



**HAL**  
open science

# Lyophilized tomato plant material: Validation of a reliable extraction method for the analysis of vitamin C

Sylvie Serino, Guy Costagliola, Laurent Gomez

## ► To cite this version:

Sylvie Serino, Guy Costagliola, Laurent Gomez. Lyophilized tomato plant material: Validation of a reliable extraction method for the analysis of vitamin C. *Journal of Food Composition and Analysis*, 2019, 81, pp.37 - 45. 10.1016/j.jfca.2019.05.001 . hal-02620717

**HAL Id: hal-02620717**

**<https://hal.inrae.fr/hal-02620717>**

Submitted on 25 Oct 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

## **Lyophilized Tomato Plant Material: Validation of a Reliable Extraction Method for the Analysis of Vitamin C**

Sylvie Sérino, Guy Costagliola, Laurent Gomez\*,

UR 1115 Plantes et Systèmes de Culture Horticoles (PSH), Domaine Saint-Paul, INRA,  
228 route de l'Aérodrome, CS-40509, F-84914, Avignon Cedex 9, France;

\*Author to whom correspondence should be addressed:

Laurent Gomez, Tel.: +33 (0)432722464. E-mail address: [laurent.gomez@inra.fr](mailto:laurent.gomez@inra.fr)

1 **Abstract**

2 Vitamin C is widely studied for its protective role in humans and plants, but its  
3 quantification in fresh vegetable matrices is delicate, especially because of the unstable  
4 nature of its reduced form. Facing this aspect and with unavoidable extraction  
5 constraints, we developed and validated a method for extracting vitamin C from the  
6 lyophilized powder of tomato fruits and leaves. Easy and quick to implement by  
7 removing most of the cold constraints needed for the use of fresh powder, this method  
8 proved to be precise, accurate, and linear at the same time, across a large vitamin C  
9 concentration range (160–740 mg 100 g<sup>-1</sup> for dried fruits; 190–1140 mg 100 g<sup>-1</sup> for  
10 dried leaves). A simultaneous quantification of total and reduced vitamin C levels was  
11 performed by spectrophotometry, using a microplate reader. The evaluation of the  
12 impact of the storage conditions of the lyophilized powders on the vitamin C  
13 concentrations made it possible to optimize certain parameters of the method and to  
14 evaluate its robustness as well as the remarkable biochemical stability of the lyophilized  
15 sample.

16

17 **Keywords:** vitamin C; lyophilized powder; tomato; cold chain; spectrophotometry

18

## 19 **1. Introduction**

20 Vitamin C is of major interest for its health value, both to the plant (Truffault et  
21 al., 2014; Zaoui et al., 2016) and to humans who consume it (Camarena & Wang, 2016;  
22 Moser & Chun, 2016; Pearson et al., 2017). These characteristics and quality are largely  
23 related to the antioxidant properties of the reduced form of the molecule (ascorbic acid,  
24 AsA). In vegetables, vitamin C is naturally present in the form of AsA and in its  
25 oxidized form (dehydroascorbic acid, DHA). The sum AsA + DHA constitutes the total  
26 vitamin C (T-AsA) and DHA is recyclable in AsA under certain conditions (Truffault et  
27 al., 2016; Zaoui et al., 2016). It is essential to determine the proportion of AsA in the  
28 plant precisely, so as to be able to calculate the redox ratio (AsA/T-AsA), which is a  
29 frequently used indicator to assess its development stage or the impact of different  
30 stresses on its metabolism (Bernard et al., 2009; Burkey et al., 2003; El Airaj et al.,  
31 2013; Garchery et al., 2013; Gest et al., 2013; Massot et al., 2013). The literature refers  
32 to numerous techniques for the extraction and assay of vitamin C, but they are generally  
33 long to implement, expensive, analyze only one form, or require specific material (Liu  
34 et al., 2010; Munyaka et al., 2010; Tarrago-Trani et al., 2012). Stevens et al. (2006)  
35 were thus inspired by the method described by Kampfenkel et al. (1995) to propose a  
36 method of extraction and colorimetric assay of vitamin C both fast and inexpensive for  
37 assaying simultaneously T-AsA and AsA by microplate in about 20 samples of small  
38 size (< 1 g fresh matter). The extraction was performed by homogenization with a  
39 solution of trichloroacetic acid at 6% and the determination was based on the  
40 spectrophotometric detection of the dipyriddy-Fe<sup>2+</sup> complex formed after the reduction  
41 of Fe<sup>3+</sup> to Fe<sup>2+</sup> by the AsA present in the extracts. Since then, we have successfully used  
42 such methodology on powders of fresh tomato fruit and leaf (Gautier et al., 2008;  
43 Gautier et al., 2009; Massot et al., 2010; Massot et al., 2012; Ripoll et al., 2016a; Ripoll

