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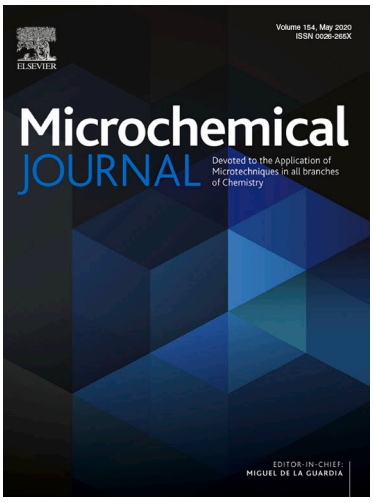

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María Victoria Salomon, Patricia Piccoli, Ariel Fontana

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Simultaneous determination of carotenoids with different polarities in tomato products using a C_{30} core-shell column based approach

María Victoria Salomon[a,b], Patricia Piccoli[a,b], Ariel Fontana[a,b]*

[a]Grupo de Bioquímica Vegetal, Instituto de Biología Agrícola de Mendoza, Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de Cuyo (UNCuyo), Almirante Brown 500, M5528AHB Chacras de Coria, Argentina.

[b]Cátedra de Química Orgánica y Biológica, Departamento de Biomatemática y Fisicoquímica, Facultad de Ciencias Agrarias-UNCuyo, Chacras de Coria, Mendoza, Argentina

*Corresponding author: Ariel Fontana

E-mail address: afontana@mendoza-conicet.gob.ar; fontana_ariel@yahoo.com.ar

Tel: +54-0261- 4135010, ext. 1228

Fax: +54-0261- 496-0469

Abstract

A rapid and cost-effective chromatographic approach based on a core-shell column with high-performance liquid chromatography diode array detection (HPLC-DAD) is proposed for the quantification of carotenoids in tomato fruits and its derived products. Separation of analytes with different polarities, like lutein, ß-carotene and lycopene was achieved in less than 16.5 min (total run time of 20 min) by using an Accucore C_{30} column (3.0 mm x 150 mm, 2.6 µm) with a gradient of methanol, methyl *tert*-butyl ether (MTBE) and ultrapure water, a temperature of 10 °C and a flow rate of 0.4 mL min^{-1}. The maximum backpressure reached was 270 bar, making the developed method suitable for standard HPLC instruments commonly found in routine laboratories. After a thorough evaluation of the solvent for re-suspending the extracts, the mixture methanol:MTBE (1:1) was the most adequate to allow accurate quantification and avoid underestimation of analytes. The limits of detection (LODs) ranged from 0.03 to 0.46 mg kg^{-1} dry weight of the sample, with overall recoveries (accuracy) between 86% and 116% for target compounds. The developed method was applied for the determination of carotenoids in 7 fresh and processed tomato samples confirming its suitability for quantification of analytes in different matrixes.

Keywords: ß-carotene; lutein; lycopene; method validation; tomato products

1. Introduction

Tomato (*Solanum lycopersicum*) is one of the most worldwide cultivated and consumed vegetables due to its good taste and wide variety of forms and colors, being consumed both fresh and processed into a variety of manufactured products [1, 2]. Furthermore, tomato is a source of nutrients and antioxidant compounds including carotenoids, vitamins, phenolics, compounds and sugars that are important not only due to current consumer preferences but also from human health benefits [3, 4]. Carotenoids are a diverse kind of 40-C isoprenoids produced by plants, which comprise two major groups: xanthophylls with a cyclized structure containing one or more oxygenated functions, as lutein; and carotenes, which are hydrocarbon compounds composed of only carbon and hydrogen, either linear or cyclized, such as lycopene and ß-carotene [5]. They are natural pigments, having also an important role in different physiological processes such as light-harvesting in photosynthesis and giving the bright colors to flowers and fruits, mainly red, orange and yellow [6, 7]. In tomato ripe fruit, the most abundant pigment is lycopene that gives the characteristic red color (reaches >80% approximately). Also smaller amounts of lutein and α and ß-carotene, responsible for yellow and orange colors are found. Other carotenoids present during tomato ripening are the carotene phytoene and the xanthophylls neoxanthin and violaxanthin [8-10]. From a human health point of view, carotenoids are important because some are precursors of vitamins as the case of α and β-carotene, and due to their high antioxidant capacity because they are able to protect cells from reactive oxygen species. Several *in-vitro* studies reported that mainly lycopene, as well as to a lesser extent other carotenoids, are very efficient scavengers of singlet oxygen and peroxyl radicals [1, 11]. In addition, a positive link between higher dietary intake and tissue concentrations of carotenoids with a lower risk of chronic diseases such as cardiovascular syndromes and certain cancers has been suggested [12, 13]. In this sense, and considering the potential preventive effects in human chronic disorders, the consumption of foods rich in

carotenoids or dietary supplementation with these bioactive compounds has been recommended [13].

