JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Changes in Phenolic Content of Tomato Products during Storage

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ABSTRACT: The effect of storage on the total polyphenol content and individual phenolic compounds as well as on the hydrophilic antioxidant capacity of ketchups and tomato juices was studied. The total polyphenol content was determined using the Folin−Ciocalteu assay, and the antioxidant capacity of the hydrophilic fraction was determined using DPPH and $ABTS^+$ assays. Individual polyphenols were identified and quantified using liquid chromatography/electrospray ionization tandem mass spectrometry on a triple quadrupole. All analyses were carried out for ketchups and tomato juices after storage for 3, 6, and 9 months. The total polyphenol content and antioxidant capacity of the hydrophilic fraction decreased during storage of ketchups and tomato juices. Ketchups, in general, showed a slightly greater stability during storage than tomato juices. The most significant decrease was observed for quercetin followed by caffeic and ferulic acids, whereas glycosilated polyphenols showed greater stability during storage.

KEYWORDS: Polyphenols, HPLC-MS/MS, antioxidant capacity, storage, stability

INTRODUCTION

Tomato consumption has greatly increased worldwide over the past 2 decades, mostly due to a growing demand for tomato-based products such as ketchups and tomato juices. Ketchups and tomato juices, as well as raw tomatoes, possess antioxidative, anti-inflammatory, antimutagenic, and anticarcinogenic properties and the capacity to modulate some key cellular enzyme functions, this being ascribed in part to their many phenolic compounds.[1,2] When considering the importance of the role of polyphenols as health-protecting factors, the consumption of tomato and tomato-based products could be seen as a nutritional indicator of good dietary habits and a healthy lifestyle.

Tomato-based products may potentially contain the same range of phenolic compounds as the tomatoes from which they are derived; however, because of differences between varieties of tomatoes and their origin,[3,4] the total polyphenol (TP) content can vary considerably. The nutritional quality of tomato products is affected by storage conditions as well as by thermal treatments during processing. In particular, thermal treatments are generally believed to be the main cause of the depletion in natural antioxidants.[5,6]

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.[7,8] During the storage of tomato-based products, various changes may occur, for example, as a result of browning and degradation reactions. It is well-known that naturally occurring antioxidants are significantly lost as a consequence of processing and storage.[9]

The literature data for individual and/or TP content, as well as the antioxidant activity of commercial juices, are limited. Although some data on the content of hydroxycinnamic acids and flavanone derivatives in fresh and stored juices are available,[10−12] storage studies lasting longer than 6 months are scarce. Therefore, further studies are necessary to determine the nutritional quality of their bioactive compounds during storage. Moreover, there is no information concerning changes in flavonols, flavanones, and hydroxycinnamic acids in ketchups and tomato juices during storage.

The aim of this study was to evaluate the stability of the hydrophilic antioxidant compounds present in six commercially available tomato juices and ketchups over a storage period of 9 months. Both products were kept at storage temperature. The hydrophilic antioxidant capacity, TP, and content of flavonols (kaempferol-3-*O*-rutinoside, rutin, and quercetin), flavanones (naringenin and naringenin-7-*O*-glucoside), and hydroxycinnamic acids (chlorogenic, caffeic, caffeic-*O*-hexoside, ferulic, and ferulic-*O*-hexoside acids) were analyzed.

MATERIALS AND METHODS

Standards and Reagents. All samples and standards were handled without exposure to light. Caffeic and chlorogenic acids, rutin, quercetin, Folin−Ciocalteu (F-C) reagent, ABTS [2,2′azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], PBS (phosphate-buffered saline, pH 7.4), Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid 97%], manganese dioxide, and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma Chemical Co (St. Louis, MO); naringenin, naringenin-7-*O*-glucoside, and kaempferol-3-*O*-rutinoside were purchased from Extrasynthèse (Genay, France). Ethanol and formic acid, high-performance liquid chromatography (HPLC) grade, were obtained from Scharlau Chemie, S.A. (Barcelona, Spain); and ultrapure water (Milli-Q) was from Millipore System (Bedford, MA).

Received: May 29, 2011
Accepted: August 5, 2011
Revised: July 29, 2011
Published: August 05, 2011

Processing Conditions and Ingredients of Commercial Samples. In this study, a 9 month storage trial was designed to investigate the stability of the main bioactive antioxidant compounds of six commercially available tomato juices and six commercial ketchups. All of the studied brands contained the same ingredients, but in some cases, the amount of each ingredient was not known. Each brand of ketchup and tomato juice was analyzed at the start of the trial (month 0). Three cartons of each brand of ketchup and tomato juice were stored for 3, 6, and 9 months. Ketchups and tomato juices were kept in Tetra Paks at a storage temperature of 4.0 ± 0.1. Both products analyzed were available in Barcelona markets.

Extraction and Isolation of Phenolic Compounds. Samples were treated in triplicate in a darkened room with a red safety light to avoid oxidation of the analytes, following the procedure of Vallverdú-Queralt et al. with some modifications.[13]

Ketchup samples (1.0 g) were weighed and homogenized with 80% ethanol in Milli-Q water (4 mL); they were then sonicated for 5 min and centrifuged (4000 rpm at 4 °C) for 20 min. The supernatant was transferred into a flask, and the extraction was repeated. Both supernatants were combined and evaporated under nitrogen flow. Finally, the residue was reconstituted with Milli-Q water (0.1% formic acid) up to 1.5 mL.