44 et al., 2016b; Truffault et al., 2015). However, the technique of extraction used always  
45 brought along the major disadvantage of the constraints related to the necessary  
46 preservation of the biochemical integrity of the fresh sample. Indeed, vitamin C is very  
47 sensitive to temperature and AsA tends to oxidize quickly to DHA (Truffault et al.,  
48 2014), which complicates the reliability of extraction in fresh vegetables. This led us to  
49 search for and then validate a less restrictive alternative, consisting of extracting vitamin  
50 C from lyophilized vegetable material (DM). Studies already conducted in this regard  
51 have yielded contradictory results on the relevance of this approach. For example,  
52 Chang et al. (2006) and Stevens et al. (2006) attributed an 8% to 20% loss of vitamin C  
53 content in tomato to the freeze-drying process, unlike George et al. (2011), who  
54 observed no significant impact.

55         Based on the aforementioned context, the present study aims to report the  
56 validation of a new method of extraction of vitamin C from lyophilized tomato plant  
57 powder, which we named "new method", by verifying its accuracy compared to the  
58 results obtained with the fresh plant powder (reference methods), its precision, the  
59 linearity of its response, and the absence of a matrix effect. We also tested robustness  
60 criteria, namely the influence of the storage temperature of the lyophilized powder  
61 (room temperature,  $-20\text{ }^{\circ}\text{C}$ , and  $-80\text{ }^{\circ}\text{C}$ ), the storage time, or the impact of the mass of  
62 the analytical tested sample (TS).

63

## 64 **2. Material and methods**

### 65 **2.1. Plant material**

66         The validation of the "New method" was carried out on samples stored at  $-80$   
67  $^{\circ}\text{C}$ , resulting from a 2010 greenhouse experiment on the impact of the genotype on the  
68 texture and firmness of the tomato fruit under hydric stress (Aurand et al., 2015).

69 Samples of leaves (final stage but not senescent) and tomato fruits at mature stage (red  
70 ripe stage and seven days after postharvest storage at 20 °C) and at green stage (20 days  
71 after anthesis) were selected from six genotypes (Cervil, Levovil, VilB, NIL-L4, NIL-  
72 L9, and NIL-V9) described by Aurand et al. (2015). A wide range of vitamin C  
73 concentrations was obtained from fruits (around 160–450 mg 100 g<sup>-1</sup> DM or 10–30 mg  
74 100 g<sup>-1</sup> fresh matter) and from leaves (around 190–450 mg 100 g<sup>-1</sup> DM or 25–65 mg  
75 100 g<sup>-1</sup> fresh matter), in order to highlight a possible matrix effect. Samples were  
76 harvested in 2010, cut into pieces (the fruits), immediately immersed in liquid nitrogen,  
77 and stored at -80 °C. Then, they were finely ground in the presence of liquid nitrogen  
78 and stored at -80 °C. For validation purposes, part of this powder was freeze-dried in  
79 2015 (GENESIS 25ES; Virtis Company, Gardiner, NY) under the following conditions:  
80 freezing at -25 °C, followed by a primary freeze-drying at 0.4 mbar to eliminate free  
81 water (ending at 3 °C) and by two subsequent cycles of secondary freeze-drying with a  
82 pressure variation of 1.6 mbar at 0.001 mbar to remove the bound water (ending at 10  
83 °C). At the end of the freeze-drying process and upon return to atmospheric pressure,  
84 the dry powders were stored at -80 °C.