The content of compounds may vary according to environmental conditions where the tomato plants are grown, also during the post-harvest stage and the processing conditions to manufacture other products [14]. These changes can influence the nutritional characteristics of the products (fresh and manufactured) as well as the preferences of consumers due to changes in taste or visual appearance. Due to the relevance of carotenoids content in tomato and its derived products, and their importance on bioactive properties, it is of utmost significance to determine their composition. These data could help provide valuable information for the characterization of samples and equally increase the value of the product by addressing a specific group of consumers. In fact, during the last decade consumers showed an increasing interest in healthy food and value-adding food ingredients, particularly related to the content of bioactive compounds such as carotenoids. On this matter, highly efficient analytical methodologies for the identification and quantification of carotenoids in both fresh and processed tomato products are required for these objectives to succeed. At the same time, laboratories of all over the world are demanding simple, rapid and economically affordable methods for quality control of products.

HPLC coupled to different detectors such as ultraviolet, PDA, and mass spectrometry (MS) has been the choice for the analysis of carotenoids [15-19]. Currently, novel separation techniques based on ultrahigh-pressure systems (UHPLC) allow rapid, multipurpose and high-throughput separations, that successfully meet the analysis needed for complex samples such as those coming from food and plant extracts [16, 20]. Technological developments in HPLC resulted in UHPLC instruments capable of superior resolutions, but with the disadvantage of being sometime expensive to average laboratories enrolled on quality control in developing countries, or difficult to adapt from known procedures [21].

The use of superficially porous particles, also named core-shell, has gained popularity in recent years representing an alternative to increase HPLC separation efficiencies, resolution and speed, with back pressures significantly lower than those associated with UHPLC. These particles can overcome the ultrahigh backpressure resulted from that porous sub-2 µm due to their reduced resistance to mass transfer and the high particle uniformity [22]. Their advantage relies on the possibility of achieving UHPLC performance on regular HPLC instruments, increasing reproducibility, resolution, sensitivity and flexibility along with shorter analysis times and lower solvent consumption respect to traditional HPLC methods.

Despite the ample possibilities of this column technology, very limited use of C_{30} stationary phase with superficially porous particles has been reported. The majority of applications were for lipid analysis and the comparison of their performance with different C_{18} columns were reported [23, 24]. The results showed that superficially porous C_{30} column has excellent peak capacity and retention time reproducibility, allowing to separate a higher number of peaks than their C_{18} counterparts. The characteristics of the C_{30} column with core-shell particles also offer the possibility to obtain high shape selectivity for long-chain hydrophobic and structurally related isomers, such as carotenoids and vitamin K derivatives [25].

The application of core-shell technology for carotenoids determination has been reported in two previous works using C_{18} stationary phases for the determination of the pairs lutein/ß-carotene in broccoli [26] and lycopene/ß-carotene in tomatoes [27]. For C_{30} phases there are also some applications for the rapid quantification of carotenoids in starchy staples and peppers [28, 29]. Besides the interesting results presented in these papers, the determination of a relevant compound with a low polarity such as lycopene was not performed. This compound is very important for several food samples, particularly for tomato products, in which lycopene is the most abundant carotenoid. This compound should not be omitted in any method aimed to characterize carotenoid composition. Additionally, lycopene needs to be considered when a C_{30}

phase is used because its strong retention on these material, increasing the analysis time of the method. In fact, to achieve a suitable comparison, it is essential to include this compound during chromatographic method development. The polarities of lutein and carotene derived compounds are different because the former compound is a molecule containing oxygen, as all xanthophylls derived carotenoids. In the case of carotenes (non-oxygenated molecules) such as ß-carotene and lycopene, they are isomeric forms. Lycopene is an acyclic molecule while ß-carotene has a cyclic structure. This cyclization results in steric hindrance between the methyl group at C-5 of the ring and the hydrogen located at C-8 in the polyene chain, taking the Π-electrons of the ring double bond out of the plane with those of the chain [30]. Thus, bicyclic ß-carotene, although having the same number of bonds as lycopene, has different chemical properties including lower solubility than lycopene. Considering the importance of determining carotenoids of different polarity, particularly in tomato products, and the lack of methods quantifying simultaneously the compounds using a C_{30} column approach, the development of a more versatile chromatographic approach is required. Particularly important is to find an alternative to UHPLC systems which may be too expensive for some quality control laboratories. In this sense, the core-shell columns could be capable to undertake rapid and economically affordable results in most laboratories. The quantification of the major carotenoids in tomato products and other matrices, avoiding consuming much time and expenses, could allow improving the quality control of food products having a key impact on the development of regulatory policies in developing countries. In this context, due to the need for high-throughput methods available for average laboratories and the complexity that represents the simultaneous quantification of carotenoids with different polarities, like lutein, ß-carotene and lycopene, the aim of this work was to develop a rapid, simple, and cost-effective HPLC analytical method, using a C_{30} core-shell chromatography approach coupled to DAD for the determination of carotenoids in tomato products. The optimized method was validated and

its applicability demonstrated by analyzing the carotenoids profile in different tomato fruits and processed tomato samples.

