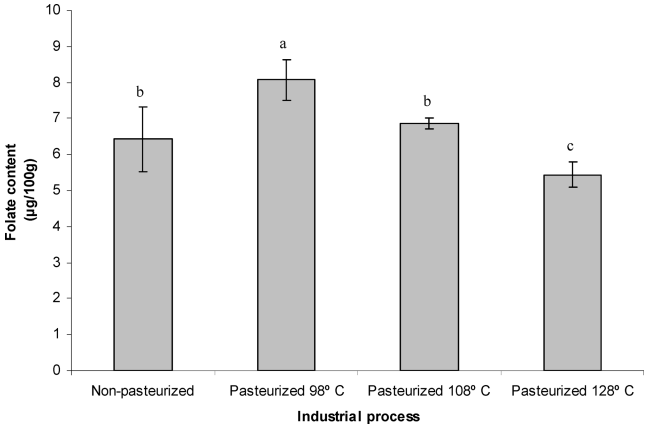


Figure 4. Folate content (5-CH_3-H_4-folate in μg/100 g of fresh weight) in tomato puree before and after pasteurization at 98, 108, or 128 °C. All results are means of quadruplicates, and the error bars show standard deviations. Different letters on bars show significant differences ($p < 0.05$) among the nonpasteurized and pasteurized tomato puree samples.

28.6 and 18.0 μg/100 g of FW, respectively. Romario, Zoco, Tina, and Malva cultivars yielded folate contents that were not significantly different and varied from 11.2 to 13.0 μg/100 g of FW. On the other hand, the lowest folate contents were found in Cherry Pera and Heinz H-9776 (6.5 and 6.0 μg/100 g of FW, respectively). The consumption of light red and fully ripe stages of cultivar Ronaldo therefore should make a greater contribution to meeting the recommended daily allowance (RDA) of folates; the estimated average folate intake from one tomato per day (serving size = 150 g) is 10.7% of the RDA.

Effects of Pasteurization, Storage, and Packaging on Folate Content in Tomato Puree. The effect of pasteurization on folate content was studied in a hot-break tomato puree using different temperatures (**Figure 4**). 5-CH_3-H_4-folate was the only folate form detected in both the nonpasteurized and pasteurized HTP samples, with a mean value of 6.4 μg/100 g of FW in the former. 5-CH_3-H_4-folate concentration in HTP pasteurized at 98 °C increased significantly to 8.1 μg/100 g of FW. However, in the other two samples the content of 5-CH_3-H_4-folate decreased significantly to a