85

## 86 **2.2. Reagents and standards**

87 The AsA (ref. A0278-25G) used as standard and the reagents 2,2-dipyridyl  
88 (DPD), dithiothreitol (DTT), *N*-ethylmaleimide (NEM), and FeCl<sub>3</sub> were provided by  
89 Sigma (Saint-Quentin Fallavier, France). The reagents NaH<sub>2</sub>PO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub> (for  
90 phosphate buffer preparation), trichloroacetic acid (TCA), H<sub>3</sub>PO<sub>4</sub> and ethanol were  
91 obtained from VWR (Fontenay-sous-Bois, France).

92

## 93 **2.3. Extraction methods**

94 Two different reference methods were used to validate the accuracy of the “new  
95 method” (Figure 1).

96

### 97 **2.3.1. “New method”**

98 The sampling steps of each tested sample (TS) and vitamin C extraction took  
99 place at room temperature and were dissociable. Samplings consisted of weighing TS of  
100 lyophilized leaf (20 mg) or fruit (40 mg) powder in 2 mL microtubes and storing them  
101 at  $-80\text{ }^{\circ}\text{C}$  (preferably) if the extraction and analytical determination for vitamin C was  
102 not performed rapidly. After adding the extraction solvent in each microtube (1 mL  
103 TCA at 6%), the extraction by homogenization was carried out in three series of eight  
104 TS using a vibratory oscillating mill (MM301, Retsch; 30 s; 30 oscillations  $\text{s}^{-1}$ ) and  
105 accessories for agitation of up to 10 microtubes. After a first centrifugation (16,110 g, 5  
106 min at  $4\text{ }^{\circ}\text{C}$ ; Model 5415R; Eppendorf, Le Pecq, France), the 24 microtubes (limit of the  
107 centrifuge) were again homogenized and centrifuged under the same conditions.

108

### 109 **2.3.2. Reference method 1 on fresh leaf powder**

110 Vitamin C was extracted according to the method of Stevens et al. (2006), with  
111 the only significant modification consisting of intensifying the cold chain. For this  
112 purpose, the samples of fresh leaf powders ( $-80\text{ }^{\circ}\text{C}$ ) were kept on a bed of liquid  
113 nitrogen placed inside a cold bank ( $\sim -20\text{ }^{\circ}\text{C}$ ). Within this low-temperature environment,  
114 a TS of fresh leaf powder (approximately 100 to 200 mg) was weighed and introduced  
115 into a microtube containing the extraction solvent (0.6 mL TCA at 6%). Vitamin C was  
116 immediately extracted by homogenization with a vortex (at room temperature), then the  
117 microtube was put back on hold in crushed ice, still in the cold bank. This process was  
118 repeated to obtain one series of 24 microtubes. At the end of the series, the microtubes

119 were again stirred for a few seconds in a vortex, weighed to determine the mass of each  
120 TS and centrifuged (16,110 g, 15 min at 4 °C; model 5415R; Eppendorf, Le Pecq,  
121 France).

122

### 123 **2.3.3. Reference method-2 on fresh fruit powder**

124 For this reference method, vitamin C was extracted from the fresh fruit powder  
125 according to a modified version of the method of Stevens et al. (2006). The first step  
126 was to introduce the TS of fruit powder (approximately 400 to 600 mg) into previously  
127 weighed and frozen microtubes. The integrity of the vegetable powders and TS were  
128 ensured by the presence of liquid nitrogen in a box placed at room temperature. The  
129 microtubes containing the TS were then stored at -80 °C until analysis (extraction and  
130 analytical determination), carried out in a series of 24 samples (three series of eight TS)  
131 as follows. The first series of eight fresh TS were placed on a frozen rack (~ -20 °C);  
132 the extraction solvent (0.6 mL TCA at 6%) was immediately added and vitamin C was  
133 extracted simultaneously in the eight TS using a vibratory oscillating mill (1 min; 30  
134 oscillations s<sup>-1</sup>; MM301, Retsch). After a slight vortex agitation, the microtubes were  
135 then placed on hold in crushed ice for the necessary time to carry out the analogous  
136 extraction of two new series of eight TS. The 24 microtubes were then weighed to  
137 determine the mass of each TS and centrifuged (16,110 g, 15 min at 4 °C; model  
138 5415R; Eppendorf, Le Pecq, France;).

