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HPLC method and Antioxidant activity for bioactive component determination of Lycopersicon esculentum Mill. varieties from a coastal area of southern Spain

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Abstract:	The tomato (Lycopersicon esculentum Mill.) is one of the most widely consumed vegetables, and is a component of the so-called "Mediterranean diet." It represents a significant source of antioxidants in the human diet, with an important biological function (lycopene and β-carotene), as well as other components with antioxidant properties. Antioxidant contents differ according to the variety of tomato, and environmental and agronomic conditions of growth. The aim is to optimize an HPLC method for the determination of bioactive compounds, and to use different antioxidant tests to determine free-radical scavenging activity. The analytical parameters, recovery assays higher than 97%, satisfactory precision with R.S.D values below 8%, good linearity ($r > 0.999$), good sensitivity and appropriate limits of detection and quantification show that the technique used is satisfactory for measuring these compounds. The tomato samples examined contain highly bioactive components, and have high antioxidant capacity with high correlation between phenolic compound contents and antioxidant activity.

HPLC method and Antioxidant activityfor bioactive component determination of *Lycopersicon esculentum Mill.*varieties from a coastal area of southern Spain

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Abstract

The tomato (*Lycopersicon esculentum Mill*.) is one of the most widely consumed vegetables, and is a component of the so-called "Mediterranean diet."It represents a significant source of antioxidants in the human diet, with an important biological function (lycopene and β-carotene), as well as other components with antioxidant properties. Antioxidant contents differ according to the variety of tomato, and environmental and agronomic conditions of growth. The aim is to optimize an HPLC method for the determination of bioactive compounds, and to use different antioxidant tests to determine free-radical scavenging activity.The analytical parameters, recovery assays higher than 97%, satisfactory precision with R.S.D values below 8%, good linearity (r>0.999), good sensitivity and appropriate limits of detection and quantification show that the technique used is satisfactory for measuring these compounds. The tomato samples examined contain highly bioactive components, and have high antioxidant capacity withhigh correlation between phenolic compound contents and antioxidant activity.

*Keywords:*Tomato; Lycopene; β-carotene; Antioxidant capacity; HPLC

Introduction

The Mediterranean Diet is perhaps the healthiest food modelin the world, and is strongly associated with a reduced risk of chronic degenerative diseases, a fact corroborated by numerous studies[1]. It is characterized by a complete, balanced combination of fresh, local and seasonal food. The diet's high contents of vegetables, fresh fruit and olive oil guarantee an adequate intake of antioxidant compounds and explain its beneficial effects on health[2,3]. An exogenous supply of antioxidants in the diet is needed to strengthen endogenous human antioxidant defenses and to confront situations in which excessive free radicals are produced. It is important to know the antioxidant capacity (AC) of foods in order to determine their resistance to oxidation with the subsequent loss of quality and nutritional value, and also to predict their antioxidant potential for human intake.

The tomato (*Lycopersicon esculentum Mill*.) is one of the most widely consumed vegetables, and is a component of the so-called "Mediterranean diet" [4]. As such it is a major component of daily meals in many countries. Due to its high consumption and versatility in culinary preparations, the tomato represents a significant source of antioxidants in the human diet, with an important biological function, especially lycopene, responsible for the red colour[5] and β-carotene, a precursor of vitamin A[6], as well as other components with antioxidant properties. Antioxidant contents differ according to the variety of tomato, and environmental and agronomic conditions of growth[7], and also with variations in microclimatic environments[8].

Spectrophotometry, HPLC and colorimetry are techniques used to determine bioactive compounds in food products[9]. HPLC is a good technique for β-carotene and lycopene quantification and identification, with determinations of both components usually carried out simultaneously following the same procedure[10]. Different

methodologies have been used to evaluate the in-vitro antioxidant capacity of foods, with the results obtained depending on the method used[11-13], as each method measures different aspects of the antioxidant activity of food extracts. The chemical nature of phenolic compounds, the extraction method used and the assay method can all affect the quantification of antioxidant contents and capacity[14]. Most methods are based on the use of a large range of radical generating systems. The sample for which the antioxidant capacity is to be analyzed can inhibit the generation of the radicals. Each component of a sample should be measured, but it is hard to determine the number and concentration of antioxidants.

