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ORIGINAL PAPER

An improved and fast UHPLC-PDA methodology for determination of *L*-ascorbic and dehydroascorbic acids in fruits and vegetables. Evaluation of degradation rate during storage

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Abstract This study provides a versatile validated method to determine the total vitamin C content, as the sum of the contents of *L*-ascorbic acid (L-AA) and dehydroascorbic acid (DHAA), in several fruits and vegetables and its degradability with storage time. Seven horticultural crops from two different origins were analyzed using an ultra-high-performance liquid chromatographic–photodiode array (UHPLC-PDA) system, equipped with a new trifunctional high strength silica (100% silica particle) analytical column (100 mm×2.1 mm, 1.7 μm particle size) using 0.1% (*v*/*v*) formic acid as mobile phase, in isocratic mode. This new stationary phase, specially designed for polar compounds, overcomes the problems normally encountered in HPLC and is suitable for the analysis of large batches of samples without L-AA degradation. In addition, it proves to be an excellent alternative to conventional C18 columns for the determination of L-AA in fruits and vegetables. The method was fully validated in terms of linearity, detection (LOD) and quantification (LOQ) limits, accuracy, and inter/intra-day precision. Validation experiments revealed very good recovery rate of 96.6±4.4% for L-AA and 103.1±4.8 % for total vitamin C, good linearity with r^2-values >0.999 within the established concentration range, excellent repeatability (0.5%), and reproducibility (1.6%) values. The LOD of the method was 22 ng/mL whereas the LOQ was 67 ng/mL. It was possible to demonstrate that L-AA and DHAA concentrations in the different horticulture products varied oppositely with time of storage not always affecting the total amount of vitamin C during shelf-life. Locally produced fruits have higher concentrations of vitamin C, compared with imported ones, but vegetables showed the opposite trend. Moreover, this UHPLC-PDA methodology proves to be an improved, simple, and fast approach for determining the total content of vitamin C in various food commodities, with high sensitivity, selectivity, and resolving power within 3 min of run analysis.

Keywords Ascorbic acid · Dehydroascorbic acid · Total vitamin C · Ultra-high-performance liquid chromatography · Validation · Fruits and vegetables

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Introduction

Vitamin C (2,3-endiol-*L*-gulonic acid-γ-lactone) is one of the most important vitamins for human nutrition, supplied by diet. Synthesis of collagen, carnitine, and hormones; immune response; iron absorption; and cell protection against free radicals are some of its crucial physiological roles [1–3]. It is also used by the food industry, as a nutritional food additive or antioxidant, preventing oxidation of foods [3, 4]. Since humans cannot synthesize vitamin C, an adequate intake of vitamin C from foods and/or supplements is vital for normal functioning of the human body [3, 5, 6]. *L*-ascorbic acid (L-AA) is a water-soluble vitamin widely distributed in plant material. Vegetables and fruits, particularly citrus and tropical fruits, green leafy vegetables, broccoli, and peppers, are important sources of vitamin C [4, 7–9]. However, fruits such as pears, apples, and plums contain only a very modest concentration of this vitamin.

According to the literature, vitamin C concentration varied considerably among the type of plant and cultivar, being influenced by genetic factors, maturity stage, environmental and cultural practices, and post-harvest conditions [7, 10].

"Vitamin C" refers to all compounds exhibiting equivalent biological activity of L-AA, including its oxidation products, esters, and synthetic forms [5, 10]. L-AA is the main biologically active form of vitamin C and is reversibly oxidized to dehydroascorbic acid (DHAA), due to the presence of two hydroxyl groups in its structure. This reaction is a principal step in the antioxidant activity of L-AA, due to its high electron donating power and ready conversion back to the active reduce form [2, 3, 8]. Further oxidation originates diketogulonic acid, which has no biological function, and the reaction is no longer reversible [1, 10].

Various factors, including the presence of oxygen and metal ions (especially Fe^{3+}, Ag^{+}, Cu^{2+}), alkaline pH, high temperature, and enzymatic reactions induce oxidation reactions of L-AA in raw products [1, 3, 7]. DHAA exhibits equivalent biological activity to L-AA, so it is important to measure both molecules to know total vitamin C content in foodstuffs. In many horticultural crops, DHAA represent less than 10% of total vitamin C, but DHAA tends to increase during storage [10].

The content of vitamin C in food commodities (the sum of the contents of L-AA plus DHAA) is used as an index of the health-related quality of products, since, as compared with other beneficial compounds, it is more sensitive to degradation by processing and storage. Therefore, interest in the simultaneous analysis of these molecules has also increased greatly in food analysis [1, 5, 9]. However, some authors do not take into account DHAA, when reporting vitamin C content in food commodities [1].

An accurate and specific determination of vitamin C content on foodstuffs is extremely important to understand the relationship of dietary intake and human health [11]. To ensure that the subsequent analysis is effective, it is very important to optimize sample extraction when analyzing vitamin C in complex samples, such as fruits and vegetables [4, 11]. Due to the labile nature of vitamin C, preparation procedures are designed to avoid loss of vitamin [9]. Light, pH, and temperature, among others, must be controlled during preparation of samples for analysis, especially if the procedures involve maceration or other disruption of cells. Failure to assess stability of vitamin C in raw products during sample processing, and analysis could result in significant errors in analytical results [3]. Methods for the extraction of L-AA are based on the use of solvents with different properties, being metaphosphoric acid the most common solvent used, which prevents L-AA oxidation, inhibits ascorbate oxidase and metal catalysis, and, in addition, precipitates proteins [4, 9, 12].