139

### 140 **2.4. Analytical determination of vitamin C**

141 Regardless of the extraction method applied, the vitamin C analytical  
142 determinations by colorimetry with a microplate-reader described by Stevens et al.  
143 (2006) were carried out immediately with minor modifications. The determination was



144 based on spectrophotometric detection of the dipyrridyl-Fe<sup>2+</sup> complex formed after the  
145 reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> by the AsA present in the extracts. Part of the 96-well  
146 microplate allowed dosing the AsA naturally contained in the sample extract. The other  
147 part of the microplate allowed determining the T-AsA after the reduction of the DHA  
148 contained in the sample extract by incubation with a reducing agent (DTT). The  
149 analytical determination was performed by external calibration with a Multiscan Ascent  
150 MP reader (Labsystems, Thermo Fisher Scientific, Courtaboeuf, France). The stock  
151 solution of AsA (1 mg mL<sup>-1</sup> in 6% TCA) used to prepare standards for external  
152 calibration was stored at -80 °C as 1-mL aliquots in microtubes. In contrast, the  
153 standard solutions (seven from 0.02 to 0.4 mg mL<sup>-1</sup> AsA in TCA at 6% and one without  
154 AsA) were prepared at each assay from an aliquot of the stock solution of AsA. In order  
155 to assay vitamin C, 20 µL of standard solutions were distributed in the T-AsA part of  
156 the microplate and 20 µL of extracts were distributed in both the T-AsA and AsA parts.  
157 For all the following steps, the microplate was shaken (~1 min) after the addition of  
158 each reagent. For T-AsA determination, 20 µL of 5 mM DTT (in 0.5 M phosphate  
159 buffer, pH 7.5) were added into the T-AsA part and the covered microplate was  
160 incubated for 20 min at 37–39 °C to reduce DHA. Then, 10 µL of NEM at 0.5% (w/v in  
161 water) were added to eliminate the excess of DTT. Subsequently, 80 µL of color reagent  
162 were added (see below) and the covered microplate was incubated for 60 min at 37–39  
163 °C. After incubation, the absorbance was read at 550 nm using a microplate reader. For  
164 the simultaneous AsA assay, the DTT and NEM were omitted and replaced by the same  
165 volumes of 0.5 M phosphate buffer, pH 7.5, in the AsA part of the microplate and the  
166 procedure was carried out as described above. The color reagent consisted of the  
167 following: solution **A**: 31% orthophosphoric acid, 4.6% w/v TCA, and 0.6% w/v iron

168 chloride; solution **B**: 4% 2,2-dipyridyl (w/v made up in 70% ethanol); solutions **A** and **B**  
169 were mixed, 2.75 parts **A** to 1 part **B**.

170 *Calculation.* The T-AsA and AsA contents (in mg 100 g<sup>-1</sup> DM) were calculated using  
171 the following equation:

$$172 \quad \text{T-AsA or AsA content} = (A - b)/a \times [V_{\text{TCA}} + \text{TS} \times (1 - \text{DM}_{(\%)}/100)] \times 100/\text{TS} \times \\ 173 \quad 100/\text{DM}_{(\%)}$$

174 Simplified equation for lyophilized powder (DM<sub>(%)</sub> = 100):

$$175 \quad \text{T-AsA or AsA content} = (A - b)/a \times V_{\text{TCA}} \times 100/\text{TS}$$

176 For an extract absorbance, A (for T-AsA or AsA calculation, depending on the part of  
177 the microplate); external calibration curve parameters a and b (for  $y = ax + b$ ); a mass of  
178 test sample (in g), TS; a percentage of dry matter of the sample, DM<sub>(%)</sub>; a solvent  
179 extraction volume of TCA at 6% (in mL),  $V_{\text{TCA}}$ .