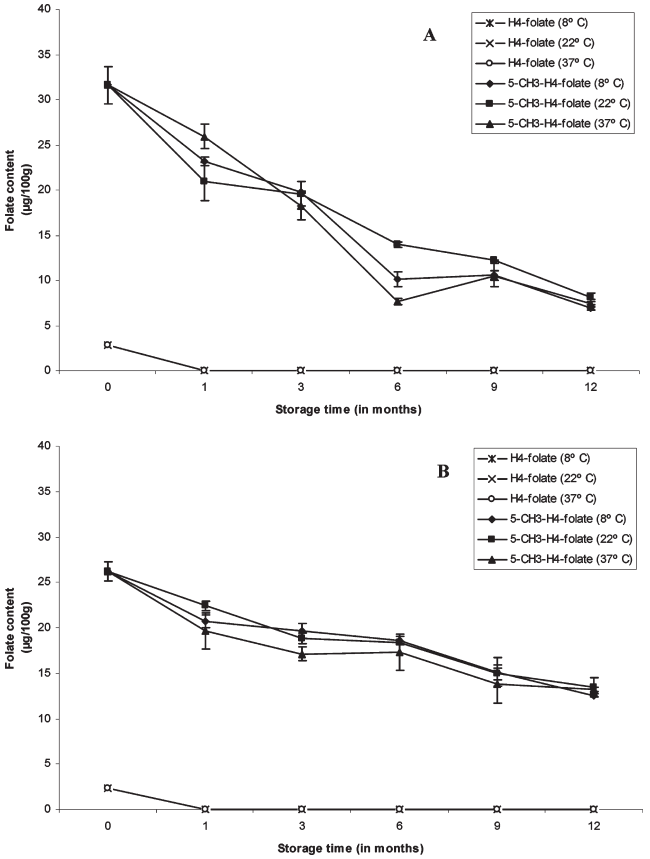


Figure 5. Folate content (H_4-folate and 5-CH_3-H_4-folate in μg/100 g of fresh weight) in tomato juice contained in glass bottles (**A**) or Tetra Paks (**B**) during 12 months of storage at 8, 22, and 37 °C. All results are means of quadruplicates, and the error bars show standard deviations.

final concentration of 5.44 μg/100 g of FW as temperature increased when compared to the nonpasteurized HTP sample.

The effects of storage temperature (8, 22, or 37 °C) and type of packaging (Tetra Pak or glass bottle) on folate content are shown in **Figure 5**. H_4-folate was detected in both Tetra Pak and glass

bottle samples only at time zero in low concentrations (2.3−2.8 μg/100 g of FW) and was significantly ($p < 0.005$) lower in the tomato juice contained in a Tetra Pak. From the 1st month until the 12th month of storage, H_4-folate was not detected at any of the tested temperatures. The initial content of 5-CH_3-H_4-folate in glass-bottled tomato juice (**Figure 5A**) was 31.61 μg/100 g of FW, which decreased significantly ($p < 0.001$) as storage time increased, regardless of the temperature. All samples showed a similar content of 5-CH_3-H_4-folate (6.96−8.11 μg/100 g of FW) after 12 months of storage. The content of 5-CH_3-H_4-folate in tomato juice contained in a Tetra Pak (**Figure 5B**) was initially lower than that in the glass-bottled tomato juice. A similar tendency for the 5-CH_3-H_4-folate concentration to decrease during storage in a Tetra Pak was observed. This concentration reached the same level at all temperatures (12.5−13.5 μg/100 g of FW) by the end of the shelf life test, but it was clearly higher than that in glass-bottled tomato juice.

DISCUSSION

Raw Tomatoes. Fruits and vegetables are good sources of naturally occurring folate, primarily 5-CH_3-H_4-folate. However, the natural folate content does not remain constant because it is affected by cultivar (*19*). Jägerstad et al. (*19*) reported that the variation of folate content in different cultivars of vegetables did not exceed 30%, with the exception of tomatoes. We agree with these authors because in this study the content of 5-CH_3-H_4-folate among all samples showed variability of >30%. The Ronaldo cultivar showed a significantly higher 5-CH_3-H_4-folate content at all ripeness stages compared to the other cultivars evaluated. This is in agreement with previous investigations on Ronaldo, Siena, and Copo tomato cultivars harvested in 2002 (*11*, *13*). Besides, in one of these studies, the Ronaldo cultivar also showed the highest concentration of vitamin C. This may suggest that ascorbic acid could serve as protective agent against folate degradation after harvesting. However, no relationship was found between the contents of folates and ascorbic acid in the different tomato cultivars in this study (data not shown).

Knowledge of the folate content in different tomato cultivars is very useful for selecting the best tomato cultivars. However, other variables have to be considered, because 5-CH_3-H_4-folate concentration was clearly dependent on ripeness as well as year of harvest. The results obtained in the present study for raw tomatoes agree with those reported by Strålsjo et al. (*20*) for the folates content in strawberries and by Piironen et al. (*21*) for folates in wheat genotypes.

As previously mentioned, we found differences in the folate content in the four ripeness stages in the tomato cultivars, but they did not share a common pattern. Despite that, we observed that folate content decreased during ripening in all cultivars except Ronaldo and Cherry Pera. Previous studies have also shown that folate content drops as tomatoes ripen, turning from green to red (*11*, *22*). Periago et al. (*11*) reported that the folate content decreased by >50% from green to red tomatoes. Although folate content may vary with the maturation process in food plants, not all tomato cultivars studied in this investigation followed the trend reported by other researchers. Consequently, the effect of ripeness on the folate content in tomatoes is not easy to evaluate, as was previously found by Strålsjo et al. (*20*) for the different maturity stages of strawberries.