Tomato juices (10 mL) were centrifuged (4000 rpm at 4 °C) for 10 min. The supernatant was discarded, and 4 mL of 80% ethanol in Milli-Q water was added to 1 g pellets. They were sonicated for 5 min and centrifuged (4000 rpm at 4 °C) for 20 min following the procedure described above for the extraction of ketchups.

Solid-phase extraction (SPE) of these extracts was carried out following the procedure of other authors.[14,15] First, 1 mL of methanol and subsequently 1 mL of sodium acetate 50 mmol/L pH 7 were loaded into Oasis MAX cartridges from Waters to equilibrate it; then, 1 mL of each extract was diluted with 1 mL of Milli-Q water and acidified with 34 μL of 35% hydrochloric acid before being loaded into the cartridges separately. These were rinsed with sodium acetate 50 mmol/L pH 7 (5% methanol). The polyphenols were eluted with 1800 μL of methanol (2% formic acid). The eluted fractions were evaporated under nitrogen flow, and the residue was reconstituted with water (0.1% formic acid) up to 250 μL and filtered through a 13 mm, 0.45 μm PTFE filter (Waters) into an insert-amber vial for HPLC analysis. Samples were stored at −20 °C until analysis.

Analysis of TPs. The content of TP was analyzed using the F-C method. All ketchup and tomato juice extracts (20 μL of the eluted fractions) were mixed with 188 μL of Milli-Q water in a thermo microtiter 96-well plate (Nunc, Roskilde, Denmark). Then, 12 μL of F-C reagent and 30 μL of sodium carbonate (200 g/L) were added following procedures described previously.[16,17] The mixtures were incubated for 1 h at room temperature in darkness. After the reaction period, 50 μL of Milli-Q water was added, and the absorbance was measured at 765 nm in a UV/vis Thermo Multiskan Spectrum spectrophotometer (Vantaa, Finland). Gallic acid was used as a calibration standard, and the TP content was expressed as mg of gallic acid equivalents (GAE)/100 mg fresh weight (FW).

Antioxidant Capacity of the Hydrophilic Fraction. The antioxidant capacity of the hydrophilic fraction in tomato juices and ketchups was measured by $ABTS^+$ and DPPH assays following the procedure described previously.[18−20]

$ABTS^+$ Assay. A 1 mM concentration of Trolox (antioxidant standard) was prepared in methanol once a week. Working standards were prepared daily by diluting 1 mM Trolox with methanol. Solutions of known Trolox were used for calibration.

An $ABTS^+$ radical cation was prepared by passing a 5 mM aqueous stock solution of ABTS (in methanol) through manganese dioxide powder. Excess manganese dioxide was filtered through a 13 mm 0.45 μm filter PTFE (Waters). Then, 245 μL of $ABTS^+$ solution was added to 5 μL of Trolox or to tomato samples, and the solutions were stirred for 30 s. The homogenate was shaken vigorously and kept in darkness for 1 h. Absorption of the samples was measured on a UV/vis Thermo Multiskan Spectrum spectrophotometer at 734 nm, and methanol blanks were run in each assay. Results were expressed as mmol Trolox equivalent (TE)/100 g DM.

DPPH Assay. The antioxidant activity was also studied through the evaluation of the free radical-scavenging effect on DPPH radical. Solutions of known Trolox were used for calibration. Five microliters of tomato samples or Trolox was mixed with 250 μL of methanolic DPPH (0.025 g/L). The homogenate was shaken vigorously and kept in darkness for 30 min. The absorption of the samples was measured on the spectrophotometer at 515 nm. Results were expressed as mmol TE/100 g DM.

HPLC-ESI-MS/MS Analysis. To evaluate the effect of storage on the content of flavonols, flavanones and hydroxycinnamic acids, polyphenols were quantified using HPLC-electrospray ionization tandem mass spectrometry (ESI-MS/MS) following the procedure of Vallverdú-Queralt et al.[16] An API 3000 (PE Sciex, Concord, Ontario, Canada) triple quadrupole mass spectrometer equipped with a Turbo Ionspray source in negative-ion mode was used to obtain MS and MS/MS data.

For quantification purposes, data were collected in the multiple reaction monitoring (MRM) mode, tracking the transition of parent and product ions specific for each compound. In particular, we selected 12 transitions corresponding to ferulic acid-*O*-hexoside m/z 355 → 193; ferulic acid m/z 193 → 134; chlorogenic acid m/z 353 → 191; caffeic acid m/z 179 → 135; caffeic acid-*O*-hexoside m/z 341 → 179; quercetin m/z 301 → 151; rutin m/z 609 → 300; naringenin m/z 271 → 151; naringenin-7-*O*-glucoside m/z 433 → 271; kaempferol-3-*O*-rutinoside m/z 593 → 285; and ethyl gallate (internal standard) m/z 197 → 169.

Quantification of polyphenols was performed by the internal standard method. Polyphenols were quantified with respect to their corresponding standard. When standards were not available, as in the case of caffeic-*O*-hexoside and ferulic-*O*-hexoside acids, they were quantified with respect to the corresponding hydroxycinnamic acid (caffeic and ferulic acids).