There are many methods, based on different analytical techniques, to measure L-AA content in foodstuffs, such as spectrophotometric, titration, or enzymatic methods, but the liquid chromatographic (LC) with ultraviolet (UV) or diode array methods are the preferred, due to their separation capability, simplicity, short analysis time, high sensitivity, and accuracy [4, 13]. Moreover, unlike other methods, LC allows the determination of L-AA and DHAA in various matrices. However, simultaneous detection of both L-AA and DHAA is quite a complicated analytical problem [1]. Because of the low absorptivity of DHAA in the UV range of the spectrum, usually, DHAA is determined indirectly as the difference between the total vitamin C after DHAA reduction and L-AA content of the original sample, by the so-called subtraction method. Various reducing agents, such as homocystein, DL-1,4-dithiothreitol (DTT) and dimercaptopropanol (BAL) have been studied [9, 11, 14, 15]. In addition to UV detection, simultaneous determination of L-AA and DHAA can be achieved by fluoresce detection (derivatization of DHAA with *O*-phenyldiamine) or by mass-spectrometry detection [1, 11, 12]. Recently, charged aerosol detection has been presented as an alternative detection system in HPLC for the direct analysis of L-AA and DHAA [16].

The ultra-high-performance liquid chromatography (UHPLC) is the latest advance of chromatographic separation techniques and is based on the same principles of high-performance liquid chromatography (HPLC). However, it has some features that distinguish it from the HPLC and make its application more advantageous, namely higher efficiency and resolution, shorter time analysis, and increased sensitivity [17]. The development of columns of very small particle size allows for the use of much lower flows of mobile phase at very high pressures. UHPLC has been used for the determination of fat-soluble vitamins but less so for hydrosoluble ones (apart from some mentions of manufacturers brochures) [18, 19]. Xingnan and Adrian [20] developed and validated an UHPLC method for the quantification of serum L-AA and DHAA, by the subtraction method. The obtained results showed the effectiveness of separation of the method, with higher sensitivity and accuracy and reduced analysis time compared with HPLC.

Because Madeira Island is a Portuguese region with excellent climate conditions for the production of some exotic fruits and vegetables [8] but not self-sufficient, importing some of these commodities, we developed a small comparative evaluation of vitamin C contents and its degradation on locally produced and imported foodstuffs.

In the present work, a simple, rapid, and improved methodology for the analysis of L-AA, DHAA, and total vitamin C content in fruits and vegetables by ultra-high-performance liquid chromatographic–photodiode array (UHPLC-PDA), using as stationary phase a new advanced T3 bonding process (HSS T3), which utilizes a trifunctional C_{18} alkyl phases bonded at ligand density that promotes polar

compound retention and aqueous mobile phase compatibility, in order to analyze a large batch of samples in a short period of time, is reported. This phase allows a fast analysis of vitamin C and L-AA, without using of buffer solution and pH adjustments, using an isocratic mode elution. This method was optimized and carefully validated by evaluating the selectivity, the linear range, the limits of detection and quantification, the accuracy, and repeatability. A comparative assessment of vitamin C losses in different vegetables and fruits was performed.

Experimental

Chemicals and reagents

All reagents and standards were of analytical grade. *L*-ascorbic acid (L-AA), metaphosphoric acid (MPA) and formic acid were purchased by Panreac (Madrid, Spain). Acetic acid, ethylendiaminetetraacetic acid disodium salt (EDTA), Tris buffer were obtained from Merck (Darmstadt, Germany), DTT from Acros-Organics (Geel, Belgium), and sulfuric acid from Riedel-de Haen (Seelze, Germany). All solutions were prepared with water from a Milli-Q Direct 8 ultrapure water purification system (18 M Ω cm at 23 °C) (Millipore, USA). A standard stock solution of L-AA (200 μg/mL) was freshly prepared on each day of analysis in 3% MPA–8% acetic acid–1 mM EDTA solution and stored in amber flasks at 4 °C prior to chromatographic analysis. Solutions of variable concentrations were prepared by diluting the standard stock solution with mobile phase (0.1% (*v*/*v*) formic acid).

Raw material

The edible portion of fruits, passion fruits (*Passiflora edulis Sims*), papayas (*Carica papaya L.*), strawberries (*Fragaria× ananassa*), lemons (*Citrus limon (L.) Burm.F.*), and vegetables, broccoli (*Brassica oleraceae L. var. Italica plenk*) and green and red peppers (*Capsicum annuum L.*), grown in Madeira Island and abroad was analyzed. Food commodities were supplied (at least 3 kg of samples) by a national food distributor (Sonae MC) with connections to local registered producers, from February until May 2011. Local products were delivered by Sonae to Organic Chemistry and Natural Products Laboratory (Madeira Chemical Center-CQM) 1 or 2 days after harvest. All foodstuffs were immediately stored in a common refrigerator at 4 °C before analysis.