Folate contents in Malva and Heinz cultivars were differently affected by the year of harvest. The content of 5-CH_3-H_4-folate was significantly higher in all tomatoes studied during 2008 than in 2006 and 2007. All cultivars were harvested from the same region of Spain, Badajoz, during 2006, 2007, and 2008 using the same agricultural practices. The average temperature and rainfall in Badajoz were 17.8 °C and 428.0 mm in 2006, 16.5 °C and 283.5 mm in 2007, and 16.8 °C and 436.9 mm in 2008 (*23*). In 2006, the temperature was higher than in the other two years; thus, an indirect influence may be established between temperature and higher content of 5-CH_3-H_4-folate in tomatoes. Comparison of the folate content between samples cultivated in 2007 and 2008 shows that both years registered a similar average temperature, but the higher rainfall in 2008 may be related with a larger synthesis of 5-CH_3-H_4-folate in that year. However, as has been mentioned above, other factors may play an important role in determining the folate content, and further studies have to be undertaken to determine the effect of environmental conditions.

Processed Tomatoes. Many plant foods are processed before consumption, potentially affecting the folates present. However, some dietary constituents may enhance folate bioavailability by increasing the stability of folates during food processing, for example, folate-binding protein and antioxidants such as ascorbic acid (*24*). The results of this work indicated that the pasteurization of HTP at 98 °C for 40 s significantly increased the extractability of folate. In general, the plant folate biosynthesis pathway is distributed among three subcellular compartments. In plant cells, the cytosol, mitochondria, and chloroplasts contain folates predominantly in the form of polyglutamylated derivatives (*25*). Thus, these conditions of heat treatment improved the total folate extractability by causing a breakdown of the cellular organelles and allowing the conversion of polyglutamate to monoglutamate derivatives of the folate (*6*, *26*). In our opinion, the results obtained were not affected by the water content of the different tomato purees, as all samples contained a similar content, 94.1−94.2%. Therefore, the increase in the folate content after pasteurization was due to the reasons mentioned above. In processed tomato products, but not in raw tomato, we observed H_4-folate besides 5-CH_3-H_4-folate. This could be due to the fact that tomato processing (especially homogenization and pasteurization) can break tomato cells and therefore more folate vitamers are released from the inner cell compartments (*26*). 5-CH_3-H_4-folate was the most abundant vitamer, contributing around 90%, whereas H_4-folate was only 10% of the total folate. Thermal treatment at 108 °C for 40 s did not cause changes in the 5-CH_3-H_4-folate content of HTP, whereas an increase in the pasteurization temperature to 128 °C for 40 s led to 15.4% losses of the initial content of this vitamer. Our data are in agreement with a previous study, in which 5-CH_3-H_4-folate in a model food system was relatively stable up to 120 °C (*27*). That study also reported that antioxidants, especially ascorbic acid, had a protective effect and strongly retarded folate degradation during thermal treatments. Tomatoes contain moderate amounts of ascorbic acid, but the content varies due to genetic, agronomic, and environmental factors (*11*, *28*). Besides ascorbic acid, tomato contains other antioxidant compounds such as lycopene and phenolic compounds, which could have a synergistic effect to prevent folate oxidation. In addition, thermal treatments have been observed to increase the overall antioxidant potential of tomato juice (*29*). This effect could also be responsible for the low losses in the total content of folates of HTP after pasteurization.

The tomato juice used in the shelf life test was prepared from HTP belonging to a different batch from the HTP used in the pasteurization assay. For this reason a large difference in the total folate content and in the relative abundance of the folate vitamers was observed between the HTP subjected to pasteurization and the tomato juice. In the tomato juice two folate vitamers (5-CH_3-H_4-folate and H_4-folate) were detected, so the total folate content corresponded to the sum of these two

compounds. These findings concur with the scientific literature, because 5-CH_3-H_4-folate is the predominant natural form of folates in vegetables (45−65%), whereas H_4-folate and methylene forms represent only 10−15% of the total pool of folates (*25*). In a recent study, it was observed that processing of peas resulted in a conversion of 5-CH_3-H_4-folate to H_4-folate (*30*). In addition, the total folate content of these compounds was significantly higher in glass-bottled tomato juice than in Tetra Pak, although both tomato juices were produced from the same batch of HTP. This variability could be due to the destruction of folates by hydrogen peroxide oxidation, because the packaging is sanitized with this oxidative agent before filling the brick with the tomato juice.