The liquid chromatograph was an Agilent series 1100 HPLC instrument (Agilent, Waldbronn, Germany) equipped with a quaternary pump, an autosampler, and a column oven set to 30 °C. Mobile phases consisted of 0.1% formic acid in Milli-Q water (A) and 0.1% formic acid in acetonitrile (B). A Luna C_{18} column 50 mm × 2.0 mm i.d., 5 μm (Phenomenex, Torrance, CA), was used. The injection volume was 20 μL, and the flow rate was 0.4 mL/min. Separation was carried out in 20 min under the following conditions: 0 min, 5% B; 16 min, 40% B; 17 min, 95% B; 19 min, 95% B; and 19.5 min, 5% B. The column was equilibrated for 5 min prior to each analysis.

Statistical Analysis. The significance of the results was analyzed using the Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, MD). Analysis of variance (ANOVA) was used to compare the means of groups of measurement data.

RESULTS AND DISCUSSION

Effect of Storage on the Total Phenolic Content and Antioxidant Capacity of the Hydrophilic Fraction. Generally, thermal processing of tomato-based products may lead to gains or losses in total phenol content.[6,20] Thermal processing such as traditional pasteurization applied to ketchups and tomato juices might release more bound phenolic compounds in the breakdown of cellular constituents. However, cellular disruption can release oxidative enzymes that could destroy phenolic compounds. The total phenolic content observed in these samples is in the range of those reported in tomato and tomato products (between 100 and 500 mg/kg).[9,21,22] As shown in Figure 1A,B, time of storage slightly affected the TP content as determined by F-C assay. Although there was a slight variation in the TP content

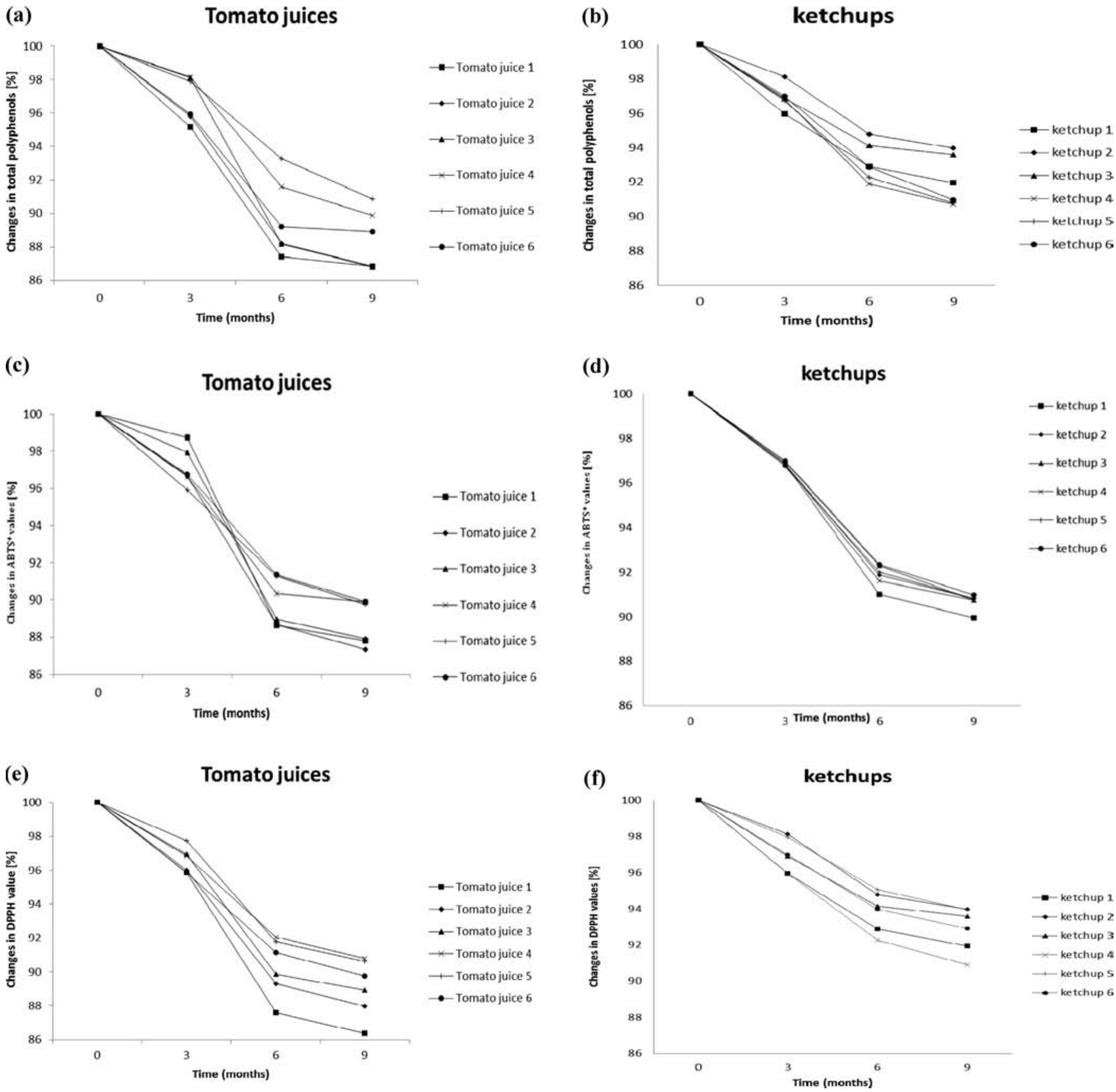

Figure 1. Changes in TPs in tomato juices (A) and ketchups (B). Changes in $ABTS^+$ [%] in tomato juices (C) and ketchups (D). Changes in DPPH [%] in tomato juices (E) and ketchups (F).