2. Experimental

2.1. Chemicals and standards

HPLC-grade methanol and methyl *tert*-butyl ether (MTBE) were acquired from Sigma-Aldrich (Steinheim, Germany). The absolute ethanol and *n*-hexane were purchased from Mallinckrodt, Baker Inc. (Phillispsburg, NJ, USA), and ultrapure water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA).

Lutein (≥96%), ß-carotene (≥93%), and lycopene (≥85%) standards were obtained from Sigma-Aldrich. For the preparation of stock solutions, the respective carotenoids were weighed and dissolved in ethanol (lutein) and *n*-hexane (ß-carotene and lycopene). The preparation of stock solutions was performed under darkness. The final concentration of the stock solutions was 40, 200, and 40 mg L^{-1} for lutein, ß-carotene and lycopene, respectively. Additional dilutions were prepared weekly in the corresponding solvent for each compound and stored in dark glass bottles at -80 °C. The standards used during the optimization and validation of the method were prepared in methanol:MTBE (1:1) and also stored at -80 °C.

2.2. Samples

Fresh samples of cherry and pear tomatoes (pear, *Solanum lycopersicum*), as well as the industrial tomato sauce, were purchased from different local grocery stores of Mendoza, Argentina. Homemade sauce and roasted tomatoes were prepared following traditional recipes. Briefly, the homemade sauce was prepared from peeled and seedless tomato obtained with a blanching and crushing process and sterilized by boiling them for 40 min. Roasted tomato was prepared by baking them for 2 hours at 180 ºC. Then, all samples (including fresh tomatoes) were ground into smaller particles with liquid N_2 and freeze-dried during 72 h. After that, dried samples were maintained at darkness until processing.

2.3. Carotenoids extraction

The extraction procedure was developed in the laboratory, but previously the selection of the extraction solvent was made based on the available bibliography [4, 18, 31]. Then, to extract carotenoids from different samples, 3 sequential extractions were performed as follows. A 100 mg portion of the previously freeze-dried material was macerated with 1 mL of ultrapure water and 5 mL of ethanol:*n*-hexane (60:40, v/v) and simultaneously ground to a fine powder using a mortar and pestle. Then, the mixture was transferred to a conical glass tube (screw cap), sonicated for 15 min, and then centrifuged 15 min at 1344 g (4000 rpm). To avoid carotenoid degradation and isomerization during sonication, the temperature was carefully monitored to ensure that it did not reach 30 ºC. The solvent phase was collected and transferred to another glass tube to be evaporated to dryness by vacuum centrifugation. Two additional extractions were done by adding 3 mL aliquots of *n*-hexane each one to the conical tube containing the pellet of the previously extracted sample and repeating the same process described above. All solvent phases from the same sample were collected and dried in the same glass tube by vacuum. Once the aliquots of extract were dried, it was re-suspended with a mixture of methanol:MTBE (1:1, v/v) and injected in the HPLC-DAD. The entire procedure was performed protecting the samples and extracts from light.

During method development, the optimization of injection solvent was done to get the complete re-suspension of compounds. For doing this study, an extraction from 500 mg of freeze-dried tomato sample following the same procedure explained above (adjusting the volume of solvent and water for the amount of material) was performed. The liquid extract was divided into 5 equal and homogeneous fractions and separately evaporated to dryness by vacuum. Then, each fraction was dissolved in 1 mL of the following solvents and mixtures to be tested: methanol, MTBE, *n*-hexane, methanol:MTBE (1:1, v/v), and methanol:MTBE:ultrapure water (70:26:4, v/v/v; initial mobile phase of the optimized chromatographic method).

It is convenient to mention that sample extracts and standards were analyzed on the same day of its preparation. When it was not possible, the re-suspended extracts were stored in dark vials at -80 °C. Additionally, and based on previous work [32], we avoided the addition of an antioxidant to sample extracts. These authors compared the stability of carotenoids with and without adding antioxidants to extracts, concluding that no statistically significant difference between the individual and total carotenoid content in samples, despite using an antioxidant, were observed.