The stability of folate during its shelf life depended on the vitamer and folate losses increasing as storage time increased in all of the conditions tested. The initial total folate losses (in the first month of storage) ranged between 24.6 and 39.1% in glass-bottled tomato juice, but they were never higher than 30.9% in Tetra Pak tomato juice. Total folate losses reached values of 76.4−79.7% in glass-bottled tomato juice after 12 months of storage and slightly above 50% in Tetra Pak tomato juice, irrespective of temperature (8, 22, and 37 °C). H_4-folate rapidly degraded within 1 month of storage in the three temperature conditions. This is not surprising because H_4-folate is a very labile folate form (*31*), and its low content was probably quickly degraded by light and/or temperature during storage. On the other hand, the 5-CH_3-H_4-folate content losses in Tetra Pak tomato juice were significantly lower than those found in glass-bottled tomato juice. 5-CH_3-H_4-folate is a folate form more stable than H_4-folate, and the opaque packaging might have protected it from the degradative effect of light. Additionally, the tomato juice in Tetra Paks was fortified with ascorbic acid during manufacturing. The content of ascorbic acid found in tomato juice contained in Tetra Paks was 68 mg/100 g, while no ascorbic acid was detected in the tomato juice in glass bottles on day 0 (data not shown). The ascorbic acid contained in Tetra Paks might have protected 5-CH_3-H_4-folate from oxidative degradation during its shelf life, allowing it to decrease at a slower rate than in glass bottles.

In conclusion, the tomato can be considered as a moderate source of folate, but its content is undoubtedly influenced by the cultivar, ripeness stage, and environmental conditions during growth. Pasteurization of HTP resulted in a significant increase of the extractability of folates from the cell compartments, when the temperature did not exceed 108 °C for 40 s. However, due to the lability of folate to different factors, pasteurization at temperatures above 108 °C and storage during its shelf life caused important losses in the folate content. Considering that glass bottles led to higher losses of 5-CH_3-H_4-folate due to the effect of light, tomato juice should be packed in opaque material to prevent the photodegradation of folate during its shelf life. Therefore, agronomic as well as industrial parameters (thermal processing, packaging, and storage conditions) should be considered to optimize the folate content of tomato products.

ABBREVIATIONS USED

HTP, hot break tomato puree; H_4-folate, tetrahydrofolate; 5-CH_3-H_4-folate, 5-methyltetrahydrofolate; 5-HCO-H_4-folate, 5-formyltetrahydrofolate.

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LITERATURE CITED

(1) de Bree, A.; van Dusseldorp, M.; Brouwer, I. A.; Van het Hof, K. H.; Steegers-Theuniessen, R. P. M. Folate intake in Europe: recommended, actual and desired intake. *Eur. J. Clin. Nutr.* **1997**, *51*, 643–660.

(2) Van Guelpen, B.; Hultdin, J.; Johansson, I.; Stegmayr, B.; Hallmans, G.; Nilsson, T. K.; Weinehall, L.; Witthöft, C.; Palmqvist, R.; Winkvist, A. Folate, vitamin B12, and risk of ischemic and hemorrhagic stroke: a prospective, nested case-referent study of plasma concentrations and dietary intake. *Stroke* **2005**, *36*, 1426–1431.

(3) De Wals, P.; Tairou, F.; Van Allen, M. I.; Uh, S. H.; Lowry, R. B.; Sibbald, B.; Evans, J. A.; Van den Hof, M. C.; Zimmer, P.; Crowley, M.; Fernandez, B.; Lee, N. S.; Nivonsenga, T. Reduction in neural-tube defects after folic acid fortification in Canada. *N. Engl. J. Med.* **2007**, *357*, 135–42.

