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Nutritional characterisation of commercial traditional pasteurised tomato juices: carotenoids, vitamin C and radical-scavenging capacity

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Abstract

Commercial conventional thermal pasteurised tomato juices were nutritionally evaluated, at the same point in their commercial shelf lives, for their carotenoid contents, vitamin C, and antioxidant activities and physical and physicochemical characterisation. Higher lycopene epoxide, lycopene, γ-carotene, β-carotene contents, and vitamin A values were found in those juices obtained from a concentrated tomato source, suggesting structural changes in the tomato tissue due to the concentration process. The stability of vitamin C seems to be affected by the type of container. Radical-scavenging capacity, measured on the basis of the DPPH· stable radical, was higher in the aqueous (AQ) fractions than in the organic (OR) fractions of tomato juices. Results suggest that vitamin C was mainly responsible for the DPPH· radical-scavenging of the AQ tomato fractions, whereas lutein and lycopene were the individual carotenoids responsible, not only for the EC_{50}, but also the kinetic changes of the DPPH· of the OR tomato fractions. These results extend the research in processed tomato products to other bioactive components, different from the most generally studied lycopene.
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Keywords: Processed tomato juice; Thermal pasteurisation; Colour; Bioactive compounds; Carotenoids; Vitamin C; Radical-scavenging capacity

1. Introduction

Reactive oxygen species (ROS) could be important causative agents of a number of human diseases, including coronary heart disease and cancer. It has been suggested that a high intake of fruits and vegetables, the main sources of antioxidants in the diet, could decrease the potential stress caused by ROS (Pellegrini, Riso, & Porrini, 2000). Tomato (*Lycopersicon esculentum* Mill.) is one of the world's major food crops (Frusciante et al., 2000). Specifically, tomatoes are the second highest produced and consumed vegetable in the USA today (Willcox, Catignani, & Lazarus, 2003). Besides, this fruit has remarkably high concentrations of vitamin C and carotenoids, such as lycopene and the provitamin A β-carotene (Beecher, 1998). Consequently, tomatoes and tomato-based foods may provide a convenient matrix by which nutrients and other health-related food components can be supplied to humans. In the USA diet, tomato is the most important source of the carotene lycopene (Clinton, 1998), the third leading contributor of vitamin C and the fourth leading source of vitamin A (Willcox et al., 2003). Accordingly, protective activity of tomato products on in vivo markers of lipid oxidation (LDL oxizability, and 8-iso-$PFG_{2\alpha}$) have been reported (Visioli, Riso, Grande, Galli, & Porrini, 2003). Moreover, the consumption of tomato products has been associated with a lower risk of developing digestive tract and prostate cancers (Giovannucci, Rimm, Liu, Stamp-

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fer, & Willett, 2002). These protective effects of tomato products may be due to the ability of lycopene and other antioxidant components to prevent cell damage through synergistic interactions (Friedman, 2002; George, Kaur, Khurdiya, & Kapoor, 2004).

Tomatoes are consumed, either as fresh or as industrially processed products. Processed tomato products include canned and sun-dried tomatoes, juices, ketchup, pastes, purees, salads, sauces and soups (Shi & Le Maguer, 2000). In the USA, more than 80% of the tomato annual consumption is consumed in the form of processed products (Willcox et al., 2003). In contrast, knowledge about the activity of antioxidants of commercial processed tomato products is scarce, and is generally limited to the lycopene content (Anese, Falcone, Fogliano, Nicoli, & Massini, 2002; Shi & Le Maguer, 2000; Takeoka et al., 2001; Yaping et al., 2002).

One major characteristic of antioxidant vitamins, carotenoids and polyphenols is their antioxidant potential, either in lipophilic or hydrophilic compartments (Arnao, Cano, & Acosta, 2001). Thus, selection of a suitable extraction procedure using solvents with different polarities may allow the evaluation of different potential antioxidants (Schwarz et al., 2001). From the methodological point of view, the widespread use of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) radical-scavenging model is recommended as fast, easy and accurate, for measuring the antioxidant activity of plant foods (Da Porto, Calligaris, Celotti, & Nicoli, 2000; Espín, Soler-Rivas, Wichers, & García-Viguera, 2000; Pinelo, Rubilar, Sineiro, & Núñez, 2004). The protocol used by our group (Sánchez-Moreno, Plaza, De Ancos, & Cano, 2003a, 2003b) takes into account, not only the antioxidant concentration, but also the reaction time to reach the plateau of the scavenging reaction. This feature could be an advantage over other methods using only the antioxidant concentration (Martínez-Valverde, Periago, Provan, & Chesson, 2002; Sánchez-Moreno, 2002).