2.4. HPLC-DAD analysis

Target compounds were determined using an HPLC-DAD system (Dionex Softron GmbH, Thermo Fisher Scientific Inc., Germering, Germany). The instrument was a Dionex Ultimate 3000 comprising a vacuum degasser unit, an autosampler, a quaternary pump, and a chromatographic oven. The detector used was a Dionex DAD-3000. It consists of an analytical flow cell set to scan from 200 nm to 500 nm, operated with a data collection rate of 5 Hz, a bandwidth of 1 nm and a response time of 1.000 s. The wavelengths selected for quantification of analytes were: 445 nm, 450 nm, and 480 nm, for lutein, ß-carotene, and lycopene, respectively. The control of all the system acquisition parameters and the process of data were performed with the Chromeleon 7.1 software.

Chromatographic separations were done in an Accucore C_{30} column (3.0 mm x 150 mm, 2.6 µm) Thermo Scientific (Bellefonte, PA, USA) including an Accucore C_{30} guard column (10 mm x 2.1 mm). The mobile phase consisted of methanol (A), MTBE (B), and ultrapure water (C). Separation of analytes was performed with the following gradient: 0 min, 26 % B; 0-10 min, 76 % B; 10-14 min, 90 % B; 14-16 min, 26 % B; 16-20, 26% B. The percent of C remained constant at 4% throughout the chromatographic run. The mobile phase flow was 0.4 mL min^{-1}. The column temperature was 10 °C and the injection volume 5 µL. The autosampler temperature was maintained constant at 15 °C. Carotenoids were identified and quantified based

on the comparison of their retention times (tR) and absorbance values of detected peaks in tomato samples with those obtained from the injection of each pure standard. Furthermore, in order to verify the peak identification and the absence of interferences at analytes tR, samples were added with known concentrations of target compounds. Quantification was performed by means of an external calibration prepared with pure standards of each carotenoid.

2.5. Evaluation of method performance

The developed method was validated in terms of linearity, accuracy and precision. Linearity was evaluated for each compound at 8 different levels. The standards were prepared in methanol:MTBE (1:1). The ranges of concentration were between 0.0625 and 10 mg L^{-1} for lutein, from 0.0625 to 20 mg L^{-1} for ß-carotene, and from 0.5 to 75 mg L^{-1} for lycopene. The limit of detection (LOD) and limit of quantification (LOQ) were estimated from the S/N values corresponding to chromatographic peaks at the lower level of the linearity study. LOD and LOQ were defined as the signal-to-noise ratio equal to or greater than 3 and 10, respectively. In all studies, standards and samples were injected in triplicate.

For the recovery study, two different concentration levels for each compound were evaluated. Levels were selected based on the expected concentration of analytes in tomatoes and its derived products, also considering the linear range of the method. To 50 mg of freeze-dried tomato sample a mix of standards was added. The concentrations in level 1 were: 5 mg kg^{-1} for lutein and ß-carotene and 20 mg kg^{-1} for lycopene. For concentration in level 2 were: 20 mg kg^{-1} for lutein and ß-carotene and 100 mg kg^{-1} for lycopene. Then, using the same procedure described above, spiked samples were extracted. The absolute recoveries (R%) of the proposed method were calculated as the difference between the concentrations measured for extracts from spiked and non-spiked aliquots of tomato, divided by the theoretical concentration added to the sample, and multiplied by 100. The precision was evaluated over inter-day (reproducibility) and intra-day (repeatability) studies. Intra-day precision was evaluated by analyzing in the same

day 3 replicates of samples spiked with compounds at concentrations of level 1 and 2. The inter-day precision was assessed with the same portion of samples, spiked also at levels 1 and 2, and processed in triplicate during 3 consecutive days. Using the two sets of data, the percent relative standard deviations (RSD) were calculated.

3. Results and discussion

3.1. Development of the chromatographic method

A separation and quantification method for carotenoids with different polarities and chemical moieties, namely lutein, ß-carotene, and lycopene was optimized by means of a core-shell column. The particular characteristics that columns packed with core-shell particles have, still when they are used in conventional HPLC equipment, justify its selection. These facts include the speed of analysis, good efficiency, and theoretical plates with reduced heights while the ability of maintaining sample loading capacity and backpressure at moderate values. Taking into account, core-shell columns represent a balanced option for most laboratories, particularly those involved in quality control of food products.

The chromatographic separation of carotenoids is usually performed using RP-HPLC. Although C_{18} stationary phases have been highly applied for carotenoid analysis, the use of C_{30} phases is a convenient alternative due to their superior selectivity. Besides that, its application has been limited because of the longer separation times that they normally have. In recent years, with the introduction of core-shell column technology, the time of analysis has been substantially reduced without resigning from separation performance. That is, this technology allows UHPLC performance on regular HPLC instruments with increased reproducibility, resolution, sensitivity, and flexibility along with shorter analysis times and lower solvent consumption than traditional HPLC methods [33]. In this study, separation conditions were optimized using an Accucore C_{30} column (3.0 mm x 150 mm, 2.6 µm). Departure separation conditions were taken from previous articles dealing with the HPLC determination of carotenoids [28, 34]. The