(4) Cole, B. F.; Baron, J. A.; Sandler, R. S.; Haile, R. W.; Ahnen, D. J.; Bresalier, R. S.; McKeown-Eyssen, G.; Summers, R. W.; Rothstein, R. I.; Burke, C. A.; Snover, D. C.; Church, T. R.; Allen, J. I.; Robertson, D. J.; Beck, G. J.; Bond, J. H.; Byers, T.; Mandel, J. S.; Mott, L. A.; Pearson, L. H.; Barry, E. L.; Rees, J. R.; Marcon, N.; Saibil, F.; Ueland, P. M.; Greenberg, E. R. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *JAMA−J. Am. Med. Assoc.* **2007**, *297*, 2351–2359.

(5) Khanna, D.; Park, G. S.; Paulus, H. E.; Simpson, K. M.; Elashoff, D.; Cohen, S. B.; Emnery, P.; Dorrier, C.; Furst, D. E. Reduction of the efficacy of methotrexate by the use of folic acid: post hoc analysis from two randomized controlled studies. *Arthritis Rheum.* **2007**, *52*, 3030–3038.

(6) Scott, J.; Rébeillé, F.; Fletcher, J. Folic acid and folates: the feasibility for nutritional enhancement in plant foods. *J. Sci. Food Agric.* **2000**, *80*, 795–824.

(7) Rébeillé, F.; Ravanel, S.; Jabrin, S.; Douce, R.; Storozhenko, S.; Van der Straeten, D. Folates in plants: biosynthesis, distribution, and enhancement. *Physiol. Plant.* **2006**, *126*, 330–342.

(8) Pennington, J. A. T. Food composition databases for bioactive food components. *J. Food Compos. Anal.* **2002**, *15*, 419–434.

(9) Willcox, J. K.; Catignani, G. L.; Lazarus, S. Tomatoes and cardiovascular diseases. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 1–18.

(10) Gahler, S.; Otto, K.; Böhm, V. Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products. *J. Agric. Food Chem.* **2003**, *51*, 7962–7968.

(11) Periago, M. J.; García-Alonso, J.; Jacob, K.; Olivares, A. B.; Bernal, M. J.; Iniesta, M. D.; Martínez, C.; Ros, G. Bioactive compounds, folates and antioxidant properties of tomatoes (*Lycopersicum esculentum*) during vine ripening. *Int. J. Food Sci. Nutr.* **2008**, *12*, 1–15.

(12) Dewanto, V.; Wu, X.; Adom, K. K.; Liu, R. H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 3010–3014.

(13) Olivares, A. B.; Bernal, M. J.; Ros, G.; Martínez, C.; Periago, M. J. Processing effect on folates in green peas and gazpacho. In *Proceeding of the First International Conference on Folates—Analysis, Bioavailability and Health*; University Press: Warsaw, Poland, 2004; pp 61−67.

(14) Pfeiffer, C. M.; Rogers, L. M.; Gregory, J. F. Improved analysis of folate in cereal-grain food products using tri-enzyme extraction and combined affinity and reverse phase liquid chromatography. *J. Agric. Food Chem.* **1997**, *45*, 407–413.

(15) Konings, E. J. A validated liquid chromatography method for determining folates in vegetables, milk powder, liver, and flour. *J. AOAC Int.* **1999**, *82*, 119–127.

(16) Vahteristo, L.; Finglas, P. M.; Witthöft, C.; Wigertz, K.; Seale, R.; De Froidmont-Görtz, I. Third EU MAT intercomparison study on food folate analysis using HPLC procedures. *Food Chem.* **1996**, *57*, 109–111.

(17) Gregory, J. F.; Sartain, D. B.; Day, B. P. F. Fluorometric determination of folacin in biological materials using high performance liquid chromatography. *J. Nutr.* **1984**, *114*, 341–353.

(18) Finglas, P. M.; Scott, K. J.; Witthöft, C. M.; Van den Berg, H.; De Froidmont-Görtz, I. *The Certification of the Mass Fraction of Vitamins in Four Reference Materials: Wholemeal Flour (CRM 121), Milk Powder (CRM 421), Lyophilised Mixed Vegetables (CRM*

485) and Lyophilised Pig'S Liver (CRM 487); Office for Official Publications of the European Communities: Luxembourg, 1999 (EUR report 18320).