Therefore, the aim of this work was to evaluate the bioactive antioxidant components, carotenoids and vitamin C, and the radical-scavenging capacity, measured using solvents of different polarities, of traditional pasteurised commercial tomato juices. This work could clarify the role, in tomato products, of antioxidants other than the extensively studied lycopene.

2. Materials and methods

2.1. Chemicals

Anhydrous disodium hydrogen phosphate, L(+)-ascorbic acid, *meta*-phosphoric acid and sodium dihydrogen phosphate monohydrate were purchased from Merck (Darmstadt, Germany). Butylated hydroxytoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), DL-dithiothreitol, lycopene, and *trans*-β-apo-8′-carotenal were obtained from Sigma Chemical Co (St. Louis, MO, USA). Anhydrous sodium sulphate, citric acid monohydrate, magnesium hydroxide carbonate 5-hydrate, sodium chloride (NaCl) and sodium hydroxide (NaOH) (0.1 N) were purchased from Panreac Química (Barcelona, Spain). Hoffman-La Roche (Basel, Switzerland) kindly provided β-carotene and lutein. Acetic acid was obtained from Hopkin & Williams (Essex, UK). Acetonitrile, dichloromethane, diethyl ether, methanol, *n*-hexane 95%, and tetrahydrofuran (THF) were obtained from Labscan Ltd (Dublin, Ireland).

2.2. Tomato juice samples

Freshly made tomato juice was obtained from tomatoes (*Lycopersicon esculentum* Mill. cv. Daniella (Solanaceae)) (Almería, Spain) by homogenisation of the whole fruit (flesh and peel) using a blender (Osterizer, NC, USA). Based on previous work (Plaza, Muñoz, De Ancos, & Cano, 2003), we added 2% citric acid and 0.6% sodium chloride to obtain optimal sensorial and microbiological characteristics. The freshly made tomato juice was coded as A. Additionally, six different brands of commercial tomato juices were purchased in Spanish supermarkets and were coded as B, C, D, E, F and G. Three samples of each brand, from different commercial batches, were bought. All juices were produced by traditional pasteurisation with a shelf life at room temperature up to 6 months, and had sodium chloride added as declared on the labels by the producer. All samples were analysed at the same point in their commercial shelf life.

2.3. Physical and physicochemical assays

2.3.1. pH and titratable acidity

Tomato juice (10 g) was blended with 20 ml of deionised water in an ultrahomogeniser (Omni mixer, model ES-207, Omni International Inc, Gainesville, VA, USA). The mixture was heated to 100 °C, then 20 ml of deionised water were added and the resulting mixture was cooled to 20 °C. pH was measured at this temperature with a pH meter (Microph 2000, Crison, Barcelona, Spain). After determination of pH, the solution was titrated with 0.1 N NaOH to pH 8.1 and the results were expressed as percentage of citric acid (g of citric acid per 100 g fresh weight (fw)).

2.3.2. Soluble solids

Soluble solids of tomato juice were determined using a digital refractometer (ATAGO, Tokyo, Japan) at 20 °C and results were reported as degrees Brix.

2.3.3. Total solids

The AOAC method (AOAC, 1984) was modified as described previously (Cano, Marín, & Fuster, 1990). Total solids were measured with a microwave oven operating at 200 W for 20 min by drying to constant weight and results were expressed as g of dry weight (dw) per 100 g fw.

2.3.4. Viscosity

Viscosity was measured on approximately 35 ml of tomato juice with a model DV-II viscometer (Brookfield Engineering Laboratories, Inc, Stoughton, MA, USA). Viscosity was expressed as centipoise (cP).