We have developed a modified HPLC method to simultaneously identify and quantify the antioxidant compounds present in tomatoes (lycopene andβ-carotene).The aim of the present research is to study the bioactive contents using the modified HPLC method, and the antioxidant activity using different suitable methods of several varieties of tomato from a coastal area in Southern Spain.The characterization of this vegetable should contribute to knowledge of its antioxidant capacity and bioactive components.

Material and Methods

Sampling and sample preparation

We chose several different commercial varieties of tomato, *Lycopersicon esculentum Mill*., (Cherry, Sugary, Applause and Bond varieties) commonly cultivated on the coast of Granada (Spain) and obtained from a local farming cooperative. The varieties differed in fruit shape/typology (round, oval, and cherry fruit) and maturity (green and red). The samples are described in Table 1, with more details below. Tomatoes were prepared as appropriate by removal of the outer leaves, peeling, etc.The

fresh fruit were processed in order to separate endocarp (seeds), mesocarp (pulp) and exocarp (peel, husk, skin, etc.), only the pulp was considered for analysis and the peel and seeds were discarded. All extracted samples were stored in an inert nitrogen atmosphere away from light in amber-coloured glass bottles at 4ºC until analysis to avoid possible alterations. All the samples were analyzed by reversed-phase high-performance liquid chromatography techniques (RP-HPLC) and different spectrophotometric methods for measuring antioxidant capacity, to respectively determine the bioactive components (lycopene and β-carotene) and total antioxidant compounds.

Determination of lycopene and β-carotene

A precise amount (5g) of sample was weighed and placed in a test-tube with a screw top, we then added 25 mL n-hexane/methanol/acetone (2:1:1) mixture with 0.5 % of BHT to prevent degradation of the analytes[15], and the test-tube was homogenized by continuous agitation for 30 minutes. Thetest-tubes were then centrifuged at 5000*g* for 15 min and the organic layer recovered. Duplicate extractions were prepared from each sample and all extracts were measured three times in each assay. The chromatographic injection must take place as early as possible to avoid oxidation and decomposition of the β-carotene and lycopene. Otherwise, the sample can be stored at -20ºC for a maximum of one week.

Determination of antioxidant capacity

The edible portions (pulp) of the fruit were stored at –4ºC in polyethylene pouches until required for analysis. For the analysis of antioxidant activity and the total phenol contents, the edible portions were evaluated on the pulp methanolic extract: 5 g of fresh pulp fruits and 20 mL methanol were homogenized in an Osterizer blender for 30 min.

The tubes were centrifuged at 5000*g* for 15 min and the supernatant was recovered and used for bioactive components analysis. Duplicate extractions were prepared from each sample and all extracts were measured three times in each assay.

Reagents and standards

All chemicals were analytical reagent grade unless otherwise stated and water was obtained from a Milli-Q purification system (Millipore, Bedford, MA). Anhydrous sodium carbonate (Na_2CO_3), acetone and hexane were all purchased from Carlo-Erba (Rodano, Milan, Italy). Methanol, FolinCiocalteu, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), 2,2-azinobis-(3-ethylbensothiazoline)-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), N,N- Dimethyl-p-phenylenediaminedihydrochloride (DMPD), Buthyl-hydroxyl-toluene (BHT), β-Carotene type II synthetic, Lycopene and gallic acid were provided by Sigma-Aldrich (Milan, Italy) and potassium peroxodisulphate ($K_2S_2O_8$), sodium acetate 3-hydrate, Acetic acid glacial, sodium acetate anhydrous, Ferric chloride 6-hydrate and hydrochloric acid (37%) and Iron (III) Chloride anhydrous 97 % by Panreac (Barcelona).2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) for FRAP method was from Fluka Chemicals (Madrid, Spain). All reagents were of analytical-reagent grade unless otherwise specified.

Instrumentation

Reserved-phase HPLC was used in the determination of β-carotene and lycopene. HPLC separation was performed with a Perkin-Elmer liquid chromatographic system equipped with a Perkin-Elmer Serie 200 quaternary pump, diode-array detector S-200

P-E, autosampler Serie-200 and peltier Serie-200 oven (PE, Madrid, Spain). The software TotalChrom v6.2 with LCI was used. The column was a C 18 (250 x 4.6 mm, 5 µm particle size) (Teknokroma, Barcelona, Spain). A Büchi 144 rotavapor and a P-Selecta (Barcelona, Spain) bath were also used for sample preparation.