180

## 181 **2.5. Investigating a matrix effect**

182 The internal standards were prepared and added to 20 lyophilized tomato  
183 samples (four green fruits, nine mature fruits, and seven leaves). For each sample, the  
184 analysis was carried out with no addition and then with addition of three levels of AsA  
185 corresponding to 20, 100, and 150 µg per TS (TS = 40 mg for fruits and 20 mg for  
186 leaves). The extraction of TS with the internal standards addition was carried out  
187 according to the proposed "new method" but replacing the extraction solvent (1 mL  
188 TCA at 6%) with 1 mL of standard solution (respectively, 0.02, 0.1, and 0.15 mg mL<sup>-1</sup>  
189 in TCA at 6%), each being prepared by dilution of the stock solution of AsA.

190 The lowest concentration of added standard was equivalent to the lowest point of  
191 the external calibration curve. The highest standard concentration (7.5 times the lowest)

192 was chosen so that the overall concentration of the extracts would remain within the  
193 calibration range.

194

## 195 **2.6. Evaluation of the robustness of the "new method"**

196 The effect of storage temperature on the stability of freeze-dried powders was  
197 evaluated by comparing the AsA and T-AsA contents of 40 TS stored for 3.5 months at  
198  $-20\text{ }^{\circ}\text{C}$  or at  $-80\text{ }^{\circ}\text{C}$  (eight green fruits, 20 mature fruits and 12 leaves). The long-term  
199 stability of mature fruit powders was verified after the storage of 58 TS for 20 months at  
200  $-80\text{ }^{\circ}\text{C}$ . The stability of the freeze-dried powders was also evaluated after 24 hours of  
201 storage of the TS at room temperature and exposed to room lighting (three green fruits,  
202 seven mature fruits and three leaves). This test was repeated over a period of 34 days  
203 with three samples (one green fruit, one mature fruit, and one leaf).

## 204 **2.7. Statistical analysis**

205 The "new method" has been validated in T-AsA and AsA by checking its  
206 precision, linearity and accuracy. For each test, the series were compared using the  
207 Wilcoxon signed rank test and their correlation was verified with the Spearman rank test  
208 (Scherrer, 1984). According to the definitions of Kennedy and Neville (1986), the term  
209 precision refers to the closeness to which measurements agree with each other, while the  
210 term accuracy expresses the closeness of measurements to the true value.

211 In order to study the precision of the "new method", two series of analyses of 44  
212 lyophilized tomato powders (eight green fruits, 25 mature fruits, and 11 leaves) were  
213 conducted from different TS, with several days apart (between six and 15 days).  
214 According to ISO 5725 (1994) standard regarding results obtained under these  
215 conditions, the reproducibility value  $R$  is the value below which there is 95%  
216 probability of finding the absolute difference between two results from a single assay.

217 The lower the  $R$  value, the better the reproducibility. This value was calculated using the  
218 following equation:

$$S_r = \left( \frac{1}{2q} \sum_{i=1}^q w_i^2 \right)^{\frac{1}{2}}$$

$$R = 2.8S_r$$

219 Where  $q$  is the double analyzed sample number and  $w_i$  is the absolute difference  
220 between pairs of results. In order to take into account the order of magnitude of the  
221 values obtained during the precision test, we also calculated the relative root mean  
222 square error (RRMSE).

223 The regression parameters obtained using the internal standards addition made it  
224 possible to evaluate the linearity of the method and a possible matrix effect was sought  
225 by comparing the concentrations of T-AsA and AsA obtained by internal and external  
226 calibrations.

227 The accuracy of the "new method" was checked on the same 44 samples selected  
228 to evaluate its precision by comparing the results obtained by this method (lyophilized  
229 powders) with those obtained with "reference method 1" for fresh leaf powders and  
230 "reference method 2" for fresh fruit powders.

231

### 232 **3. Results and discussion**

233 All the validation results of the statistical analysis are presented in Table 1.

234 As a preamble to the validation of the "Nnew method", here are some observations on  
235 its development. The major constraint of the extraction of vitamin C from fresh powder  
236 is the cold chain imposed by the high lability of this compound. Very easy to handle and  
237 much less sensitive to temperature and oxidation, the "new method" using lyophilized  
238 powder reduced this cold chain significantly by making the use of liquid nitrogen and