separation conditions were optimized using ternary mixtures of methanol, MTBE, and ultrapure water. The initial gradient was the following: 0 min, 70% B; 10 min, 20% B; 20 min, 6% B; 21 min, 6% B; 23 min, 70 % B. The flow rate was 1 mL min^{-1} and the column temperature 20 °C. Under these conditions, an early elution of carotenoids was observed, with the additional drawback of lutein peak was not detected. Thereafter, modifications of flow rate, mobile phase gradient composition and column temperature were evaluated. Reducing the flow rate to 0.4 mL min^{-1} allowed achieving a good compromise between separation and total run time. The progressive increase of MTBE (26 - 90%) between 0 to 16 min allowed better sensitivity and separation of lycopene among ß-carotene. MTBE is essential to facilitate the elution of lycopene, which is strongly retained in a methanol environment and C_{30} stationary phase So the gradual increasing of this solvent allowed the successful elution of lycopene. Another important evaluated condition was the column temperature, studying values between 10 and 30 °C. It was observed that at lower temperatures (10-20°C), better separation between ß-carotene and lycopene pair was achieved; while higher temperatures caused pairing between these compounds, affecting their correct resolution in the chromatographic run. The analytes peaks in the chromatograms of standards and tomato extracts were well-resolved by using a flow rate of 0.4 mL min^{-1} and 10 °C column temperature. Using the optimized conditions, simultaneous separation and determination of carotenoids with different polarities were rapidly and selectively achieved in less than 16.4 min (20 min from injection-to-injection), showing baseline resolution for the studied analytes (See Fig. 1).

3.2. Selection of injection solvent

Due to the different polarity of studied carotenoids and to avoid the possibility of incomplete dissolution of analytes, special attention during the dissolving and re-suspending the extracts prior to HPLC-DAD analysis was taken. Typically, the dried extracts of samples or standards are dissolved in the initial mobile phase or on a high percent of it since this compatibility is

critical for achieving good peak shape and sensitivity during analysis. Most carotenoids are insoluble in water and soluble in organic solvents such as acetone, alcohols, tetrahydrofuran, ethyl ether, chloroform, hexane and ethyl acetate. Nevertheless, their solubility depends on the presence of different functional groups [15]. The complete solubilisation of these pigments is critical to avoid the incompatibility of the injection solvent with the mobile phase and also to prevent blocking the column or guard column. As well, the correct selection of solvent or solvent mixture for dissolving dried extracts is essential to ensure the accurate quantification and avoid underestimation of analytes concentration. Different solvents and combinations based on the chemical nature of studied carotenoids were evaluated as dissolving/injection solvents. The studied options included: dissolution of extracts in the initial mobile phase (methanol:MTBE:ultrapure water; 70:26:4), methanol:MTBE (1:1), methanol, MTBE and hexane. For performing the study, a homogeneous freeze-dried tomato sample was used for all experiments, and extracted according to the description presented before. After extraction, independent and equal aliquots of the same homogenous extract were first dried and then dissolved in 1 mL of each evaluated solvent/mixture. Identical injection volume and chromatographic method, were used in all cases with the aim to facilitate the comparison between the resulting chromatographic peaks and, hence, assess the effect of the studied solvents.

Table 1 shows the effect of the different injection solvents on the concentration of carotenoids. No significant differences were observed in the retention times for the studied analytes by using different solvents or solvents mixtures. When the mixture of methanol:MTBE (1:1) was used as the injection solvent,the highest concentrations for all studied carotenoids were found. By using the initial mobile phase or methanol, better results for the more polar lutein, but a considerable deterioration of sensitivity for the less polar ß-carotene and lycopene were observed. Besides of that, the levels of lutein achieved with these solvents remained nearly

below without statistically significant differences of those achieved with methanol:MTBE (1:1). This may be due to the higher polarity of these injection solvents which make unable to achieve the adequate dissolution of the less polar carotenoids. An opposite behavior was noticed using MTBE, where lycopene and ß-carotene had statistically comparable concentrations with methanol:MTBE (1:1), but lutein was considerably underestimated (75 % lower level than those obtained with the mixture). When hexane was used for re-suspending the extracts, ß-carotene and lycopene were less affected, but a high decrease of concentration of lutein was observed (82 % lower level than those obtained with the best condition). Therefore, the above results indicated that an adequate combination of solvents is more advantageous to improve sample solubility than a single solvent. On one side, methanol allowed to increase the solubility of the most polar carotenoid (lutein). On the other hand, MTBE ensured the adequate solubilization of the less polar compounds (ß-carotene and lycopene), making able the accurate quantification of the different compounds independently of their polarities.