2.3.5. Colour measurements

The colour of tomato juices was measured using a tristimulus reflectance colorimeter (HunterLab, model D25 A9, Hunter Associates Laboratory, Inc., Reston, VA, USA) calibrated with a white standard tile ($X = 82.51$; $Y = 84.53$; $Z = 101.23$). Samples were placed in Petri dishes and filled to the top and colour was recorded using the CIE*Lab* uniform colour space. L^* (lightness), a^* (green-red tonality) and b^* (blue-yellow tonality) values were recorded and results were expressed as: hue angle $h = \tan^{-1}(b^*/a^*)$ and saturation (or chroma) $C = (a^{*2} + b^{*2})^{1/2}$.

2.4. Extraction, separation, identification and quantification of carotenoids

The extraction was carried out according to previous methods with minor modifications (Sánchez-Moreno, Plaza, De Ancos, & Cano, 2003c). Triplicates of each sample (50 g) were extracted with 50 ml of THF stabilised with BHT (0.01%) until the extracts were colourless. One ml of a solution of β-apo-8′-carotenal (5 mg/ml) as internal standard, was added. The combined THF extracts were concentrated to 10 ml in a rotatory evaporator at 35 °C, and partitioned between diethyl ether (150 ml) and salt water (25 ml) and transferred to a separating funnel. The organic and aqueous layers were separated. The organic layer (upper layer) was washed with water to remove water-soluble materials. The water layers were combined and washed with diethyl ether until the diethyl ether extract was colourless. The organic layers were combined and dried over anhydrous sodium sulphate. The ethereal solution was evaporated to dryness and the residue dissolved in 2 ml of dichloromethane. All steps were performed under diminished light. Samples were filtered through a 0.45-μm membrane filter and duplicates of 20 μl for each extract were analysed by HPLC.

HPLC analysis was based on a method described elsewhere (Takeoka et al., 2001). The HPLC system consisted of a Hewlett–Packard (Palo Alto, CA, USA) Model 1050 quaternary solvent delivery pump equipped with a manual injector (Rheodyne) with a 20-μl sample loop and a Hewlett–Packard 1040A rapid scanning UV–Visible photodiode array detector. Separation of carotenoids was performed on a C_{18} Hypersil ODS stainless steel column (250 × 4.6 mm i.d.; 5 μm particle size) (Technochroma, Barcelona, Spain). The solvent system used was a gradient of acetonitrile (solution A), methanol (solution B), dichloromethane (solution C) and hexane (solution D). The programme began with an isocratic gradient of 85% A, 10% B, 2.5% C and 2.5% D for 10 min, followed by a linear gradient to 45% A, 10% B, 22.5% C and 22.5% D for the next 30 min. The flow rate was 0.8 ml/min and the runs were monitored with the UV–Visible photodiode array detector at 470 nm. The data were stored and processed using a Hewlett–Packard (Palo Alto, CA, USA) ChemStation and related software. Identification of the carotenoids was acheived by HPLC, comparing the retention time and UV–Visible absorption spectrum with those of the standards previously referred to and quantification was achieved according to the procedure described previously (Hart & Scott, 1995). Total carotenoid content was expressed as μg of lutein plus μg of lycopene epoxide plus μg of lycopene plus μg of γ-carotene plus μg of β-carotene. Results were calculated as μg of the corresponding carotenoid per 100 ml of tomato juice.

Vitamin A value was calculated as retinol activity equivalents (RAE) per 100 ml of tomato juice, according to the equation (Institute of Medicine, 2001): RAE = [μg of β-carotene/12] + [μg of other provitamin A carotenoids (γ-carotene)/24].

2.5. Determination of vitamin C

Ascorbic acid and total vitamin C (ascorbic acid + dehydroascorbic acid) were determined by HPLC as described previously (Sánchez-Moreno et al., 2003c). The procedure employed to determine total vitamin C was the reducing of dehydroascorbic acid to ascorbic acid, using DL-dithiothreitol as reductant reagent, according to a modification of the procedure of other authors (Sánchez-Mata, Cámara-Hurtado, Díez-Marqués, & Torija-Isasa, 2000). Forty ml of tomato juice were homogenised with 25 ml of extraction solution (3% *meta*-phosphoric acid + 8% acetic acid). The resulting mixture was centrifuged, filtered and adjusted up to 75 ml with distilled water. Samples were filtered through a 0.45-μm membrane filter and duplicates of 20 μl for each extract were analysed by HPLC. Results were expressed as mg of ascorbic acid per 100 ml of tomato juice.