239 cold bank unnecessary. The powders were lyophilized without taking any special  
240 precautions but respecting a cold chain sufficiently rigorous to conserve their integrity  
241 between the  $-80\text{ }^{\circ}\text{C}$  freezer where they were stored and the freeze-drier. The choice of a  
242 vibratory oscillation mill was self-evident to extract vitamin C from the lyophilized TS  
243 of fruits and leaves because it allows up to 10 simultaneous extractions as well as a  
244 more efficient homogenization compared to vortex (lyophilized powder tends to  
245 flocculate with a vortex agitation). It should also be highlighted that an improved  
246 version of this mill may handle more extractions. We opted for TS masses of 40 mg for  
247 the fruits and 20 mg for the leaves, in order to maintain a ratio dry mass of  
248 sample/volume of extract comparable to that obtained by analyzing the fresh powder.  
249 However, by doubling the masses of lyophilized TS (an impossible procedure with fresh  
250 TS due to the capacity of the microtube), the results were equally satisfactory, giving  
251 the possibility of quantifying vitamin C levels up to two times lower than in the fresh  
252 TS (data not shown).

253

### 254 **3.1. Precision**

255 The two series of analyses are very strongly correlated ( $\rho > 0.97$ ) and no bias  
256 was detected ( $p > 0.01$ ) either for T-AsA or for AsA (Figure 2). The reproducibility  
257 values reflect the excellent precision of the "new method", with the high values of  $R$   
258 (21.6 and 18.6, respectively) being explained by the extent of the concentration range  
259 ( $\sim 170$  to  $450\text{ mg } 100\text{ g}^{-1}\text{ DM}$ ). The RRMSE calculated to overcome this variable are  
260 between 3.8% and 3.9%. This result is highly satisfactory because the two series of  
261 analyses were performed several days apart and it shows that the conservation of the  
262 powders at  $-80\text{ }^{\circ}\text{C}$  has no impact on the contents of T- AsA and AsA, regardless of the  
263 tissue and stage of maturity. The results are only slightly better when the two series of

264 analysis are performed on the same day on the same microplate (RRMSE = 3.3% for T-  
265 AsA and 3.6% for AsA; data not shown).

266

### 267 **3.2. Response linearity and matrix effect**

268 The standard additions carried out on 20 samples (0 to 150 µg per TS), of which  
269 three examples (green fruit, mature fruit, and leaf) are presented in Figure 3a, provide  
270 linear regression coefficients of the order of 0.99. This fact testifies the very good  
271 linearity of the "new method" for both T-AsA and AsA. The measured absorbance is  
272 proportional to the vitamin C concentration, irrespective of the tissue (fruit or leaf) and  
273 the stage of maturity of the tomato (green or mature fruit). Considering the standard  
274 additions, it can be deduced that the "new method" is linear at least over the ranges  
275 170–630 mg, 160–740 mg and 190–1140 mg of vitamin C for 100 g DM of green fruits,  
276 mature fruits and leaves, respectively.

277 A possible matrix effect was sought by comparing the values obtained by  
278 internal and external calibration on the 20 samples (Figure 3b). The excellent  
279 correlation between the two series ( $\rho = 0.97$ ) and the similarity of results ( $p > 0.01$ )  
280 testify to the absence of a matrix interference.

281 These results are highly important from a practical point of view because they  
282 validate the external calibration of the "new method" for both T-AsA and AsA.

283

### 284 **3.3. Accuracy**

285 The "new method" and reference methods for fresh powder are significantly  
286 correlated ( $\rho > 0.97$ ) for both T-AsA and AsA. The absence of bias ( $p > 0.01$ ) reflects  
287 the accuracy of the "new method" (Figure 4). In order to obtain such an excellent  
288 correlation, we mainly improved the performance of fresh powder methods, which tends