A Lambda 25 UV/VIS spectrophotometer (Perkin-Elmer®, Madrid, Spain) was used for $ABTS^{\bullet+}$ (at 734 nm for 30 min), DPPH (515 nm was recorded for 60 min), FRAP(at 593 nm for 10 min) and DMPD (505 nm was recorded for 10 min), antioxidant measurement methods and total phenol content determination. An Orion ® pH-metre was used to prepare the buffered aqueous phosphate solution.

Chromatographic conditions

The lycopene and the β-carotene were evaluated following the modified method described by Olives Barba (2006)[16]. An extract solution of tomato in hexane was analyzed by reserved-phase HPLC. The mobile phase consisted of methanol/acetonitrile (70:30 v/v) at a flow of 1.5 mL/min, maintained for 25 min. A DAD was used, the measurements taken at 472 nm and quantified by the external standard method. The mobile phases and samples were previously filtered through a 0.45 µm membrane, and degassed ultrasonically prior to use. After this, 10 µL was injected.

Standard solutions

Peaks were identified by comparing the retention times with those obtained with a standard solution of β-carotene and lycopene (Fig. 1A, 1B) and with spectroscopic analysis. As external standard, we used individual stock standard solutions prepared every day of around 2.5-50 (µg/mL) for β-carotene and 0.25-100 (µg/mL) for lycopene.

The stock standard was added to several problem samples to verify the increase in area of the chromatographic peak. Standard solutions were stored in the dark at – 20ºC.

Linearity, Precision and Detection Limit

The linearity of standard curves was expressed in terms of the determination of coefficient plots of the integrated peak area versus concentration of the same standard. The linearity of the method was confirmed by regression statistics.

The method's precision was satisfactory. We carried out six replicate determinations on the same day, with the same sample, same reagents and instruments (intra-day reproducibility). Inter-day reproducibility could not be evaluated for tomato samples because the carotenoid content cannot be kept at its initial levels for several days. Relative standard deviations (RSD) were obtained. The LOD, LOQ[17]and Recovery were studied for lycopene and β-carotene in order to check the sensitivity of the methods used (Table 2).

Spectrophotometric conditions

We used different spectrophotometric methods for measuring antioxidant capacity. Total antioxidant capacity was determined following the ABTS and DPPH method described by Samaniego *et al.* (2007)[11], and DMPD[18] and FRAP[19]adapted to samples of tomatoes. ABTS, DPPH and DMPD methods are based on formation of a coloured radical. Post-addition tests were used, with formation of the radical in the absence of the sample until a stable signal was reached. In the FRAP assay, excess Fe^{III} was used, and the rate limiting factor of FeII-TPTZ, and hence colour formation is the reducing ability of the sample [18].

We then added the sample and measured the resulting change in absorbance (discoloration of the radical), which was proportional to the concentration and

antioxidant activity of the substance analyzed. In all the methods applied, we determined the dilution of tomato methanolic extract that gave a linear response. The absorbance signal was translated into antioxidant activity using Trolox as standard antioxidant. Different calibration curve ranges were used depending on the method (Table 3).

Total Phenolic Content

Total phenolic content was determined using a Folin Ciocalteu colorimetric method described by Singleton and Rossi (1965)[20] and modified in our laboratory. We added 2.5 mL of deionized water and 500 μL of Folin-Ciocalteu reagent to 500μL of methanolic pulp extract. The mixture was allowed to stand for 5 min, and then 2 mL of a 10 % aqueous Na_2CO_3 solution was added. The final volume was adjusted to 10 mL. Samples were allowed to stand for 90 min at room temperature before measurement at 700 nm versus the blank using a Beckman spectrophotometer. The amount of total phenolics is expressed as gallic acid equivalents (μg gallic acid/g of pulp fruit) through the calibration curve of gallic acid. The calibration curve range was 0.5- 7.5 ppm (r = 0.999) (Table 3).

Statistical analysis

The SPSS®*20.0* program was used to interpret the data obtained. Duplicate extractions were prepared from each sample and all extracts were measured three times in each assay. Values were expressed as means ± standard deviation (SD). Pearson's correlation coefficients were calculated. Differences of $p < 0.01$ were considered significant.