Sample preparation

The methodology applied was developed according to Fenoll [5], with slight modifications: A representative amount of each type of foodstuff (between 100 and 200 g, depending on species) was homogenized, in a prechilled blender (Moulinex), to ensure the sample homogeneity. There were used approximately ten specimens in each analysis of passion fruits, strawberries, and broccoli (florets). In case of papayas, peppers, and lemons, slices from the specimens were taken for each analysis. For extraction of L-AA, an aliquot of 3 mL of fresh pulp of target fruits and vegetables was added to 10 mL of extraction solution (3%MPA–8% acetic acid–1 mM EDTA). The homogenate was thoroughly vortexed and then centrifuged at 10,000 rpm (refrigerated at 2–4 °C) for 10 min, using a centrifuge Sigma 3K30 Bioblock Scientific. The pellet was discharged and the supernatant filtered through 0.22 μm PTFE filters (Millipore, USA). Extractions were performed in triplicate on the day of delivery and within five consecutive days, in order to evaluate the stability of L-AA. Passion fruits, the first to be studied, were subject to extraction periodically during 15 days. After its analysis, we decided to change the methodology because of the shorter shelf-life of the other food commodities. Therefore, instead of periodic extractions within 15 days of storage, we changed for consecutive extractions during a 5-day period.

The resulting extracts were stored at −80 °C, immediately after extraction until analysis (within 1 week). Before analysis, the extracts were thawed in a cold water bath, and afterwards the solutions were diluted with mobile phase (0.1%, *v*/*v*, formic acid) in the range of 1/10–1/20, depending on the sample matrix. Subsequently, the samples were stored at 4 °C until injection.

Determination of total vitamin C was performed as proposed by Campos [21]: Tris buffer containing DTT was added to the extracts to obtain a final concentration of 20 mM DTT. The mixture was kept protected from light at room temperature for 30 min in order to convert any DHAA to L-AA. The reaction was interrupted by adding 0.4 M H_2SO_4, and the injection was made immediately afterwards. The DHAA content of the sample was calculated by subtracting the initial L-AA content from the total vitamin C content, after reduction with DTT. All samples were analyzed in triplicate (n=3). To prevent oxidation of L-AA from standard solutions and samples, all the operations were performed under reduced light (using amber flasks), minimal oxygen exposure, and low temperature.

Chromatographic conditions

The analysis were carried out on an Acquity UPLC system (Waters Corp., USA), equipped with a Waters Acquity UPLC photodiode array (PDA) detection system. The detection signal was recorded, and the peak areas were quantified and processed with Empower™ software (also from Waters, USA). The chromatographic system was equipped

with a Acquity HSS T3 analytical column (100×2.1 mm, 1.8 μm particle size), supplied by Waters (Sacavém, Portugal), using an isocratic mobile phase composed of aqueous 0.1% (*v/v*) formic acid at a flow rate of 250 μL/min. The injection volume was 2 μL. The detection wavelength for the PDA was set at 245 nm (maximum wavelength absorbance), and the analytical column was kept at room temperature. All standard solutions and extracts were filtered through 0.22 μm PTFE membrane filters (Millipore, USA) before injection in the chromatographic system. The L-AA identification in samples was carried out not only by comparison with the standard retention time but also matching with the UV absorption spectrum. Quantification was carried out with the external standard method (L-AA standards with various concentrations) using the following expression to calculate the L-AA concentration (C_A) on samples:

$$C_A(\mathrm{mg}/100\mathrm{g}) = \frac{A_a \times D \times RF \times 100}{m}$$

where A_a is the peak area of the analyte, D is the sample factor dilution, RF is the response factor, and m is the sample weight. The RF is a way to adjust the proportionality of the detector response to the concentration of L-AA and is calculated by the following expression:

$$RF = \frac{Cst}{Ast}.$$

Where Cst is the standard working solution concentration (50 μg/mL) and Ast is the corresponding peak area.

Method validation

The reliability of UHPLC-PDA method was validated through its selectivity, linearity, sensitivity, precision, and recovery.

The selectivity of the method was evaluated by comparison of retention times and UV–vis spectrum of L-AA standards peaks with those of fruits and vegetables extracts.

Linearity was tested with the external standard method, and calibration graphs were constructed by least-squares regression of peak area (AUs) versus concentration (micrograms per milliliter) of the calibration standards. The calibration line was divided in two linear ranges based on six concentration levels of L-AA standards (Table 1). Each point was the average of three peak area measurements (n=3).

The method sensitivity was evaluated through evaluation of LODs and LOQs, calculated from the calibration lines that defined linearity, using the following equations:

$$\mathrm{LOD} = \frac{3.3 \times \sigma}{m} \quad \mathrm{LOQ} = \frac{10 \times \sigma}{m}$$

Where σ is the standard error of the intercepted point and m is the slope of the calibration line.

Intra-day precision (repeatability) of the method was determined by analyzing six replicates of fortified samples of each foodstuff with L-AA standard at one concentration level (10 μg/mL) on the same day and expressed as the percentage of the relative standard deviations (RSD) (n=6).