(19) Jägerstad, M.; Piironen, V.; Walker, C.; Ros, G.; Carnovale, E.; Holasova, M.; Nau, H. Increasing natural food folates through bioprocessing and biotechnology. *Trends Food Sci. Technol*. **2005**, *16*, 298–306.

(20) Strålsjö, L. M.; Witthöft, C. M.; Sjöholm, I. M.; Jägerstad, M. I. Folate content in strawberries (*Fragaria* × *ananassa*): effects of cultivar, ripeness, year of harvest, storage and commercial processing. *J. Agric. Food Chem*. **2003**, *51*, 128–133.

(21) Piironen, V.; Edelmann, M.; Kariluoto, S.; Bedo, Z. Folate in wheat genotypes in the HEALTHGRAIN Diversity Screen. *J. Agric. Food Chem*. **2008**, *56*, 9726–9731.

(22) Basset, G. J.; Quinlivan, E. P.; Ravanel, S.; Rébeillé, F.; Nichols, B. P.; Shinozaki, K.; Seki, M.; Adams-Philips, L. C.; Giovannoni, J. J.; Gregory, J. F.; Hanson, A. D. Folate synthesis in plants: the *p*-aminobenzoate branch is initiated by a bifunctional PabA−PabB protein that is targeted to plastids. *Proc. Natl. Acad. Sci. U.S.A*. **2004**, *101*, 1496–1501.

(23) Ministerio de Medio Ambiente. Instituto Nacional de Meteorología. Resumen anual climatológico del año 2006, 2007 y 2008; http://www.aemet.es/es/elclima/datosclimatologicos/resumenes (accessed Jan 24, 2009).

(24) Andersson, A.; Oste, R. Loss of ascorbic acid, folate and vitamin B12, and changes in oxygen content of UHT-milk, II. Results and discussion. *Milchwissenschaft* **1992**, *47*, 299–302.

(25) Cossins, E. A. The fascinating world of folate and one-carbon metabolism. *Can. J. Bot*. **2000**, *78*, 691–708.

(26) Melse-Boonstra, A.; Verhoef, P.; Konings, E. J. M.; Van Dusseldorp, M.; Matser, A.; Hollman, P. C. H.; Meyboom, S.; Kok, F. J.; West, C. E. Influence of processing on total, monoglutamate and polyglutamate folate contents of leeks, cauliflower, and green beans. *J. Agric. Food Chem*. **2002**, *50*, 3473–3478.

(27) Indrawati; Arroqui, I.; Messagie, I.; Nguyen, M. T.; Van Loey, A.; Hendrickx, M. Comparative study on pressure and temperature stability of 5-methyltetrahydrofolic acid in model systems and in food products. *J. Agric. Food Chem*. **2004**, *52*, 485–492.

(28) Martínez-Valverde, I.; Periago, M. J.; Provan, G.; Chesson, A. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). *J. Agric. Food Chem*. **2002**, *82*, 323–330.

(29) Sánchez-Moreno, C.; Plaza, L.; de Ancos, B.; Cano, M. P. Nutricional characterisation of commercial tradicional pasteurizad tomato juices: carotenoids, vitamin C and radical-scavenging capacity. *Food Chem*. **2006**, *98*, 749–756.

(30) Stea, T. H.; Johansson, M.; Jägerstad, M.; Frølich, W. Retention of folates in cooked, stored and reheated peas, broccoli and potatoes for use in modern large-scale service systems. *Food Chem*. **2006**, *101*, 1095–1107.

(31) Jones, M. L.; Nixon, P. F. Tetrahydrofolates are greatly stabilized by binding to bovine milk folate-binding protein. *J. Nutr*. **2002**, *132*, 2690–2694.

(32) Institute of Medicine. *Dietary Reference Intakes for Thiamine, Rivoflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline*; National Academy Press: Washington, DC, 1999; pp 196−305.

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