Results and Discussion

Determination of lycopene and β-carotene: HPLC method evaluation

The HPLC method developed was evaluated by linearity, precision, sensitivity, recovery and limits of detection (LOD) and quantification (LOQ) for lycopene and β-carotene. The analytical parameters (Table 2), recovery assays higher than 97%, satisfactory precision with R.S.D values below 8%, good linearity ($r > 0.999$), good sensitivity and appropriate limits of detection (LOD) and quantification (LOQ) show that the technique used is satisfactory for measuring these bioactive compounds (lycopene and β-carotene) in samples of *Lycopersicon esculentum Mill.* The linearity of standard curves was expressed in terms of the determination of coefficient plots of the integrated peak area versus concentration of the same standard. Standard calibration curves were established by plotting peak areas against concentrations of lycopene and β-carotene, excellent linearity of the calibration curve was observed in the range tested (Table 2). The systems were linear in all cases (R^2>0.999).The linearity of the method was confirmed by regression statistics. The methods' precision was satisfactory. Six replicate determinations on the same day, with the same sample, same reagents and instruments were carried out (intra-day reproducibility). Inter-day reproducibility could not be evaluated for tomato samples because the carotenoids content cannot maintained in its initial levels for several days. Relative standard deviations (RSD) with results less than 2.5% for lycopene and 3.5 % for β-carotene were obtained (Table 2). All the RSD values obtained were below the maximum 11% limit for substances around 1 μg/ml recommended by AOAC (1993)[21]. Mean recovery percentages for the HPLC method ranged between 96.36% (lycopene) and 100.62 % (β-carotene) (Table 2). Values accepted by AOAC (1993)[21]. Recovery was better for β-carotene, probably due to the lower stability of lycopene[22]. The LOD and the LOQ were studied[17] for lycopene and β-carotene in order to check the sensitivity of the

methods used (Table 2). The technique had excellent sensitivity to analyze this sample.

Table 4 summarizes the bioactive compounds of the tomatoes measured by HPLC methods. Mean and standard deviation were calculated from data of triplicate analysis. Bioactive compounds were identified by comparing retention time (t_R) with standard records and with spectroscopic analysis. For lycopene and β-carotene we added the standard to several problem samples to verify the increase of the chromatographic peak area. Fig. 1C shows an HPLC chromatogram for an analyzed tomato sample. The lycopene contents of tomato were found to be high, ranging from 16.97 ± 1.25 to 210.46 ± 25.12 mg/100g ($p<0.01$). The differences may be due to varieties, climate, ripeness, method, etc[23]. The β-carotene concentrations expressed in mg/ 100g ranged from 97.84 ± 15.23 to 521.12 ± 21.21 mg/100g ($p<0.01$). Similar or lower values have been reported in the literature with lycopene content ranging from 5.22-9.49 mg/100g[4], 4.7-8.8 mg/100g[10], 2.8-4.5 mg/100g[16] and 1.69-15.78 mg/100g[24]and for β-carotene content ranging from 0.30-0.51 mg/100g[4] , 0.7-3.9 mg/kg[10] and 0.6-1.2 mg/100g[16].

Antioxidant capacity methods

Table 4 summarizes the antioxidant capacities of the tomatoes measured by the different methods. The $ABTS^{\bullet+}$ method was applied to all samples for 30 min, the DPPH method for 15 min, and the FRAP and DMPD methods for 10 min. The antioxidant activity values expressed in μmolTrolox/g ranged from 9.19 ± 0.34 to 42.02 ± 1.96TEAC when measured by ABTS, 0.43 ± 0.05 to 2.59 ± 0.14TEAC when

measured by DPPH, and 0.58 ± 0.04 to 2.12 ± 0.31 TEAC when the FRAP method was used. For the DMPD method the contents ranged from 18.16± 4.16 to 60.52 ± 8.12 mmolTrolox/g.

For tomato samples these results were similar to those of other authors[4,24], although very few studies have been reported on antioxidants for the fruits considered in this study.