Recovery was tested by the standard addition procedure at two levels (10 and 50 μg/mL). Samples of target fruits and vegetables were fortified with standard solutions before extraction. In each additional level, six determinations were carried out (n=6), and the recovery of L-AA and total vitamin C was determined using the UHPLC-PDA method applied in this study, as follows:

$$R(\%) = \frac{C_{\mathrm{AF}} - C_{\mathrm{AD}}}{C_{\mathrm{st}}}$$

Where C_{AF} is the concentration of L-AA in fortified samples, C_{AD} is the concentration of L-AA in samples without fortifying, and C_{st} is the concentration of standard solution added to the samples.

Results and discussion

Method validation

Chromatographic conditions were optimized to reduce the analysis time while keeping a good resolution between the peaks of L-AA and other co-extracted compounds in the samples. Good results were obtained using a mixture of water and formic acid as the mobile phase, as described by Quirós [13]. This mobile phase presents the advantages of being cheap and easy and rapid to prepare. The flow rate significantly influenced L-AA retention time: The best flow value was 250 μL/min, due to better back-pressure (approximately 5,800 psi), retention times (approximately 2 min) and resolution for L-AA peaks. To check the feasibility of the UHPLC-PDA method for the analysis of *L*-ascorbic acid, the following parameters were determined: selectivity, linearity, sensitivity, precision, and recovery. According to Fig. 2, the retention time of the L-AA peak of L-AA standard is in agreement with that of passion fruit extract, and the peak was free from interferences. Moreover, the UV spectrum obtained from the samples is identical to that of L-AA standards. Similar findings were obtained from other samples. Thus, the method is capable of separating the compound of interest from other components of the sample.

Good linearity was achieved in both ranges of concentrations tested, with correlation coefficients (r) higher than 0.999 in all cases. The proposed method showed a good sensitivity with LOD and LOQ of 22 and 67 ng/mL, respectively (Table 1). The LODs and LOQs were calculated with the calibration line on the 0.05–2 μg/mL range, since it

Table 1 Linearity and sensitivity for the assayed UHPLC-PDA method to determine L-AA

Range of linearity (μg/mL)	Regression equation[a] (y)	Correlation coefficient (r)	Standard error[b]	LOD (μg/mL)	LOQ (μg/mL)
0.05–100	27698x–2032.6	0.9999	3272.7	0.390	1.182
2–100	27735x–4569.5	0.9999	7642.5	0.909	2.756
0.05–2	27179x–146.9	0.9998	181.42	0.022	0.067

[a] y=slope±intercepted point (n=3)

[b] Standard error of the intercept point of the calibration line

showed higher sensitivity values compared with the calibration line on the 2–100 μg/mL range.

The method showed also a satisfactory precision. All the RSD values achieved for peak areas with this UHPLC-PDA method were lower than 4% (Table 2). The results obtained showed that the UHPLC-PDA method applied has a good reproducibility and that is stable and reliable.

The recovery rate of L-AA and total vitamin C was evaluated to assess the extraction efficiency of the proposed method and matrix effects. Satisfactory results were found with recoveries, as shown in Table 3. Very good mean recovery rates were obtained for L-AA and total vitamin C—96.6%± 4.4% and 103.1%±4.8%, respectively.

The proposed method was considered adequate, according to the selectivity, linearity, precision, and recovery results. Thus, it can be employed in the analysis of food commodities. In Table 4, we present a comparison between the results obtained in this work with data of other similar HPLC analysis. In general, our method showed a higher sensitivity and accuracy than most other methods presented in Table 4. Also, our retention time was lower than the others, with the exception of the method developed by Li and Franke [20].

Application of the method

Once the analytical method was validated, it was used for determine the L-AA in foodstuffs. The L-AA analysis and the preparation of the samples were realized in a short period of time because ascorbic acid is easily oxidized in solution. The type of extraction medium is very important in order to prevent the irreversible oxidation of AA to DHAA and DKG. The use of a high ionic strength acidic extraction solvent is required to suppress metabolic activity upon disruption of the cell and to precipitate proteins. A metal chelator such as EDTA is also usually required. As suggested by various authors [1, 3, 11, 12], the use of MPA is the best way to extract and stabilize L-AA. For this reason, the samples were extracted with 3% MPA–8% acetic acid–1 mM EDTA solution, which is known to limit L-AA degradation to less than 5% [4]. This was in agreement with the recovery tests, which showed that this extraction solution is effective on preventing the oxidation of L-AA. There are several factors that negatively influence vitamin C stability, thus it is necessary to keep the influences of these variables to a minimum. The adoption of a simple and rapid method is essential to avoid loss of *L*-ascorbic acid. Working at low temperatures, protecting samples from light and oxygen, provides additional protection. Figure 1b shows a typical chromatogram of L-AA obtained pre- and post-reduction under the established conditions. Initially, passion fruit extracts showed a clearly defined peak but with some minor (unidentified) shoulder peaks that were not completely resolved from the L-AA peak. This interference was eliminated effectively with a 1/20 dilution instead of the initial 1/10 dilution. The passion fruit chromatograms showed a good peak shape of L-AA without tailing and a suitable retention time.