between different brands of ketchups and tomato juices, the trends reflecting the effects of storage were similar. The polyphenol content decreased by about 12% for tomato juices and 7% for ketchups over the whole of the storage period. The most significant decrease was observed between 0 and 6 months of storage. These results are in line with those reported by Klimczak et al.[23] They found that after 6 months of storage of orange juices, there was approximately a 10−22% decline in TPs and vitamin C content. Other studies reported that phenolic compounds were stable during storage of tomato juice at 4 °C for 3 months[9] and storage of tomato pulp at 4 and 20 °C for 3−5 months.[24]

The antioxidant capacity of the hydrophilic fraction of tomato-based products was evaluated using the DPPH and $ABTS^+$ assays. Both methods are easy and accurate assays for measuring the antioxidant capacity of food. The relative contributions of individual compounds to the antioxidant capacity of the hydrophilic fraction ($ABTS^+$ and DPPH) of tomato-based products are shown in Figure 1C−F. The $ABTS^+$ and DPPH values decreased during storage. Results were similar to those reported for the TP content. It is widely known that ascorbic acid and phenolic compounds are the major contributors to the antioxidant capacity of the hydrophilic fraction. Therefore, polyphenol losses were paralleled by the decrease in hydrophilic antioxidant capacity. The most significant decrease was observed between 0 and 6 months of storage. For tomato juices, the antioxidant capacity of the hydrophilic fraction decreased between 10 and

Table 1. Changes in the Content of Hydroxycinnamic Acids (μg/g FW) in Ketchups and Tomato Juices (n = 3) during 9 Months of Storage[a]