The antioxidant activities evaluated by different methods showed similar trends with high correlations for ABTS-DPPH (r = 0.982), FRAP-DMPD(r= 0.904)and DPPH-FRAP (r= 0.802) (Table 5). There is a statistically significant relationship between DPPH and ABTS at the 99% confidence level. The correlation coefficient indicates a relatively strong relationship between the variables. Both methods used for measuring antioxidant capacity have the same behaviour based on the ability of antioxidants to scavenge the long-life radical cations $ABTS^{\bullet+}$ and DPPH. The sample for which the antioxidant capacity is to be analysed can inhibit generation of the radicals. A weak correlation ($r = 0.769$) between the FRAP value and TEAC value suggested that the compounds capable of reducing oxidants could be different from those scavenging free radicals in these tomatoes. Since the P-value in the ANOVA table is greater or equal to 0.10, there is not a statistically significant relationship between ABTS-DMPD, and DPPH-DMPD at the 90% or higher confidence level. The correlation coefficient equals 0,545 and 0.534 respectively, indicating a moderately strong relationship between the variables.

Interpretation of the several methods is different and comparisons very difficult, depending on the initial concentration of the radical and a recalculation of data with

Trolox as standard[23]. Therefore the use of different methods helps to identify variations in the response of the compounds extracted from the fruit samples.

Total Phenol Content

The total phenol content of the tomatoes measured by the Folin-Ciocalteau method is summarized in Table 4. The values expressed in µg /g gallic acid ranged from 523.11 ± 30.86 to 153.34 ± 10.87. Similar or higher values have been reported in the literature, although the information available on their phenolic contents is rather scarce[25]. For tomatoes the data were 73.51 mg/100g[25] and ranging from 34.04-60.67 mg/100g[24] and from 2.11-8.60 mg/ 100g[8]. The TP contents in the cherry tomato fruits were considerably higher compared to all others tested, and this increase can be ascribed to an increase in received solar radiation[26].The differences may be due to varieties, genotypes, climate, ripeness and maturity [8,23].

Sugary, Cherry 1 and cherry 2 samples showed high total phenol contents ranging from 444,44 µg/g and 523,11µg/g gallic acid. The high antioxidant capacity of these three extracts seems to correspond to high phenol contents (Table 4). The lowest total phenol contents (153,34 µg /g gallic acid) were found in the Cherry 3 sample, which also has the lowest values for antioxidant capacity as measured by the direct methods ABTS and DPPH (Table 4). In addition, we can link the differences in phenol contents of the extracts from Sugary, Cherry 1 and Cherry 2 from those of the extracts from Bond (green and red), Cherry 3 and Applause to the difference in variety, the size of the fruits and their degree of maturation ($p<0.01$).Total phenol contents decrease as fruit size increases, probably because of the decrease in solar radiation received. Our results

agree with those obtained by Raffo et al. (2006)[26] and Kacjan et al. (2011)[8], who explained that higher levels of total phenol contents in cherry tomatoes, compared to larger fruits, are largely due to the higher skin to volume ratio of these varieties, which could enhance their phenolic content.

The correspondence found between total phenol contents and the capacities of antioxidants in the different samples analyzed is supported by the literature, which confirms the existence of a correlation between the total phenol contents using the Folin-Ciocalteu method, and antioxidant activity measured by FRAP, ABTS and DPPH[27].

Correlation between total polyphenol contents and the different measurement methods

Table 5 summarizes the correlation between total polyphenol contents and the different measurement methods, and suggests that the phenolic compounds may contribute significantly to the overall antioxidant properties of tomato samples. The relation between the main antioxidant compounds of tomatoes (total polyphenol) and the antioxidant capacity measured by the different methods is interesting. The correlation between total phenol contents and antioxidant capacity has been widely studied in different foodstuffs: fruit and vegetables[28] wine [29], tropical fruits[23], seeds[13], green tea infusions[12] and olive oil[11]showing that where there is a high concentration of total polyphenol content, the antioxidant capacity of that food increases significantly. Our study of the tomato samples found the best correlation with different methods between total polyphenol contents and antioxidant capacity. The positive correlations obtained between phenolic content and antioxidant capacity measured as ABTS, DPPH,

FRAP, and DMPD were respectively 0.990, 0.980, 0.892 and 0.796 (Table 5). A highly positive correlation between the ABTS, DPPH, FRAP and DMPD values and total phenolic content suggested that phenolic compounds could be the main components responsible for these samples' capacity to scavenge free radicals.