An aliquot (0.5 ml) of the mixture was taken to react with 1.5 ml of a solution 20 mg/ml of DL-dithiothreitol for 2 h at room temperature and darkness. During this

time, the reduction of dehydroascorbic acid to ascorbic acid took place. Samples were filtered through a 0.45-μm membrane filter and duplicates of 20 μl, for each extract, were analysed by HPLC. Results were expressed as mg of total vitamin C per 100 ml of tomato juice.

2.6. Scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical

2.6.1. Extraction

Two fractions [aqueous (AQ) and organic (OR)] were prepared from tomato juices and used in the antioxidant assay. Triplicates of each sample (30 g) were extracted with 10 ml of a sodium phosphate buffer solution (0.1 M, pH 3.0) and centrifuged at 12,000*g* for 20 min at 4 °C. The pellet was homogenised with 20 ml of a sodium phosphate buffer solution (0.1 M, pH 7.4) and centrifuged at 10,000*g* for 15 min at 4 °C. Supernatants were combined to yield an AQ fraction. The pellet was then thrice extracted with 20 ml of THF and centrifuged at 10,000*g* for 10 min at 4 °C. Supernatants were combined to yield an OR fraction. The solvent was evaporated to dryness and the organic residue was dissolved in 3 ml of a Tween 20 solution (10% THF).

2.6.2. DPPH· radical-scavenging capacity (aqueous and organic fractions)

The determination of the radical-scavenging capacity was done using the stable radical, DPPH·. The method is described extensively elsewhere (Sánchez-Moreno et al., 2003a, 2003b). The parameters EC_{50}, which reflects 50% depletion of the initial DPPH· and the time needed to reach the steady state at EC_{50} concentration (T_{EC50}) were calculated. The antiradical efficiency ($AE = 1/EC_{50} \times T_{EC50}$), a parameter that combines both factors, was also calculated.

2.7. Statistical analysis

Results were collected as means ± SD of six independent determinations. One-way analysis of variance (ANOVA) was used to compare the means. Differences were considered significant at $P < 0.05$. All statistical analyses were performed with Statgraphics Plus 2.1 (Statistical Graphics Corporation, Inc., Rockville, MD, USA).

3. Results and discussion

3.1. Physical and physicochemical characterisation

The physical and physicochemical parameters of the commercial traditional pasteurised tomato juices B–G, along with the freshly made tomato juice A studied in this work, are shown in Table 1. These quality parame-

Table 1
Physical and physicochemical characteristics of freshly made (A) and commercial traditional pasteurised (B–G) tomato juices[a]

Sample	pH	Soluble solids (°Brix at 20 °C)	Total solids (g dw/100 g fw)	Titratable acidity (g citric acid/100 g fw)	Viscosity (cP)	Colour				
						*L**	*a**	*b**	*h*	*C*
A	2.73 ± 0.01a	7.4 ± 0.2d	8.31 ± 0.14f	2.16 ± 0.01f	540.03 ± 14.22a	26.32 ± 0.44c	16.89 ± 0.53a	17.93 ± 1.32b	46.71 ± 0.13c	24.61 ± 1.34a
B	4.15 ± 0.06d	5.2 ± 0.1a	5.28 ± 0.07a	0.35 ± 0.01b	1415.42 ± 85.42d	26.11 ± 0.26bc	18.25 ± 0.36c	20.17 ± 0.26c	47.86 ± 0.20c	27.20 ± 0.44bc
C	4.37 ± 0.01f	5.9 ± 0.1b	6.17 ± 0.03d	0.37 ± 0.00c	1065.83 ± 9.38b	24.64 ± 0.14a	17.65 ± 0.12bc	21.58 ± 0.56d	50.72 ± 0.60e	27.88 ± 0.49c
D	3.83 ± 0.05b	5.2 ± 0.1a	5.66 ± 0.03b	0.50 ± 0.01e	1436.25 ± 42.33d	25.56 ± 0.17b	17.02 ± 0.19b	19.65 ± 0.37c	49.11 ± 0.24d	26.00 ± 0.40b
E	4.39 ± 0.09f	5.7 ± 0.2b	5.80 ± 0.01c	0.35 ± 0.00b	1862.50 ± 36.42e	27.14 ± 0.22d	19.97 ± 0.10d	22.07 ± 0.29d	47.86 ± 0.39c	29.77 ± 0.23d
F	4.04 ± 0.02c	5.3 ± 0.2a	5.36 ± 0.04a	0.42 ± 0.00d	1209.58 ± 12.14c	29.54 ± 0.09e	29.49 ± 0.18e	16.20 ± 0.12ab	28.77 ± 0.32b	33.65 ± 0.10e
G	4.24 ± 0.01e	6.8 ± 0.1c	6.80 ± 0.11e	0.29 ± 0.00a	1421.67 ± 15.63d	27.40 ± 0.57d	30.93 ± 0.73f	15.70 ± 0.56a	26.92 ± 0.92a	34.70 ± 0.73e