months of storage	caffeic acid	caffeic acid-*O*-hexoside	ferulic acid	ferulic acid-*O*-hexoside	chlorogenic acid
			ketchup 1		
0	1.41 ± 0.04 a	3.87 ± 0.11 a	25.15 ± 1.21 a	29.24 ± 0.72 a	10.24 ± 0.14 a
3	1.33 ± 0.06 b	3.72 ± 0.23 b	24.97 ± 1.01 b	28.34 ± 1.34 b	9.98 ± 0.20 b
6	1.26 ± 0.05 c	3.61 ± 0.19 c	22.50 ± 1.44 c	27.50 ± 1.19 c	9.71 ± 0.39 c
9	1.22 ± 0.05 d	3.49 ± 0.22 d	22.30 ± 1.38 d	27.10 ± 1.04 d	9.32 ± 0.30 d
			ketchup 2		
0	1.34 ± 0.05 a	3.79 ± 0.14 a	21.31 ± 1.50 a	28.08 ± 0.40 a	9.38 ± 0.09 a
3	1.30 ± 0.07 b	3.72 ± 0.26 b	20.85 ± 0.89 b	27.69 ± 1.33 b	9.15 ± 0.33 b
6	1.25 ± 0.06 c	3.58 ± 0.21 c	20.22 ± 1.25 c	26.81 ± 1.21 c	8.90 ± 0.34 c
9	1.19 ± 0.05 d	3.45 ± 0.18 d	19.20 ± 1.10 d	26.54 ± 1.09 d	8.50 ± 0.31 d
			ketchup 3		
0	1.33 ± 0.05 a	3.52 ± 0.11 a	22.45 ± 1.02 a	28.32 ± 0.87 a	9.47 ± 0.15 a
3	1.29 ± 0.07 b	3.47 ± 0.17 b	21.71 ± 0.98 b	27.40 ± 0.99 b	9.10 ± 0.31 b
6	1.25 ± 0.06 c	3.38 ± 0.20 c	20.38 ± 1.05 c	26.30 ± 1.01 c	8.81 ± 0.25 c
9	1.15 ± 0.04 d	3.25 ± 0.18 d	20.11 ± 1.19 d	25.49 ± 1.22 d	8.60 ± 0.36 d
			ketchup 4		
0	1.18 ± 0.04 a	2.20 ± 0.07 a	24.11 ± 1.15 a	15.59 ± 0.36 a	5.58 ± 0.10 a
3	1.11 ± 0.03 b	2.09 ± 0.12 b	23.90 ± 1.40 b	14.92 ± 0.45 b	5.41 ± 0.19 b
6	1.08 ± 0.03 c	2.06 ± 0.16 c	22.51 ± 1.03 c	14.54 ± 0.49 c	5.20 ± 0.24 c
9	1.02 ± 0.05 d	2.03 ± 0.18 d	22.01 ± 1.22 d	14.22 ± 0.56 d	5.09 ± 0.17 d
			ketchup 5		
0	1.90 ± 0.05 a	3.92 ± 0.05 a	22.30 ± 1.09 a	25.91 ± 0.58 a	9.54 ± 0.12 a
3	1.81 ± 0.08 b	3.85 ± 0.19 b	21.15 ± 1.08 b	25.41 ± 1.15 b	9.15 ± 0.15 b
6	1.74 ± 0.09 c	3.77 ± 0.21 c	20.88 ± 1.01 c	24.41 ± 1.15 c	8.90 ± 0.19 c
9	1.65 ± 0.07 d	3.64 ± 0.13 d	20.50 ± 1.23 d	24.12 ± 1.10 d	8.69 ± 0.21 d
			ketchup 6		
0	1.24 ± 0.05 a	2.93 ± 0.08 a	21.22 ± 1.22 a	19.23 ± 0.64 a	11.04 ± 0.12 a
3	1.18 ± 0.06 b	2.86 ± 0.14 b	20.91 ± 1.09 b	19.01 ± 0.99 b	10.76 ± 0.10 b
6	1.14 ± 0.07 c	2.74 ± 0.16 c	20.09 ± 1.33 c	18.25 ± 1.11 c	10.51 ± 0.33 c
9	1.09 ± 0.06 d	2.70 ± 0.10 d	19.41 ± 1.13 d	18.02 ± 1.28 d	10.01 ± 0.39 d
			tomato juice 1		
0	2.38 ± 0.10 a	3.75 ± 0.12 a	20.65 ± 1.14 a	29.91 ± 0.96 a	9.36 ± 0.14 a
3	2.31 ± 0.13 b	3.69 ± 0.22 b	20.05 ± 1.21 b	28.49 ± 1.45 b	9.15 ± 0.41 b
6	2.20 ± 0.11 c	3.51 ± 0.19 c	19.21 ± 1.01 c	27.91 ± 1.34 c	8.50 ± 0.51 c
9	2.01 ± 0.09 d	3.44 ± 0.20 d	18.01 ± 1.10 d	27.21 ± 1.41 d	8.30 ± 0.47 d
			tomato juice 2		
0	2.29 ± 0.07 a	2.16 ± 0.10 a	21.11 ± 1.20 a	20.76 ± 0.70 a	8.41 ± 0.29 a
3	2.23 ± 0.14 b	2.11 ± 0.15 b	20.65 ± 1.33 b	20.32 ± 1.21 b	8.22 ± 0.44 b
6	2.06 ± 0.16 c	2.07 ± 0.16 c	18.97 ± 1.26 c	19.71 ± 1.15 c	7.65 ± 0.51 c
9	1.91 ± 0.10 d	2.00 ± 0.13 d	18.51 ± 1.09 d	18.89 ± 1.26 d	7.40 ± 0.36 d
			tomato juice 3		
0	1.46 ± 0.06 a	3.27 ± 0.11 a	23.46 ± 1.24 a	26.66 ± 1.08 a	9.43 ± 0.25 a
3	1.39 ± 0.13 b	3.11 ± 0.11 b	22.68 ± 1.13 b	25.80 ± 1.23 b	8.66 ± 0.39 b
6	1.28 ± 0.11 c	3.03 ± 0.21 c	21.11 ± 1.19 c	24.31 ± 1.16 c	8.37 ± 0.46 c
9	1.22 ± 0.09 d	2.96 ± 0.17 d	20.40 ± 1.25 d	24.10 ± 1.10 d	8.31 ± 0.41 d
			tomato juice 4		
0	1.81 ± 0.07 a	2.56 ± 0.05 a	19.14 ± 1.22 a	25.24 ± 0.79 a	9.26 ± 0.18 a
3	1.77 ± 0.11 b	2.49 ± 0.14 b	18.05 ± 1.12 b	24.49 ± 1.01 b	9.15 ± 0.34 b
6	1.61 ± 0.14 c	2.35 ± 0.16 c	17.38 ± 1.10 c	23.91 ± 1.12 c	8.49 ± 0.46 c

Table 1. Continued

months of storage	caffeic acid	caffeic acid-O-hexoside	ferulic acid	ferulic acid-O-hexoside	chlorogenic acid
9	1.55 ± 0.11 d	2.31 ± 0.19 d	17.16 ± 1.09 d	23.11 ± 1.41 d	8.11 ± 0.36 d
			tomato juice 5		
0	2.24 ± 0.05 a	2.83 ± 0.11 a	21.03 ± 1.36 a	19.23 ± 0.64 a	8.79 ± 0.35 a
3	2.19 ± 0.12 b	2.72 ± 0.09 b	20.34 ± 1.24 b	18.32 ± 0.99 b	8.22 ± 0.39 b
6	2.03 ± 0.09 c	2.68 ± 0.23 c	19.07 ± 1.09 c	17.99 ± 1.13 c	7.91 ± 0.44 c
9	1.92 ± 0.13 d	2.59 ± 0.25 d	18.87 ± 1.02 d	17.27 ± 1.08 d	7.65 ± 0.40 d
			tomato juice 6		
0	2.30 ± 0.09 a	2.73 ± 0.12 a	22.11 ± 1.41 a	28.75 ± 0.87 a	10.35 ± 0.12 a
3	2.17 ± 0.14 b	2.53 ± 0.11 b	21.31 ± 1.29 b	27.80 ± 1.08 b	9.86 ± 0.19 b
6	2.01 ± 0.11 c	2.68 ± 0.15 c	19.66 ± 1.38 c	26.31 ± 1.12 c	9.15 ± 0.26 c
9	1.99 ± 0.08 c	2.52 ± 0.19 d	19.14 ± 1.12 d	26.10 ± 1.23 d	8.97 ± 0.33 d

[a] Different letters in the columns represent statistically significant differences ($P < 0.05$).