Other studies report other correlations between total phenolic components and antioxidant capacity, depending on the extraction solvent, the hydrophilicity of compounds, the sample and the type of phenolic compound[23]. Raffo (2002)[30] found that the correlation between the antioxidant activity of cherry tomatoes and phenols depends on the simple phenol composition. In all foodstuffs the total polyphenol contents are related to the antioxidant capacity by contributing to their stability and by the capacity to block free radicals.

As conclusión, the high contents of vegetables, fresh fruit and olive oil guarantee an adequate intake of antioxidant compounds and explain the beneficial effects of the Mediterranean diet on human health**.** This study supplied new information about the antioxidant and bioactive components of the tomato. Tomato varieties from Southern Spain are a rich source of antioxidant compounds such as β-carotene, lycopene and phenolic compounds. The new HPLC method proposed for the determination of bioactive compounds showed adequate reproducibility, accuracy, precision, sensitive detection and quantification limits, with a simple preparation of the samples and short run times for the quantification of lycopene and β-carotene. The technique requires only a small volume of sample, no complicated or time-consuming sample preparation, and it is carried out easily and quickly. Given the complexity of oxidation processes, there is no single test method that fully reflects the antioxidant profile of a sample. In our opinion, it is important to combine several methods for determining antioxidant

capacity, given the diverse behavior of single antioxidants. In general, all the methods can be used to measure antioxidant capacity. The tomato samples examined contain high bioactive components, and have high antioxidant capacity withhigh correlation between phenolic compound contents and antioxidant activity, indicating phenolic compounds are the major contributor to the antioxidant capacities of these plants. The knowledge that this vegetable is a very good source of antioxidants should encourage and recommend their intake by the public.

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Compliance with Ethics Requirements

Conflict of Interest:

- C. Samaniego-Sanchez declares that he has no conflict of interest
- C. Stagno declares that he has no conflict of interest
- J.J.Quesada-Granadosdeclares that he has no conflict of interest
- R. Blanca-Herrera declares that he has no conflict of interest
- V. Brandolinideclares that he has no conflict of interest

This article does not contain any studies with human or animal subjects

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Figure Captions

Fig. 1. RP-HPL Cchromatogram of β-carotene (A); lycopene (B) standard solution and chromatogram of β-carotene and lycopene in a tomato extract sample (C).

Figure Captions

Fig. 1. RP-HPLC chromatogram of β-carotene (A); lycopene (B) standard solution and chromatogram of β-carotene and lycopene in a tomato extract sample (C).