[a] Values are means ± SD, $n = 6$. Means within a column with different letters are significantly different at $P < 0.05$. dw = dry weight; fw, fresh weight.

ters are related to the stability of bioactive compounds in plant-derived products.

The freshly made tomato juice had the lowest CIE a^* (16.89 ± 0.53) and C (24.61 ± 1.34) parameters, indicating the lowest green-red tonality among the tomato juices assayed. In general, the commercial tomato juices assayed had CIE*Lab* parameters in the normal range of these types of samples (Anese et al., 2002).

The freshly made tomato juice A obtained in this work had the lowest pH (2.73 ± 0.01), supported by the highest titratable acidity (2.16 ± 0.01 g citric acid/100 g fw), as expected for the addition of citric acid for microbiological reasons. The low acidity in commercial juices could be explained on the basis of the technological treatment carried out by the manufacturers, aimed to achieve low acidity-tomato juices, which have a better consumer acceptance. Manufacturers add sugar to offset acid taste and get a sweeter product, and this was shown on some of the labels of the commercial tomato juices bought for this study. Soluble solids and total solids in the commercial juices B–G studied ranged from 5.2° to 6.8° Brix, and 5.28–6.80 g dw/100 g fw, respectively. The freshly made tomato juice A had the highest soluble solids content ($7.4 \pm 0.2°$ Brix) and total solids (8.31 ± 0.14 g dw/100 g fw) among the tomato juices assayed. Viscosity ranged from 1065.83 to 1862.50 cP among the commercial juices studied B–G; however, in the freshly made juice A the viscosity was 540.03 ± 14.22 cP. Therefore, the freshly made tomato juice presented physical and physicochemical characteristics different from than the commercial traditional pasteurised tomato juices.

3.2. Carotenoid content and vitamin A value

In the commercial juices B–G tested, total carotenoid content averaged 3201 μg/100 ml (2480–4090 μg/100 ml) (Table 2). Lycopene was the main carotenoid found, averaging 2643 μg/100 ml (2129–3435 μg/100 ml). Neither the total carotenoid content (1524 μg/100 ml), nor the lycopene content (1024 μg/100 ml) of the freshly made tomato juice A, assayed for comparative purposes, were included in the respective ranges.

Vitamin A is provided from plant foods as carotenoids that can be biologically transformed to active vitamin A (Rodríguez-Amaya, 1996). In the commercial tomato juices B–G, the provitamin A β-carotene, and the vitamin A value, ranged from 182 to 321μg/100 ml and 17.6–31.5 RAE/100 ml, respectively. In the freshly made tomato juice A these values were slightly higher (369 μg/100 ml and 32.8 RAE/100 ml, respectively).

The different contents of carotenes in the commercial juices and the freshly made tomato juice could be explained in terms of the thermal treatment (pasteurisation) carried out in the commercial tomato juices according to the manufacturer label description. The juice pasteurisation process implies a thermal treatment at 90 °C for 1 min (Mermelstein, 1999). As described in the literature, there is a positive effect of the temperature on the extractability of lycopene (Anese et al., 2002; Porrini, Riso, & Testolin, 1998). Besides, lycopene in tomato is relatively resistant to degradation (Abushita, Daood, & Biacs, 2000; Nguyen & Schwartz, 1998), whereas other antioxidants (ascorbic acid, tocopherol, and β-carotene) decrease as a function of thermal processing (Abushita et al., 2000). Interestingly, juice G, which was enriched in vitamin C according to the label description, showed the highest lycopene content among the juices tested. Whether these antioxidants, or other components that are present in tomato products, play a role in preventing the degradation of lycopene has to be elucidated (Takeoka et al., 2001).