12% after 9 months of storage. For ketchups, the changes were between 7 and 9%. These results are in line with those reported by Gliszczynska-Swiglo and Tyrakowska.[25] They found that storage of apple juice at room temperature results in a decrease in the TEAC value by about 6−14%.

Effect of Storage on Individual Polyphenols in Ketchups and Tomato Juices. Tables 1 and 2 show polyphenol compounds identified and quantified by HPLC-ESI-MS/MS. Identification was aided by making comparisons with reference standards and the MS^2 fragmentation patterns.[13]

Hydroxycinnamic acids were represented by ferulic and caffeic acids, ferulic acid-*O*-hexoside, and caffeic acid-*O*-hexoside. Hydroxycinnamic acids have become a subject of much interest because they make up a significant proportion of the total phenolics ingested in a normal diet and are readily absorbed in the digestive tract.[21] Although there was a variation between tomato-based products in the content of hydroxycinnamic acids, the trends reflecting the effects of storage were similar.

The content of hydroxycinnamic acids decreased after 9 months of storage at 4 °C. The greatest decrease ($P < 0.05$) occurred in ferulic and caffeic acids in comparison to their glycosidic forms. In ketchups, ferulic and caffeic acids decreased by 9.49 and 12.84%, respectively; in tomato juices, they decreased by 12.03 and 15.18%. In the case of caffeic-*O*-hexoside and ferulic-*O*-hexoside, the 3-hydroxy-function at the C ring of the flavonoid is blocked by a sugar moiety, whereas in the case of caffeic and ferulic acids, it remains unoccupied. Thus, the blockage of the 3-hydroxyl group is perhaps one of the reasons for the greater stability of glycosidic forms toward their aglycones.[26]

Chlorogenic acid was also present in tomato-based products. Chlorogenic acid decreased by 9.10 and 12.32% for ketchups and tomato juices, respectively. Again, ketchups had a greater stability during storage than tomato juices. Chlorogenic acid may be oxidized to reactive *O*-quinones through the catalytic oxidation process. As mentioned by Odriozola et al.,[27] tomato-based products underwent a substantial loss of chlorogenic acid from 4.4 to 3.5−3.8 mg/100 mL FW after 56 days of storage at 4 °C. Peroxidase may be involved in the oxidative degradation of phenolic compounds. Therefore, the degradation of phenolic compounds during storage might be associated with the residual activity of peroxidase.

Tomato and tomato-based products have been found to be rich sources of flavonoids, with rutin and kaempferol as the main flavonols.[16] As Table 2 shows, the initial concentrations of rutin in the studied ketchups were 45.28−64.00 and 42.50−63.33 μg/g FW for tomato juices, whereas kaempferol-3-*O*-rutinoside was found at concentrations of 15.92−24.72 μg/g FW for tomato juices and 16.34−29.88 μg/g FW for ketchups. Rutin decreased by 7.20−10.61% in ketchups, whereas in tomato juices it decreased by 10.28−12.19%. These results are in line with those described previously.[28] During storage, peroxidase may be involved in the oxidative degradation of phenolic compounds that are able to interact with reactive oxygen species, transferring one or two hydrogen atoms, forming quinonoid structures, which leads to the formation of quinones.[29] In the case of rutin, the 3-hydroxy-function at the C ring of the flavonoid is blocked by a sugar moiety, whereas for quercetin it remains unoccupied. Quercetin decreased significantly throughout the storage of tomato-based products, thus reaching values of 0.21−0.51 μg/g FW for ketchups and 0.82−2.15 μg/g FW for tomato juices. Quercetin is a phenolic compound with potent antioxidant properties and, therefore, more easily oxidized. The blockage of the 3-hydroxyl group is perhaps one of the reasons for the greater stability of rutin toward oxidation.[26] Similarly, for kaempferol-3-*O*-rutinoside, the 3-hydroxy function is blocked. This explains the thermal stability of kaempferol-3-*O*-rutinoside toward quercetin. In contrast, other studies reported that glycosylation of the hydroxy substituent on C-3 decreases antioxidant activity.[30] The content of kaempferol in vegetables has not been extensively studied and there are few data about its occurrence in tomatoes. We found higher levels (between 15.92 and 29.88 μg/g FW) than those reported by Martínez-Valverde et al.[21] who reported values of kaempferol between 1.16 and 2.07 μg/g FW.

A slight decrease in naringenin and naringenin-7-*O*-glucoside was observed. Table 2 shows the changes in flavanone concentrations during storage. The small changes in individual and total flavanone content could be explained by the high stability of these compounds. The naringenin content found in ketchups and tomato juices was higher than that reported by Martínez-Valverde et al.,[21] which was 4.50 μg/g FW for raw tomatoes cv. Remate and 12.55 μg/g FW for cv. Daniella, whereas Wu M and Burrell RC[31] found naringenin levels of 40, 95, and 142.5 μg/g FW for the Haubners Vollendung, Ponderosa, and Sunny Ray varieties, respectively. The initial concentrations of naringenin in the studied ketchups were between 34.97 and 54.96 μg/g FW and between 35.97 and 52.96 μg/g FW for tomato juices, whereas naringenin-7-*O*-glucoside was found at concentrations between 1.25 and 3.27 μg/g FW for tomato juices and between 2.22 and 3.41 μg/g FW for ketchups.