Table 2 Repeatability of the assayed UHPLC-PDA method, calculated as the RSD for six replicate (n=6) injections at one concentration level (10 μg/mL)

Matrix	L-AA concentration (mg/100 g of product)	RSD (%)
Lemons	13.54±0.36	2.7
Passion fruits	11.95±0.40	3.3
Papayas	35.45±0.99	2.8
Strawberries	22.58±0.89	3.9
Broccoli	30.77±0.33	0.9
Green peppers	37.15±0.96	2.9
Red peppers	61.09±1.53	2.5

In the present method, UHPLC-PDA analysis did not apply to the direct determination of DHAA because it is a difficult task. There is always a need for redox reaction in order to reduce DHAA to L-AA, and subsequent subtraction, if simultaneous analysis of both compounds using one detection technique needs to be performed. After the reduction, no DTT and DHAA peaks were present in the chromatogram (Fig. 2b). No double peaks were observed, and the L-AA peaks were also free from interferences from other concomitants or the solvent peak. This was confirmed by recording the spectra of different peaks in the chromatograms. The reduction of DHAA to L-AA was efficiently catalyzed by DTT, since

Table 3 Recovery of the assayed UHPLC-PDA method to determine L-AA and total vitamin C in fruits and vegetables

Samples	Recovery of L-AA[a] (%)			Recovery of total vitamin C[a] (%)		
	Level I 10 (μg/mL)	Level II 50 (μg/mL)	Mean recovery	Level I 10 (μg/mL)	Level II 50 (μg/mL)	Mean recovery
Passion fruits	101.3±1.9	103.7±2.3	102.5±1.7	112.7±2.9	109.8±1.9	111.3±4.3
Strawberries	103.7±1.6	92.6±2.8	98.2±4.0	107.1±1.6	97.4±3.1	102.2±5.4
Papayas	96.0±3.7	95.5±4.7	95.7±0.5	95.1±1.3	101.5±2.8	98.3±4.0
Lemons	93.4±2.4	84.5±2.9	88.9±2.9	106.0±1.6	87.4±3.2	96.7±2.4
Broccoli	96.3±3.2	95.4±2.8	95.9±0.7	104.0±2.3	102.6±2.4	103.3±2.6
Green peppers	102.2±1.0	87.0±2.2	94.6±7.4	106.4±4.1	103.1±2.4	104.7±3.7
Red peppers	101.0±3.6	99.1±3.8	100.1±1.6	105.8±1.8	104.1±3.5	104.9±1.1

[a] Recovery mean±standard deviation (n=6 in each level)

we can observe an increase of L-AA absorbance after reduction (Fig. 2b).

The concentration of L-AA, DHAA, and total vitamin C for the analyzed foodstuffs are graphically shown in Figs. 3 and 4. Initially, the L-AA content ranged between 27.8±2.3 and 223.7±16.5 mg/100 g of edible portion in the different horticultural products. Peppers (red and green) and papayas were the species with the highest initial vitamin C contents. The food commodities with the lowest content of total vitamin C were passion fruits. Broccoli, despite showing oxidation during storage, had very little loss of total vitamin C during the period under study. Generally, vitamin C determination results are in good agreement with those reported in the literature using HPLC analysis, taking in account the normal variations due to living systems [5, 8, 9, 11, 22–32]. Those graphics also allow the observation of the oxidation trends of L-AA to DHAA and total vitamin C degradation with time of storage at 4 °C during the shelf-life of the produce.

L-AA was always the predominant form of vitamin C, but DHAA content increased during storage, being more significant on imported products. Locally produced fruits have higher concentrations of vitamin C, compared with imported ones, but vegetables showed the opposite trend. In several cases, such as peppers (red and green) and papayas, there is accentuated oxidation and degradation after day 3. These are the species which were subject to cutting in day 1. Wounding of fruits and vegetables tissues induces a series of physiological disorders that led to the degradation of L-AA.

The fact that local fruits have been harvested later than imported counterparts may justify the higher vitamin C contents obtained. The variability of total vitamin C contents within the type of foodstuff might be attributed to genetic variations, and environmental and cultural practices [7, 10]. The increase in DHAA content in foods is related to adverse post-harvest conditions that promote the degradation of L-AA. The ratio between the amount of vitamin C and total DHAA can vary greatly with the type of plant conditions and storage time [10]. Although the behavior of degradation is similar in all horticultural products, the extent of losses depended on the characteristics of each matrix. The rates of degradation of vitamin C in fruits and vegetables are affected by factors such as surface area, pH, temperature, moisture content, presence of oxygen, water activity, light and temperature rise, and the storage time. Each product has its specifications and requires different care and maintenance [3].