3.3. Performance of the methodology and validation

The analytical characteristics of the method are presented in Table 2. Quantification was performed based on linear calibration curves constructed with carotenoids standards. The calibrations gave r^2 values higher than 0.9967 in the evaluated concentration range. The LODs of the analytes were ranged between 0.03 for lutein to 0.46 mg kg^{-1} for lycopene. According to the levels of carotenoids reported in different fresh and processed tomato samples, the achieved LODs for the method showed a suitable sensitivity for routine analysis.

To evaluate the precision of the method (Table 3), inter-day (reproducibility) and intra-day (repeatability) studies were used, for that the relative standard deviation was calculated using the recoveries at the specific levels. For inter-day precision, the calculated RSDs for studied carotenoids were lower than 16.3% (lutein); while for intra-day precision were lower than 14.1% (lutein).

The method selectivity for quantification of carotenoids in different tomato derived products was evaluated by the comparison of tR and spectral comportment of analytes by analyzing a standard solution and a tomato extract (with and without analytes addition). As is presented in Fig. 1, after analyzing a positive and a spiked tomato sample, non-significant differences in tR were shown. In addition, any interference at the carotenoids tR was detected.

The accuracy of the method was estimated by the relative recoveries assessed using a freeze-dried tomato portion spiked at two different concentration levels (See Table 2). The concentrations used for recovery experiments were selected based on the expected concentration in tomato products, thus being able to evaluate the method applicability for the analysis of samples. The obtained values were between 86% and 116%, with associated standard deviations remaining below 9. These data support the efficiency of the proposed methodology for the accurate quantification of carotenoids in tomato matrix.

Previous reports for tomatoes are generally focused on determining the pair lutein/ß-carotene or lycopene/ß-carotene. Table 4 presents a comparison of different HPLC and UHPLC methods for the determination of carotenoids in different matrices. The proposed core-shell HPLC approach, as compared to other chromatographic separation procedures on C_{30} traditional columns proved to be significantly faster while maintaining its selectivity [35]. On the other hand, a total run time of 15 min was obtained by Wald et al. using a C_{30} column for the separation of major carotenoids in starchy staples from Ghana [28]. Zoccali et al. [29] also reported a shorter chromatographic method for the determination of four carotenoids in red peppers. Besides the relatively shorter analysis time compared to the present report, the separation performance is not comparable because lycopene, an important compound and the most strongly retained carotenoid on C_{30} columns, was not analyzed in these previous research. As well, higher flow rates (2 mL min^{-1}) were used in comparison with the 0.4 mL min^{-1} used in the present method. This difference is critical in reducing the organic solvent consumption,

particularly when a routine work analyzing a lot of samples will be performed. The core-shell technology allows fast equilibrium favoring mass transfer, and shortening re-equilibration times in comparison with traditional columns, thus increasing the sample throughput in routine work. To achieve reproducible results between runs, the method proposed only requires 3.5 min of stabilization with the initial condition after the elution of the last peak. In recent years, UHPLC methods have been proposed for the determination of different carotenoids classes with the associated advantages of these systems in terms of superior resolutions, shorter analysis time and lower solvent consumption. In fact, Rivera et al. achieved the separation of 13 carotenoids, including those studied in the present work, in 15 min using a RP BEH C_{18} column, with a flow rate of 0.4 mL min^{-1} [32]. Li et al. [16] separated and identified all-trans-lutein, lycopene, ß-carotene and their 22 cis-isomers by a rapid and sensitive UHPLC method using a C_{18} column in 15 min. Delpino-Rius et al. [18] also proposed the simultaneous determination of a total of 27 carotenoids, including epoxy, hydroxyl derivatives and carotenes, in fruit juices with a chromatographic analysis time shorter than 17 min. Van Hung et al. [36] proposed a fast method (within 6 min) for determining the same carotenoids as those quantified in the present work in durum wheat flours. As can be observed, the advantages of UHPLC are unquestionable because it allows determining a higher number of carotenoids in similar or even shorter times than the approach proposed here. In this sense, the main disadvantage of our method compared with UHPLC approaches is the number of compounds that have been profiled. However, UHPLC systems may be too expensive for most average laboratories. The method presented here with a core-shell column is capable to accomplish useful and cost-effective results on most laboratories. Thus, it has potential applicability for the quantification of the major carotenoids in tomato products and other matrices, avoiding consuming much time and expenses.

3.4. Application of developed method to quantify carotenoids in tomato

The applicability of the proposed methodology was evaluated in complex matrices by quantifying the content of lutein, β-carotene and lycopene in different fresh and processed tomato products (Table 5). The concentrations of the compounds obtained through the application of this method are within the range of those reported in previous studies [3, 28, 31, 37]. Fig. 1 shows representative chromatograms obtained from two fresh tomato samples with different carotenoids composition.