Table 2. Changes in the Content of Flavonols and Flavanones (μg/g FW) in Ketchups and Tomato Juices (n = 3) during 9 Months of Storage[a]

months of storage	rutin	quercetin	kaempferol-3-*O*-rutinoside	naringenin	naringenin-7-*O*-glucoside
			ketchup 1		
0	64.00 ± 1.04 a	0.56 ± 0.02 a	29.37 ± 0.82 a	44.30 ± 1.10 a	3.41 ± 0.08 a
3	62.31 ± 3.22 b	0.51 ± 0.04 b	28.91 ± 1.99 b	42.81 ± 2.50 b	3.35 ± 0.20 b
6	59.97 ± 2.56 c	0.47 ± 0.03 c	27.99 ± 1.54 c	41.10 ± 2.23 c	3.21 ± 0.11 c
9	57.21 ± 2.71 d	0.43 ± 0.01 d	26.71 ± 1.63 d	39.50 ± 2.25 d	3.10 ± 0.09 d
			ketchup 2		
0	54.50 ± 1.59 a	0.61 ± 0.01 a	29.88 ± 1.28 a	54.96 ± 1.24 a	3.34 ± 0.13 a
3	53.09 ± 3.21 b	0.57 ± 0.02 b	29.21 ± 1.22 b	53.40 ± 1.99 b	3.29 ± 0.09 b
6	51.45 ± 2.44 c	0.52 ± 0.02 c	28.51 ± 1.43 c	51.50 ± 2.40 c	3.22 ± 0.08 c
9	49.01 ± 2.88 d	0.47 ± 0.03 d	27.11 ± 1.31 d	49.81 ± 2.33 d	3.10 ± 0.12 d
			ketchup 3		
0	60.24 ± 1.33 a	0.67 ± 0.02 a	29.25 ± 0.76 a	40.13 ± 1.25 a	3.23 ± 0.13 a
3	58.01 ± 1.41 b	0.63 ± 0.04 b	28.88 ± 1.99 b	38.94 ± 1.56 b	3.17 ± 0.14 b
6	56.40 ± 3.00 c	0.57 ± 0.03 c	27.91 ± 1.23 c	37.33 ± 1.99 c	3.08 ± 0.16 c
9	54.23 ± 2.41 d	0.51 ± 0.01 d	26.60 ± 1.55 d	36.31 ± 2.31 d	2.97 ± 0.10 d
			ketchup 4		
0	55.84 ± 1.24 a	0.26 ± 0.01 a	16.34 ± 0.52 a	34.97 ± 1.43 a	2.22 ± 0.07 a
3	54.61 ± 2.20 b	0.24 ± 0.02 b	15.49 ± 0.33 b	33.55 ± 1.94 b	2.20 ± 0.09 b
6	52.20 ± 2.12 c	0.22 ± 0.01 c	15.09 ± 0.44 c	32.95 ± 1.67 c	2.11 ± 0.12 c
9	51.00 ± 1.29 d	0.21 ± 0.01 d	14.98 ± 0.31 d	32.01 ± 1.88 d	2.07 ± 0.14 d
			ketchup 5		
0	49.58 ± 1.03 a	0.61 ± 0.01 a	29.08 ± 0.99 a	45.66 ± 1.48 a	2.39 ± 0.09 a
3	49.01 ± 1.89 b	0.57 ± 0.03 b	28.50 ± 1.33 b	45.00 ± 1.99 b	2.32 ± 0.12 b
6	47.11 ± 2.15 c	0.52 ± 0.04 c	27.88 ± 1.12 c	44.01 ± 1.85 c	2.30 ± 0.08 c
9	46.01 ± 2.99 d	0.49 ± 0.03 d	27.09 ± 1.09 d	43.21 ± 1.88 d	2.27 ± 0.09 d
			ketchup 6		
0	45.28 ± 1.21 a	0.63 ± 0.02 a	21.31 ± 0.78 a	37.74 ± 3.23 a	2.70 ± 0.08 a
3	44.99 ± 1.99 b	0.60 ± 0.03 b	21.01 ± 1.96 b	37.01 ± 2.33 b	2.61 ± 0.14 b
6	43.20 ± 2.01 c	0.56 ± 0.02 c	20.35 ± 1.81 c	36.11 ± 2.41 c	2.56 ± 0.13 c
9	41.51 ± 2.67 d	0.51 ± 0.03 d	19.67 ± 1.72 d	35.01 ± 2.20 d	2.52 ± 0.16 d
			tomato juice 1		
0	62.84 ± 2.13 a	1.58 ± 0.04 a	18.54 ± 0.69 a	45.47 ± 1.66 a	1.34 ± 0.05 a
3	60.41 ± 3.01 b	1.48 ± 0.09 b	18.15 ± 0.88 b	43.00 ± 2.33 b	1.29 ± 0.09 b
6	58.97 ± 3.15 c	1.39 ± 0.07 c	17.54 ± 0.76 c	41.51 ± 2.11 c	1.26 ± 0.08 c
9	56.02 ± 2.19 d	1.26 ± 0.06 d	16.99 ± 0.59 d	40.46 ± 2.99 d	1.22 ± 0.05 d
			tomato juice 2		
0	42.50 ± 1.85 a	1.29 ± 0.05 a	18.51 ± 0.82 a	36.14 ± 0.99 a	1.25 ± 0.04 a
3	40.98 ± 1.30 b	1.21 ± 0.04 b	18.14 ± 0.97 b	33.20 ± 1.60 b	1.22 ± 0.07 b
6	39.51 ± 2.09 c	1.15 ± 0.07 c	17.50 ± 0.91 c	32.40 ± 1.78 c	1.18 ± 0.06 c
9	38.11 ± 2.21 d	1.07 ± 0.04 d	17.40 ± 0.85 d	32.10 ± 2.34 d	1.13 ± 0.05 d
			tomato juice 3		
0	61.74 ± 1.93 a	1.67 ± 0.01 a	15.92 ± 0.41 a	35.97 ± 1.26 a	2.41 ± 0.07 a
3	59.87 ± 3.01 b	1.59 ± 0.03 b	15.31 ± 1.01 b	34.45 ± 1.99 b	2.31 ± 0.08 b
6	56.32 ± 2.56 c	1.43 ± 0.02 c	14.98 ± 0.99 c	33.90 ± 2.20 c	2.29 ± 0.06 b
9	55.20 ± 2.99 d	1.28 ± 0.05 d	14.52 ± 0.73 d	32.42 ± 2.00 d	2.23 ± 0.08 c
			tomato juice 4		
0	57.58 ± 2.68 a	2.63 ± 0.04 a	16.47 ± 0.40 a	48.83 ± 1.52 a	3.27 ± 0.11 a
3	55.65 ± 2.09 b	2.46 ± 0.03 b	16.11 ± 0.55 b	46.00 ± 1.60 b	3.12 ± 0.10 b
6	53.07 ± 2.87 c	2.27 ± 0.06 c	15.37 ± 0.60 c	45.51 ± 2.01 c	3.09 ± 0.09 c