Another interesting finding was the lower lycopene epoxide, lycopene, γ-carotene, and β-carotene contents, and vitamin A value in the juices B and F compared with the rest of the commercial samples assayed. This could be explained in terms of the origin of the tomato juice in the commercial samples. According to the manufacturer label description all the juices came from concentrate except samples B and F. It is reported that the concentration process of tomato suspensions lead to a decrease in diameter of the tomato particles. Moreover, the microfibrillar network of the cellulosic cell wall is also thought to be affected by the concentration process in tomatoes (Den Ouden & Van Vliet, 2002). In this sense, it is known that the intactness of the food matrix,

Table 2
Carotenoid contents (μg/100 ml) and vitamin A values (RAE/100 ml) of freshly made (A) and commercial traditional pasteurised (B–G) tomato juices[a]

Sample	Lutein	Lycopene epoxide	Lycopene	γ-Carotene	β-Carotene	Total carotenoids	Vitamin A
A	29.4 ± 4.31a	53.2 ± 5.02a	1024 ± 98.3a	48.3 ± 4.23a	369 ± 20.0d	1524 ± 160.3a	32.8 ± 3.02d
B	33.6 ± 1.96a	78.7 ± 4.30a	2129 ± 270b	56.4 ± 5.95a	182 ± 8.79a	2480 ± 272b	17.6 ± 0.72a
C	70.3 ± 5.29cd	136 ± 19.4c	2449 ± 284bc	103 ± 16.1cd	265 ± 40.0b	3023 ± 221c	26.4 ± 3.94bc
D	51.2 ± 7.98b	183 ± 23.0d	2930 ± 297d	109 ± 16.0d	314 ± 38.4bc	3587 ± 309d	30.7 ± 3.19cd
E	61.3 ± 4.17c	133 ± 22.4bc	2544 ± 260c	101 ± 9.00cd	290 ± 40.2bc	3129 ± 187c	28.4 ± 3.69bcd
F	70.6 ± 10.09d	108 ± 21.93b	2369 ± 189bc	85.6 ± 7.49bc	265 ± 25.5b	2899 ± 249c	25.7 ± 2.39b
G	64.2 ± 4.49cd	155 ± 12.8c	3435 ± 226e	115 ± 5.47d	321 ± 14.4c	4090 ± 232e	31.5 ± 1.42d

[a] Values are means ± SD, $n = 6$. Means within a column with different letters are significantly different at $P < 0.05$.

in which carotenoids are incorporated, may be a determinant of carotenoid bioavailability in tomatoes (Van het Hof, West, Weststrate, & Hautvast, 2000). Thus, the carotenoid extraction of the commercial juices C, D, E, and G could be favoured by the concentration process. Specific carotenoid extraction research, dealing with concentrate tomato suspensions, should be done to clarify this interpretation.

Relationships among carotenoids and colour parameters were sought. In our work, hue value was shown to be related to lycopene ($r = -0.4012$, $P = 0.0714$), lutein ($r = -0.4205$, $P = 0.0577$) and total carotenoid ($r = -0.4005$, $P = 0.0720$) content, including all the tomato juices tested. It is reported that the hue value of tomato juice is a good indicator of lycopene content (Thompson et al., 2000). Also, *C*, which represents colour intensity (brightness), was shown to be related to lycopene ($r = 0.7005$, $P = 0.0004$), γ-carotene ($r = 0.5667$, $P = 0.0074$), lutein ($r = 0.7359$, $P = 0.0001$), and total carotenoid ($r = 0.6861$, $P = 0.0006$) content. We also found a positive correlation between CIE a^* (green-red tonality) and lutein ($r = 0.5662$, $P = 0.0104$), and total carotenoid ($r = 0.6861$, $P = 0.0006$) content.