Figure
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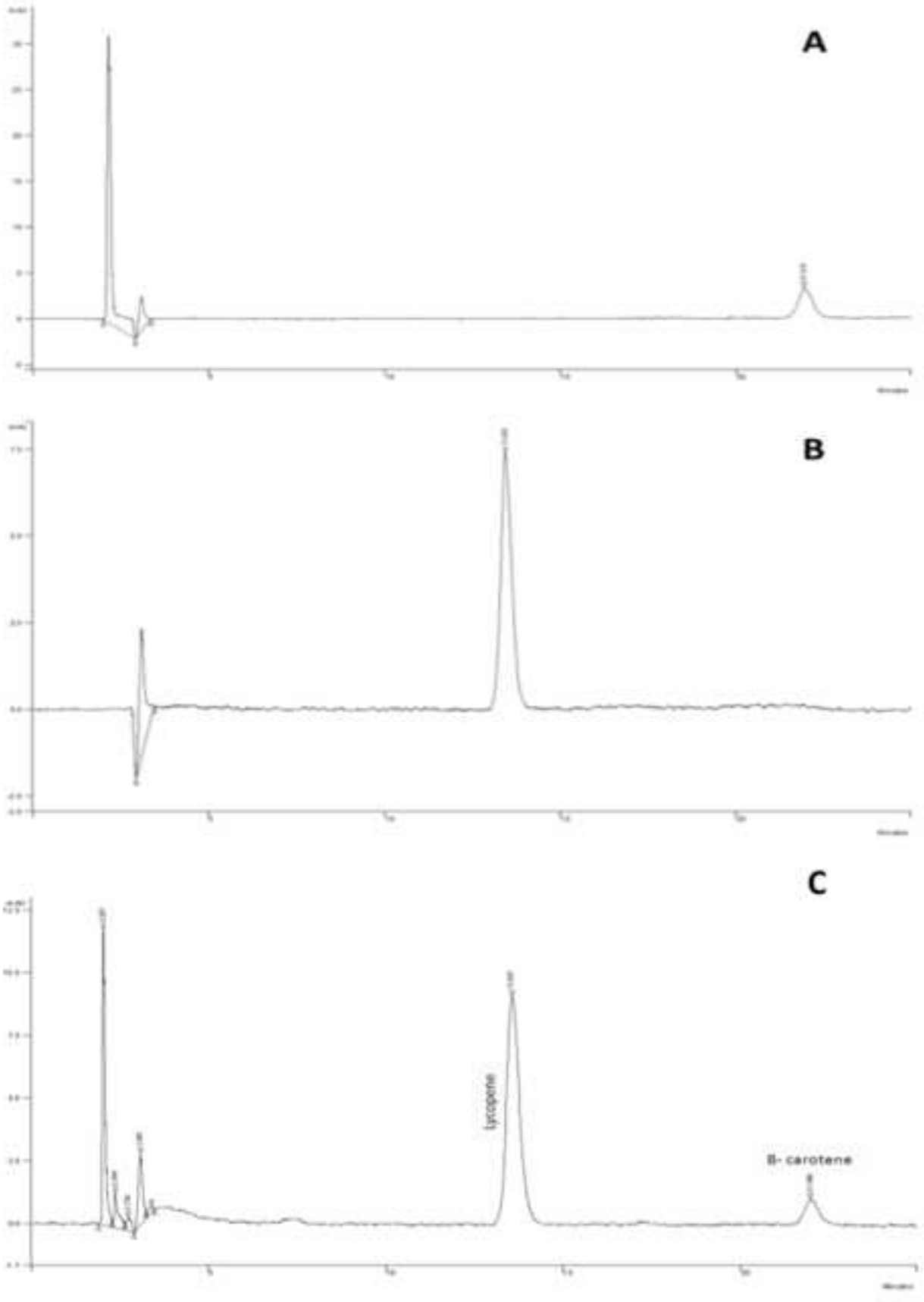

Table

Table 1

Description of tomato samples: scientific name *Lycopersicon esculentum Mill*., Solanaceae family

Samples	**Variety**	**Morphology**	**Fruit description**
Cherry	Cherry (Granada)	Cherry 1: Small size, branching growth Cherry 2: Small size, individual growth Cherry 3: Large and round	Vigorous plants with in determinate growth. Small-sized fruit with fine skin prone to splitting in bunches of 15 to 50. Sweet, pleasant taste. Some cultivar shave red and yellow fruit. The aim of this variety is to have a production that completes the annual cycle with homogeneous amounts.
Sugary	Sugary (Granada)		Plant of determinate growth, measuring less than 1.5 metres. Its fruit small and oval-shaped of a red colour, with fine skin, sweet taste and very juicy. They grow in bunches with production through out the summer season.
Applause	Applause (Granada)		Plant of determinate growth. It is very compact and with early production. The fruit are small and red, with a delicate, slightly sweet taste.
Bond	Bond (Granada)	Bond (green): Large fruit, unripe Bond (red): Large fruit, optimal ripeness	Open variety of medium vigourous plant that adapts well to both covered and open-air growth. Very firm, heavy G-GG fruit of attractive red and green colours and good taste. Highly productive and early.

Table 2

Linearity parameters, precision, recovery and limit of detection and quantification of lycopene and β-carotene determinations by HPLC methods

	Lycopene	**B-Carotene**
Concentration range (µg/mL)	0.25 – 100	2.5 - 50
Linear regression equation	$y= 3 \cdot 10^{10} x +216518$	$y= 1 \cdot 10^{9} x +29861$
R^{2a}	0.9991	0.9992
Intra-day reproducibility as RSD (n=6)	2.48 %	3.55 %
LOD [b] (µg/mL)	$9.27 * 10^{-5}$	0.0098
LOQ [c] (µg/mL)	0.00030	0.0039
Recovery % [d] (X ± SD)	96.36 ± 2.2	100.62 ± 5.2

[a] Correlation coefficients of the regression equation, [b] limit of detection, [c] limit of quantification, [d] average recoveries n = 3; X ± SD = mean ± standard deviation. y = ax + b, y = area, x = lycopene (mg/10µL) and β-Carotene concentration (mg/10 µL)

Table3

Gallic andTrolox standard curves: percentage inhibition of absorbance at 700 nm as a function of gallic concentration (gallic acid μg/g) and at 734, 515, 505 and 593 nm as a function of Trolox concentration μM and mM, respectively.