Table 4 Overview of chromatographic methods for determination of *L*-ascorbic acid

Ref.	LOD (ng/mL)	LOQ (ng/mL)	RSD (%)	L-AA recovery (%)	L-AA t_R[a] (minutes)
Present work[b]	22	67	0.9–3.9	88.9–102.5	2
Li and Franke [20]	54	270	0.6–5.4	–	0.9
	114	570	0.3–4.5	–	3.6
Hernández et al. [11]	100	–	1.97–10.9	99	6.0
Odriozola et al. [9]	17	57	0.6–3.9	93.6–104.4	–
Sanchéz et al. [14]	97	323	6.4	93.3	4.8
Valente et al. [8]	35	90	–	82.2–95.9	4.0
Fenoll et al. [5]	13	44	1.6–2.8	81–109	4.1
Frenich et al. [12]	10	50	8.7	85	4.0

[a] Retention time

[b] UHPLC analysis

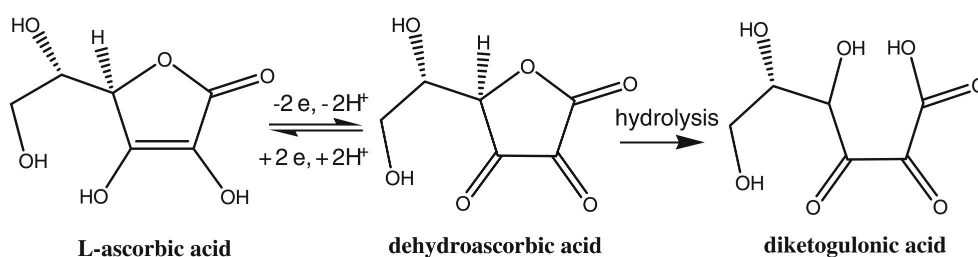

Fig. 1 Oxidation/reduction of *L*-ascorbic acid

Post-harvest losses in nutritional quality, particularly vitamin C content, can be enhanced by extend storage duration and advance state of maturity. With the exception of strawberries, imported fruits had higher losses of L-AA content than local ones. On the other hand, imported vegetables had highest losses of L-AA content that local counterpart. Foodstuffs with the highest daily average losses were local papayas (6.41%±0.17% and 6.06%±0.89% per day, respectively) and imported green and red peppers (5.29%±0.56% and 5.06%±0.49% per day). Papaya stands out for its high perishability, when subjected to cutting or slicing. The low pH of lemon pulp can justify the results obtained losses of 2.79%± 0.29% and 2.48%±0.47% per day for imported and local lemons, respectively, for the acidity of the fruit slows the oxidation of L-AA.

Storage of fruits and vegetables at 4 °C led to nutritional changes in both intact and cut foodstuffs. However, the lifetime of the latter was much smaller. We mimic the normal conditions of household packaging (common refrigerator storage at 4 °C). The results showed that the methodology was not suitable for the storage of foodstuffs wounded, as it resulted in heavy loss of L-AA contents. Fruits and vegetables that have not been kept intact should have been kept at lower temperatures with the aim of slowing the oxidation reactions.

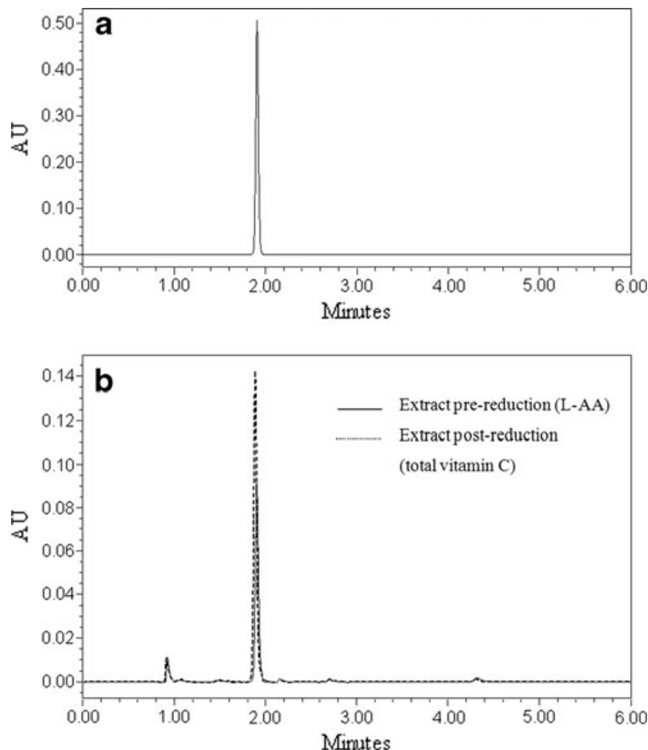

Fig. 2 Typical chromatograms of: (**a**) standard of L-AA (50 µg/mL; λ=245 nm), and (**b**) passion fruits (dilution 1/20) before and after reduction with DTT

The results obtained show the importance of determining total vitamin C content, since the evaluation of L-AA only leads to an underestimation of nutritional value. Moreover, it demonstrates that temperature and minimal processing of foodstuffs are the major factors that contribute to L-AA oxidation, during storage

Dietary reference intake (DRI) of vitamin C given for healthy males and females, >19 years, is 90 and 75 mg/day, respectively. Table 5 presents the nutritional information regarding L-AA, in accordance with the contribution of daily requirements established by the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences [33].

From the seven horticultural products studied, three can be considered rich sources of vitamin C, namely red and green peppers and papayas. Broccoli, strawberries, and lemons are good sources of this nutrient, and passion fruits have lower content of vitamin C. According to Table 4, vegetables represented a better source of this nutrient than fruits. These food commodities should be consumed regularly and preferably as fresh as possible, due to their nutritional value and especially their vitamin C content. Indeed, all the seven analyzed horticultural products provide more than 30% of DRI for vitamin C.