3.3. Vitamin C content

In the commercial tomato juices B–G tested, ascorbic acid content ranged from 7.65 to 59.4 mg/100 ml and total vitamin C content from 9.19 to 67.6 mg/100 ml (Table 3). The values of the freshly made tomato juice A, assayed for comparative purposes, were included in the corresponding range (Table 3). The extreme values in both ranges, which correspond to juices C and G, are unusual for tomato juices. However, the mean value of ascorbic acid and vitamin C content (17.9 ± 4.31 mg/100 ml and 20.0 ± 4.19 mg/100 ml, respectively) found for the other five analysed juices accorded with that described in the literature (Davey et al., 2000). It is known that packaging material influences the quality of liquid foods during storage. Specifically, permeating through packaging material is suggested to minimise detrimental effects on the retention of vitamin C (Ayhan, Yeom, Zhang, & Min, 2001). Juice C showed the lowest values of ascorbic acid and total vitamin C. This sample was the only one among the tested commercial samples which was packaged in a non-protected from light glass container, whereas the other commercial samples were packed with Tetrabrik®. This packaging material has specially designed multi-layered oxygen and light barriers to protect loss of vitamin C and flavour, and to enhance shelf life. Vitamin C is a reactive compound and it is particularity vulnerable to storage conditions (Davey et al., 2000). In our work, all commercial juices were analysed at the same point in their commercial shelf life. Thus, juice C packaging might lead to a depletion of vitamin C. Consistently, it is reported that glass-packed orange juice shows poor retention of vitamin C, losing 10% after 4 months of storage (The Ultimate citrus, 2004). On the other hand, juice G, which was enriched in vitamin C according to the label description, showed the highest ascorbic acid and vitamin C values among commercial juices tested.

Table 3
Vitamin C content (mg/100 ml) of freshly made (A) and commercial traditionally pasteurised (B–G) tomato juices[a]

Sample	Ascorbic acid	Total vitamin C
A	13.3 ± 0.13b	16.5 ± 0.81b
B	23.6 ± 1.03f	25.4 ± 1.74d
C	7.65 ± 0.15a	9.19 ± 0.52a
D	14.4 ± 0.47c	15.7 ± 0.36b
E	20.8 ± 0.22e	23.1 ± 0.65d
F	17.3 ± 0.40d	19.3 ± 0.60c
G	59.4 ± 0.71g	67.6 ± 4.55e

[a] Values are means ± SD, $n = 6$. Means within a column with different letters are significantly different at $P < 0.05$.

3.4. DPPH$^\cdot$ stable radical-scavenging

The radical-scavenging capacities of different commercially processed tomato juices were evaluated. To assess the antioxidant capacities of tomato juice constituents according to their different polarities, we extracted juices successively with phosphate buffer and THF. The results showed that the type of extraction solvent (AQ or OR) used in the antioxidant assays strongly influence the activity of tomato juices.

3.4.1. DPPH$^\cdot$ stable radical-scavenging of the aqueous fractions

In the commercial tomato juices tested, B–G, EC_{50AQ} ranged from 73.0 to 81.8 g/g DPPH$^\cdot$ and T_{EC50AQ} from 7.99 to 17.3 min (Table 4). The AE_{AQ} parameter, which includes both parameters, ranged from 7.4×10^{-4} to 1.73×10^{-3} in the commercial juices. The freshly made tomato juice A was not included in the previous range. We do not exclude the possible role of oxidative enzymes, such as ascorbate oxidase and polyphenol oxidase, in affecting the antioxidant capacity of the freshly made tomato juice. Vitamin C and polyphenols (flavonoids and hydroxycinnamic acids) are reported to be the major hydrosoluble antioxidant components in tomato (Martínez-Valverde et al., 2002; Takeoka et al., 2001). In the commercial tomato juices assayed, significant inverse correlations between the EC_{50AQ} parameter and ascorbic acid ($r = -0.8510$, $P = 0.0316$), and total vitamin C ($r = -0.8453$, $P = 0.0340$) content were found, indicating an important role of vitamin C in the antioxidant capacity of the AQ tomato juice fractions. An interesting finding of this study, was the faster kinetics in sample G (7.99 ± 0.57

Table 4
Radical-scavenging parameters of freshly made (A) and commercial traditionally pasteurised (B–G) tomato juices (aqueous fraction (AQ))[a]

Sample	EC_{50AQ} (g dw/g DPPH·)	T_{EC50AQ} (min)	AE_{AQ}[b]
A	64.3 ± 0.45a	21.2 ± 1.55b	0.00074 ± 0.00005a
B	75.8 ± 3.77ab	11.8 ± 2.60a	0.00115 ± 0.00031ab
C	80.2 ± 8.47b	17.3 ± 5.01b	0.00074 ± 0.00014a
D	81.8 ± 4.91b	11.0 ± 1.13a	0.00112 ± 0.00018ab
E	76.5 ± 4.55ab	10.3 ± 0.12a	0.00127 ± 0.00006b
F	79.9 ± 6.41b	10.3 ± 0.31a	0.00123 ± 0.00014b
G	73.0 ± 4.24ab	7.99 ± 0.57a	0.00173 ± 0.00022c