The content of lutein and β-carotene was higher in fresh samples respect to the processed one, showing that these compounds are affected by processing conditions. On the other hand, lycopene concentration was higher in processed samples, maybe because it is rather stable to storage and cooking conditions [8, 9], and also because sometimes the manufacturing process includes dehydration, and hence those compounds are concentrated after water loss. These results show that the developed technique is appropriately sensible and can be applied for nutritional analysis and quality control of tomato derived foods.

4. Conclusions

A high throughput methodology based on HPLC-DAD using a C_{30} core-shell column approach for the simultaneous determination of carotenoids with different polarities in tomato products was proposed for the first time. The resolution of target analytes was achieved in about 16 min, with UHPLC performance without build-up pressure. The achieved sensitivity was good enough to assure reliable quantification at levels commonly found in fresh and processed tomatoes, with suitable precision and linear response ranges. As well, the accuracy of the method was demonstrated when the recovery study was performed over spiked tomato samples. The applicability of the developed method was confirmed by the suitable quantification of target analytes in different tomato matrices. As result, the method is a suitable alternative because of its sensitivity, simplicity, rapidity and compatibility with conventional HPLC systems. Finally, the determination of major carotenoids with different polarities in a single run

with the method proposed here could be useful for a high throughput quality control on the food industry. Besides the method has good analytical features and a lot of potential in quality control of tomato products, future developments related to improve the proposed protocol by increasing the number of quantified carotenoids in a single injection, not only for characterization purposes but also for evaluating physiological changes of tomato plants should be performed. In addition, application in different samples and the inclusion of non-colored carotenoids should be evaluated to expand the usefulness of the method on the quantification of a high number of carotenoids of different chemical nature at the same chromatographic run.

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Table 1. Effect of the injection solvent on the carotenoids content. Values are average concentrations (mg kg^{-1} freeze-dried sample) ± standard deviations of n=3 replicates, and different letters within each column indicate statistically significant differences.

Analyte	Methanol	MTBE	Methanol:MTBE (1:1)	*n*-Hexane	Initial mobile phase
Lutein	10.1 ± 0.6 b	2.9 ± 0.2 c	11.8 ± 0.7 a	2.1 ± 0.1 c	10.5 ± 0.6 b
ß-carotene	4.1 ± 0.1 c	5.1 ± 0.2 a	5.1 ± 0.2 a	4.6 ± 0.2 b	4.1 ± 0.2 c
Lycopene	31.9 ± 0.7 d	409.1 ± 8.4 a	402.5 ± 8.2 a, b	397.1 ± 8.1 b	51.2 ± 1.1 c

Table 2. Analytical performance and absolute recoveries (%, as an estimation of accuracy) of the proposed method for freeze-dried tomatoes spiked at different concentration levels.

Analyte	**Linear range (mg L^{-1})**	**r^2**	**LOD (mg kg^{-1})**	**LOQ (mg kg^{-1})**	**Recovery (%)[a] ± RSD**	
					Level 1	Level 2
Lutein	0.0625-10	0.9996	0.03	0.08	116 ±11	93 ±6
ß-carotene	0.125-20	0.9971	0.10	0.33	109 ±3	86±4
Lycopene	0.5-75	0.9967	0.46	1.53	104 ±9	118 ±2

[a]Recoveries were calculated as described in text. n = 3 replicates. Level 1: 5 mg kg^{-1} for lutein and ß-carotene, and 20 mg kg^{-1} for lycopene. Level 2: 20 mg kg^{-1} for lutein and ß-carotene, and 100 mg kg^{-1} for lycopene.

Table 3. Intra and inter-day precision of the proposed methodology for the determination of carotenoids.

	Precision (RSD, %)			
	Intra-day [a]		**Inter-day [b]**	
Analyte	Level 1	Level 2	Level 1	Level 2
Lutein	14.1	6.1	16.3	9.1
ß-carotene	3.1	3.7	8.3	9.4
Lycopene	9.6	2.1	8.7	5.9

[a]n = 3 extractions at each concentration level in the same day.

[b]n = 9 extractions at each concentration level in 3 consecutive days.

Level 1: 5 mg kg^{-1} for lutein and ß-carotene, and 20 mg kg^{-1} for lycopene. Level 2: 20 mg kg^{-1} for lutein and ß-carotene, and 100 mg kg^{-1} for lycopene..

Table 4. Comparison of different HPLC and UHPLC methods for the determination of carotenoids in different matrices.