The major improvement of the methodology makes use of a very simple but very effective mobile phase that promotes ion suppression at very high pressures with a trifunctional high strength silica column specially designed for polar compounds, overcomes the problems normally encountered in HPLC, and is suitable for the analysis of large batches of samples without L-AA degradation. The novel stationary phase proves to be an excellent alternative to conventional C18 columns for the determination of L-AA in fruits and vegetables. Since ascorbic acid is a very polar and very small molecule, time of analysis is not a very difficult issue to overcome, since the retention time in a reverse phase column is usually very short leading to poor resolution. In fact, most HPLC methods described in the literature make use of complex mobile phases with more than

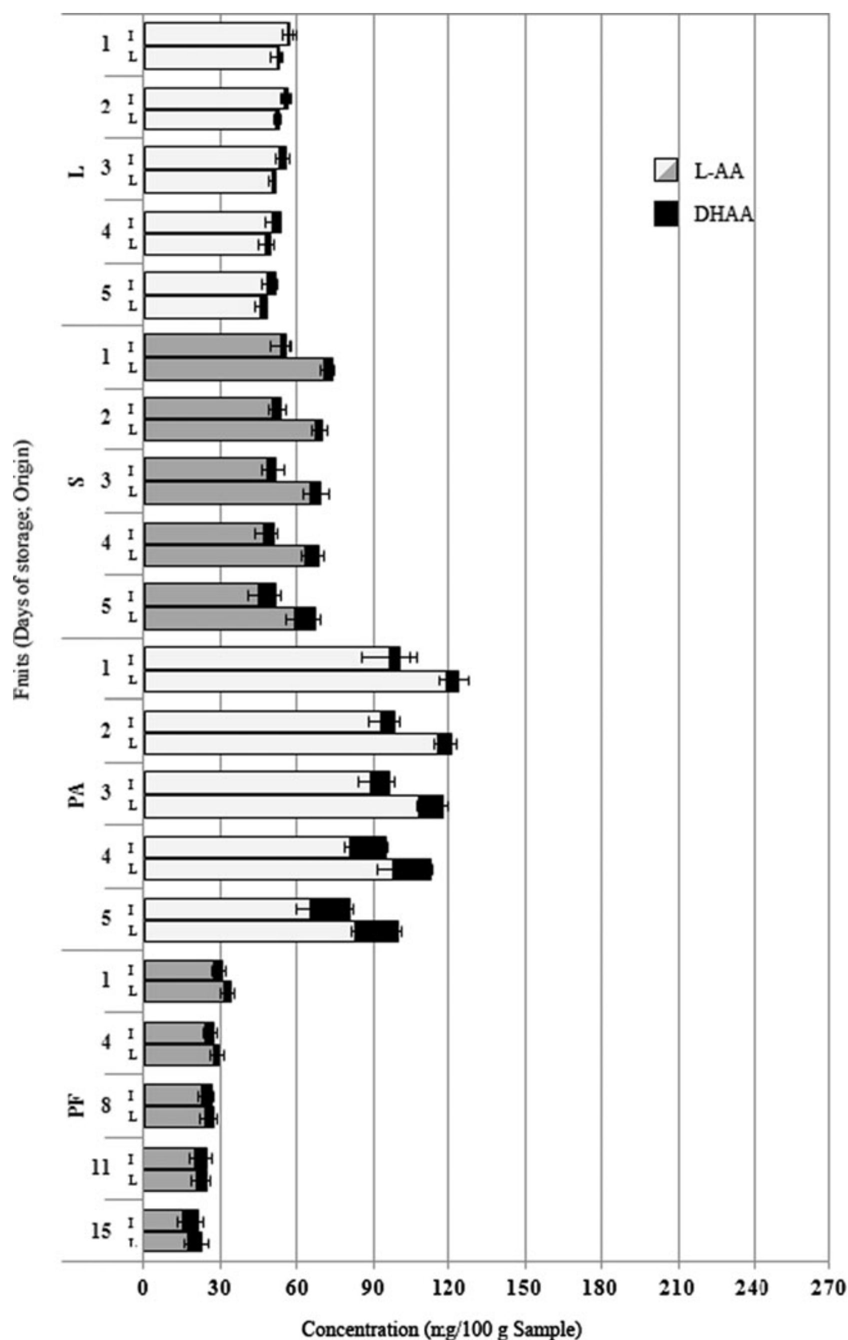

Fig. 3 Profile of total vitamin C (L-AA + DHAA) content on local and imported fruits and its degradation with storage time. *L* lemons; *S* strawberries; *PA* papayas; *PF* passion fruits; *I* imported; *L* local

two components and containing several modifiers, in order to achieve optimal retention times. Another drawback usually found is the necessity of ion suppression, i.e., working at a pH lower than ascorbic acid pKa 4.7 which often leads to degradation of columns due to silica solubility at low pH.