[a] Values are means ± SD, $n = 6$. Means within a column with different letters are significantly different at $P < 0.05$. dw, dry weight.
[b] Expressed as 1/[EC_{50} (g dw/g DPPH·) T_{EC50} (min)].

min), which was enriched in vitamin C, compared with the rest of the AQ tomato juice fractions analysed. This was in agreement with the fast kinetic reported for the ascorbic acid compared with other hydrosoluble antioxidant compounds (Sánchez-Moreno et al., 2003b). In addition, a significant positive correlation was found between the AE_{AQ} parameter and ascorbic acid ($r = 0.8829$, $P = 0.0084$), and total vitamin C ($r = 0.8665$, $P = 0.0116$) content, among all samples analysed.

3.4.2. *DPPH· stable radical-scavenging of the organic fractions*

In the commercial tomato juices, B-G tested, EC_{50OR} ranged from 226 to 295 g/g DPPH· and T_{EC50OR} from 25.2 to 33.7 min (Table 5). The AE_{OR} parameter, which includes both former parameters, ranged from 1.02×10^{-4} to 1.62×10^{-4} in the commercial juices. The freshly made tomato juice A was not included in the previous ranges. Vitamin E and carotenoids mainly constitute the lipophilic fraction in tomato products (Martínez-Valverde et al., 2002; Takeoka et al., 2001). The higher AE_{OR} of commercial tomato juices, compared with the freshly made juice, could be explained on the basis of their higher total carotenoid contents, which may be a consequence of the thermal treatment carried out by the manufacturer for the pasteurisation. Among all samples analysed, a significant inverse correlation was found between the EC_{50OR} parameter and lycopene ($r = -0.7728$, $P = 0.0416$), lutein ($r = -0.4512$, $P = 0.0401$), and total carotenoid ($r = -0.5733$, $P = 0.0066$) contents. Moreover, positive correlations between the AE_{OR} parameter and lycopene ($r = 0.7916$, $P = 0.0339$), lutein ($r = 0.5439$, $P = 0.0108$) and total carotenoid ($r = 0.7469$, $P = 0.0537$) contents were also found. This suggests that the xanthophyll, lutein, and the carotene, lycopene, were the individual carotenoids responsible, not only for the EC_{50} value, but also for the kinetic changes of the DPPH· stable radical of the OR tomato juice fractions assayed.

Table 5
Radical-scavenging parameters of freshly made (A) and commercial traditionally pasteurised (B–G) tomato juices (organic fraction (OR))[a]

Sample	EC_{50OR} (g dw/g DPPH·)	T_{EC50OR} (min)	AE_{OR}[b]
A	462 ± 63.5b	41.1 ± 0.72c	0.000053 ± 0.000006a
B	241 ± 53.0a	31.1 ± 1.51ab	0.000136 ± 0.000024bc
C	276 ± 7.47a	33.5 ± 3.08b	0.000109 ± 0.000013b
D	262 ± 23.4a	28.8 ± 1.05ab	0.000133 ± 0.000007bc
E	295 ± 35.0a	33.7 ± 2.52b	0.000102 ± 0.000020b
F	226 ± 14.9a	28.2 ± 3.30ab	0.000158 ± 0.000008c
G	249 ± 22.4a	25.2 ± 4.83a	0.000162 ± 0.000017c

[a] Values are means ± SD, $n = 6$. Means within a column with different letters are significantly different at $P < 0.05$. dw, dry weight.
[b] Expressed as 1/[EC_{50} (g dw/g DPPH·) T_{EC50} (min)].

4. Conclusion

Radical-scavenging capacity was higher in the AQ fractions than in the OR fractions of tomato juices. Vitamin C was mainly responsible for the radical-scavenging capacity of the AQ tomato juice fractions. Among others, carotenoids, lutein and lycopene, were responsible, not only for the EC_{50}, but also the kinetics of the DPPH· changes of OR tomato juice fractions.

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