Method	Analytes	Matrix	Total run time (min)	Flow (mL min^{-1})	Column type	LOQs (µg mL^{-1})	Reference
LC-DAD	lutein, ß-carotene, lycopene	Tomato and its products	35	0.5	YMC C_{30}; (150 x 4.6 mm; 5 µm)	0.12-0.14	[35]
RP-HPLC-PDA	lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene	Starchy staples	15	2	Prontosil 200-3-C_{30}; (150 x 4.6 mm; 3 µm)	0.024-0.041	[28]
UHPLC-PDA	β-Carotene, lycopene, lutein, β-cryptoxanthin, astaxanthin, canthaxanthin, zeaxanthin, phytoene, violaxanthin, neoxanthin, antheraxanthin	Maize seed	15	0.4	BEH C_{18}; (100 x 2.1 mm; 1.7 µm)	0.02-0.1	[32]
UPLC-DAD	all-trans-lutein, ß-carotene, lycopene	Tomato	18	0.3	Kinetex C_{18}; (100 x 2.1 mm; 1.7 µm)	1-10	[16]
UPLC-PDA-MS	ß-carotene, (all-E)-lutein, ß-cryptoxanthin, (all-E)-zeaxanthin, phytoene, (all-E)-violaxanthin, (9′Z)-neoxanthin, (all-E)-antheraxanthin	Fruit juices	16.6	0.5	BEH C_{18}; (100 mm x 2.1 mm; 1.7 µm)	0.07-0.52	[18]
UPLC-PDA	lutein, ß-carotene, lycopene	Wheat	6	0.6	BEH C18; (100 mm x 2.1 mm; 1.7 µm)	Not informed	[36]
HPLC-DAD	lutein, ß-carotene, lycopene	Tomato and its products	20	0.4	Accucore C_{30}; (3.0 mm x 150 mm, 2.6 µm)	0.0625-0.5	This work

Table 5. Lutein, β-carotene and lycopene contents assessed by the developed HPLC-DAD in different fresh and processed tomato samples. Values are average concentrations (mg kg^{-1} freeze-dried sample) ± standard deviations of n=4 replicates.

Sample	**Lutein**	**β-Carotene**	**Lycopene**
Yellow cherry 1	8.3 ± 0.7	8.6 ± 2.3	n.q.
Yellow cherry 2	26.2 ± 1.6	10.6 ± 2.2	n.q.
Red cherry	11.9 ± 2.9	12.1 ± 1.9	117.6 ± 14.8
Pear	7.9 ± 0.9	2.5 ± 0.3	90.9 ± 5.8
Homemade sauce	0.35 ± 0.02	5.1 ± 0.2	516.8 ± 10.2
Roasted	1.20 ± 0.04	4.7 ± 1.1	249.2 ± 6.1
Industrial sauce	0.47 ± 0.02	2.39 ± 0.09	90.1 ± 10.8

n.q.: under quantification level

Figure captions

Figure 1: Chromatograms corresponding to a standard mixture of carotenoids (0.5, 1 and 10 mg L^{-1} for lutein, ß-carotene and lycopene, respectively), and a red and yellow cherry tomato extracts recorded at 445 nm, 450 nm and 480 nm.

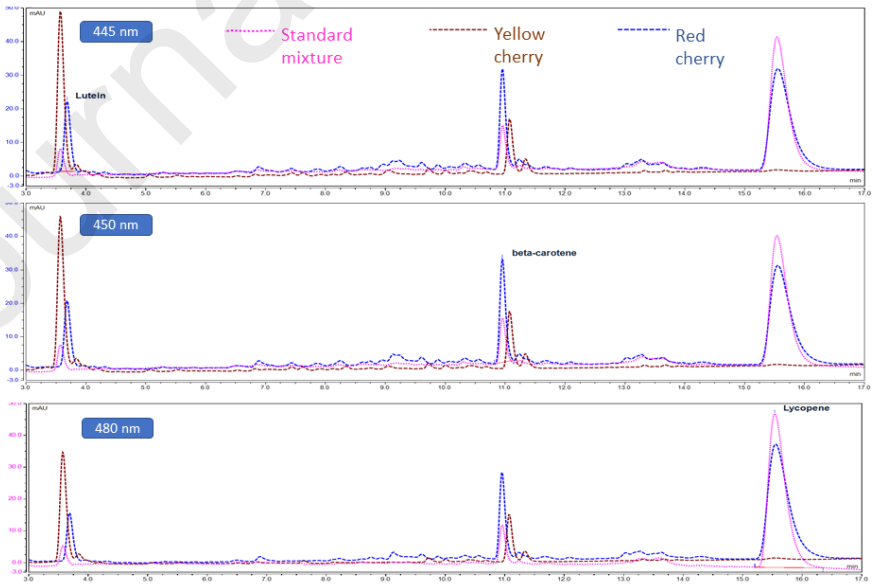
Credit Author Statement

María Victoria Salomon: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Data curation, Writing - review & editing.

Patricia Piccoli: Resources, Writing - review & editing.

Ariel Fontana: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Figure 1



Highlights

- A core-shell column approach is proposed for carotenoids determination in tomato
- Analytes with different polarities were rapidly separated without build-up pressure
- The method is rapid and economically affordable for most laboratories
- High-throughput method for quality control of carotenoids on the food industry