Conclusion

In the current study, a rapid, sensitive, and reproducible UHPLC-PDA-based methodology, using a 100 mm analytical column (Acquity HSS T3) packed with 1.8 μm particles, was developed, validated, and successfully applied to the analysis of vitamin C and L-AA in fruits and vegetables. After a careful selection of the eluent systems, it was demonstrated that the chromatographic separation of the L-AA could be achieved within 2 min. The combination of the shorter running time with a smaller flow rate also reduced drastically the solvent consumption and thus is more environmental friendly than conventional HPLC. The validated method showed a good performance with regard to selectivity, LODs, LOQs, linearity, extraction yields, accuracy, and intra/inter-day precisions.

The results demonstrated that the method revealed as an attractive and very promising approach for the analysis of

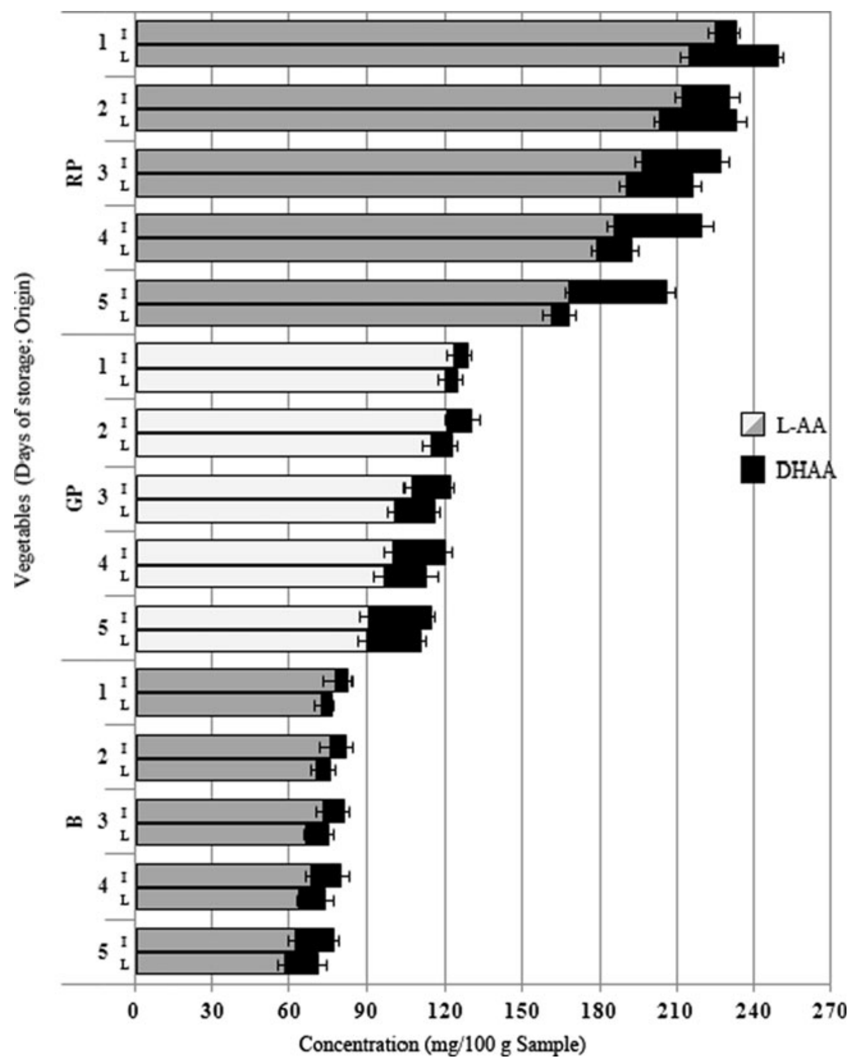

Fig. 4 Profile of total vitamin C (L-AA + DHAA) content on local and imported vegetables and its degradation with storage time. *RP* Red peppers; *GP* Green peppers; *B* Broccoli; *I* Imported; *L* Local

Table 5 Total vitamin C content in seven different horticultural products and contribution (%) for vitamin C DRI in healthy men and women, in accordance with the contribution of daily requirements established by the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences [33]

Sample	Total vitamin C (mg/100 g product)[a]	Contribution (%) for total vitamin C DRI	
		Men	Women
Fruits			
Local passion fruits	27.9±4.1	31.0%	37.2%
Imported passion fruits	26.0±3.7	28.9%	34.7%
Local strawberries	69.9±2.4	77.7%	93.3%
Imported strawberries	52.8±2.1	58.7%	70.5%
Local papayas	114.7±9.5	127.4%	152.9%
Imported papayas	93.8±7.8	104.2%	125.0%
Local lemons	50.7±2.0	56.3%	67.7%
Imported lemons	54.6±2.3	60.6%	72.8%
Vegetables			
Local broccoli	74.6±2.0	82.9%	99.5%
Imported broccoli	80.3±2.2	89.3%	107.1%
Local green peppers	116.8±6.1	130.0%	156.0%
Imported green peppers	122.0±6.5	135.6%	162.7%
Local red peppers	212.1±10.0	235.6%	282.8%
Imported red peppers	221.5±12.1	246.1%	294.7%

[a]Mean value of 5 different days (n=5)±standard deviation

total vitamin C and L-AA and for routine use in laboratory. In addition, the validated approach focused on fruits and vegetables could be extended to other type of food commodities namely processed foods.

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