Table 2. Continued

months of storage	rutin	quercetin	kaempferol-3-*O*-rutinoside	naringenin	naringenin-7-*O*-glucoside
9	51.03 ± 2.99 d	2.15 ± 0.06 d	15.09 ± 0.72 d	43.56 ± 1.50 d	2.95 ± 0.08 d
			tomato juice 5		
0	50.28 ± 2.39 a	1.05 ± 0.03 a	20.97 ± 0.85 a	38.24 ± 1.04 a	2.26 ± 0.06 a
3	48.78 ± 2.12 b	0.97 ± 0.04 b	20.01 ± 0.80 b	37.11 ± 1.23 b	2.19 ± 0.05 b
6	46.20 ± 2.09 c	0.86 ± 0.02 c	19.33 ± 0.76 c	36.89 ± 1.26 c	2.11 ± 0.07 c
9	45.11 ± 1.85 d	0.81 ± 0.01 d	19.15 ± 0.82 d	34.68 ± 1.94 d	2.06 ± 0.06 d
			tomato juice 6		
0	63.33 ± 2.43 a	1.59 ± 0.04 a	24.72 ± 0.26 a	52.96 ± 2.23 a	2.34 ± 0.08 a
3	59.66 ± 2.01 b	1.33 ± 0.03 b	23.30 ± 0.85 b	49.55 ± 1.65 b	2.19 ± 0.09 b
6	57.21 ± 2.48 c	1.29 ± 0.02 c	22.50 ± 0.96 c	48.12 ± 1.87 c	2.15 ± 0.11 c
9	55.61 ± 2.66 d	1.22 ± 0.05 d	22.31 ± 0.68 d	47.11 ± 2.00 d	2.11 ± 0.15 d

[a] Different letters in the columns represent statistically significant differences ($P < 0.05$).

Naringenin slightly decreased throughout the storage of tomato-based products. The naringenin content in ketchups decreased by 5.37−10.84% and in tomato juices by 9.31−12.18%. For naringenin-7-*O*-glucoside, the trends reflecting the effects of storage were similar to naringenin.

To our knowledge, this is the first time a study has been carried out to evaluate the stability of flavonols, flavanones, and hydroxycinnamic acids in ketchups and tomato juices during storage. Storage of tomato juices and ketchups results in a slight decrease in their polyphenol content and also in the hydrophilic antioxidant capacity, but the levels achieved would not represent a nutritional drawback. This information is of interest to consumers and nutritionists: They can expect a beneficial effect from the consumption of tomato juices and ketchups throughout storage.

The main degradation was in quercetin oxidation, whereas in the case of rutin, the 3-hydroxy function at the C ring of the flavonoid is blocked by a sugar moiety. An important fact to note is that during the storage of individual polyphenols, the glycosides of flavonols and hydroxycinnamic acids are more stable to oxidation than aglycones, probably due to the blockage of the 3-hydroxyl group at the C ring.

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ACKNOWLEDGMENT

The authors would like to express their gratitude for financial support from CICYT (AGL2010-22319-C03) and RETICS RD06/0045/0003 from the Spanish Ministry of Science and Innovation (MICINN), Spain. The CIBERobn CB06/03 is an initiative of the Instituto de Salud Carlos III, Spain. This work has been funded in part by MICINN. A. V-Q received support from MICINN and S. A. from the Sara Borrell postdoctoral program with reference CD10/00151 supported by the Institute of Health Carlos III.

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