Antioxidant activity method	Equation	Correlation coefficient (r)(n=10)	Concentration range Trolox
TPC (700 nm)	y = 0.091 x + 0.093[a]	0.999	-
ABTS•+ (734 nm)	y = 0.170 x + 6.761[b]	0.997	10 – 500 μM
DPPH (515 nm)	y = 130.9 x + 4.669 [b]	0.999	0.05 – 0.5 mM
DMPD (505 nm)	y = 48.72 x + 16.66 [b]	0.994	0.2 – 0.8 mM
FRAP (593 nm)	y = 0.004 x + 0.033 [b]	0.996	10 – 300 μM

[a] $y = bx + a$, y = absorbance (700 nm), x = Gallic acid concentration. [b] $y = bx + a$, y = percent age inhibition, x = Trolox concentration.

Table 4

Bioactive compounds content, Total phenol content and Antioxidant capacity in several varieties tomato determined by four different tests. Values are means ± S.D of 3 measurements.Different letters (a, b, c, d, e, f, g) in the same column indicate statistical differences ($p < 0.01$).

Sample	Lycopene (mg/100g)	B-carotene (mg/100g)	TPC (µg/g gallic acid)	ABTS (µmol/g Trolox eq.)	DPPH (µmol/g Trolox eq.)	FRAP (µmol/g Trolox eq.)	DMPD (mmol/g Trolox eq.)
Cherry 1	99.26 ± 10.85 (a)	521.12 ± 21.21 (a)	523.11 ± 30.86 (a)	42.02 ± 1.96 (a)	2.59 ± 0.14 (a)	1.84 ± 0.12 (a)	37.44 ± 2.17 (a)
Cherry 2	49.00 ± 2.56 (b)	273.73 ± 14.75 (b)	487.52 ± 28.53 (b)	25.22 ± 0.64 (b)	1.42 ± 0.05 (b)	2.12 ± 0.31 (b)	60.52 ± 8.12 (b)
Cherry 3	142.44 ± 12.65 (c)	254.73 ± 19.47(c)	153.34 ± 10.87 (c)	9.19 ± 0.34 (c)	0.43 ± 0.01 (c)	0.59 ± 0.04 (c)	22.94 ± 5.18 (c)
Sugary	105.96 ± 18.12 (d)	238.97 ± 21.47 (d)	444.44 ± 37.35 (d)	28.40 ± 1.05 (d)	1.29 ± 0.03 (d)	1.01 ± 0.06 (d)	35.05 ± 1.82 (d)
Bond (green)	16.97 ± 1.25 (e)	97.84 ± 15.23 (e)	172.68 ± 21.24 (e)	12.27 ± 0.49 (e)	0.61 ± 0.05 (e)	0.58 ± 0.04 (e)	18.16 ± 4.61 (e)
Bond (red)	57.92 ± 5.41 (f)	154.57 ± 17.23 (f)	196.47 ± 26.40 (f)	15.55 ± 0.78 (f), (g)	0.63 ± 0.02 (e), (f)	0.76 ± 0.09 (f)	29.76 ± 2.37 (f)
Applause	210.46 ± 25.12 (g)	209.29 ± 20.17 (g)	231.67 ± 19.64 (g)	16.31 ± 3.08 (f), (g)	0.71 ± 0.05 (g)	0.93 ± 0.05 (g)	27.93 ± 4.37 (g)

Table 5

Pearson's correlation coefficients (r) between antioxidant capacity and total phenolics, measured in the tomato extract methanolic.

	TPC	ABTS	DPPH	FRAP	DMPD
TPC	1				
ABTS	,990(**)	1			
DPPH	,980(**)	,982(**)	1		
FRAP	,892(**)	,769(*)	,802(*)	1	
DMPD	,796(*)	,545	,534	,904(**)	1

* Significant at $p < 0.05$; ** Significant at $p < 0.01$.