289 to show that the most accurate method is the "new method". Indeed, "reference method  
290 1" corresponds to the extraction method of Stevens et al. (2006) with an intensification  
291 of the cold chain (cold bank), intended to improve the precision of the results on fresh  
292 powder. With this change, the CV of fresh powder analysis decreased a lot, from 9.5%  
293 to 2.0% for T-AsA and from 16.2% to 1.4% for AsA (data not shown). However, the  
294 additional cold constraint made the homogenization step much longer and tedious (risk  
295 of musculoskeletal disorder for the operator by prolonged use of the vortex) when TS  
296 were greater than 500 mg (a frequent case with fruit powder). Therefore, we developed  
297 "reference method 2", which allowed vitamin C to be extracted from 10 TS  
298 simultaneously in just 1 min. For this we used a vibratory oscillating mill, whose  
299 efficient homogenization is much greater than that of a vortex, especially in a cold  
300 environment. Thanks to the essential presence of liquid nitrogen to handle the fresh  
301 powders and to collect the TS, we managed to keep a cold chain that would be strong  
302 enough to guarantee the reliability of the results of AsA, without resorting to the cold  
303 bank. "Reference method 2" was compared to "reference method 1" by analyzing 35  
304 samples of fresh fruit powders (20 green and 15 mature). The similarity of the two  
305 methods was verified for both T-AsA and AsA (Wilcoxon signed rank test:  $p > 0.01$  and  
306 spearman correlation:  $\rho > 0.95$ ; data not shown). However, we chose to keep "reference  
307 method 1" for the fresh leaf powder because it seems that vitamin C degrades much  
308 more quickly in this tissue than in fruits and the extraction time is quite reasonable even  
309 in the cold bank (small size of the TS).

310 The "new method" and the reference methods give comparable results over a  
311 wide range of vitamin C concentrations ( $\sim 180\text{--}450 \text{ mg } 100 \text{ g}^{-1} \text{ DM}$ ), and the correlation  
312 parameters show a very acceptable systematic and proportional bias ( $< 5\%$ ). Thus,

313 lyophilization and the subsequent extraction procedure do not lead to a loss of T-AsA or  
314 AsA.

315

### 316 **3.4. Robustness**

317         The TS weighing was carried out without any particular precaution since no  
318 degradation of vitamin C was observed in lyophilized TS of fruit or leaf after 24 hours  
319 of storage at room temperature and exposure to daylight (data not shown). As we have  
320 shown, the possibility of fractioning the preparation steps without any impact,  
321 irrespective of the tissue, contributes to the robustness of the “new method”. We also set  
322 out to evaluate the stability of the composition of lyophilized powders over time, since  
323 the excellent precision of the "new method" had already answered this question for  
324 short-term storage. In addition, we assessed long-term precision by re-analyzing 58  
325 lyophilized powders of mature fruits stored for 20 months at  $-80\text{ }^{\circ}\text{C}$  (Figure 5). After  
326 this time, the T-AsA and AsA contents had hardly changed and only a decrease in the  
327 AsA content of around 5 to 10% was observed. Nevertheless, we recommend  
328 performing the analyses quickly after lyophilization. These results reflect the  
329 adaptability of the "new method" with the possibility of postponing analysis for several  
330 days or even several months in the case of mature fruits. Storage at  $-80\text{ }^{\circ}\text{C}$  is therefore,  
331 a guarantee of preserving the integrity of the lyophilized sample over the long term. In  
332 the short term, however, other preliminary tests showed that storage at  $-20\text{ }^{\circ}\text{C}$  was  
333 satisfactory but that the nature of the sample must be considered (data not shown).  
334 Thus, 3.5 months of storage at  $-20\text{ }^{\circ}\text{C}$  did not seem to affect the T-AsA or AsA contents  
335 measured on lyophilized powders of red fruits, whereas these same contents decreased  
336 by 10% in green fruits and about 15% in the leaves. This matrix effect related to storage  
337 temperature was also verified with lyophilized powders stored for 34 days at room



338 temperature and daylight. Thus, the T-AsA and AsA contents decreased much more  
339 rapidly in the leaves (75%) than in the red fruits (20%) or in the green fruits (35%) (data  
340 not shown). Therefore, it is essential to adapt the method protocol (and in particular the  
341 timeframe of the analytical determination after lyophilization) according to the nature of  
342 the sample and storage capacity.

343

### 344 **3.5. Highlighting interfering absorbance in extracts**

345         Whatever the extraction method used, the results were obtained from the same  
346 DPD colorimetric assay technique described by Stevens et al. (2006). However, more  
347 recent work (Ueda et al., 2013) has shown a possible overestimation of vitamin C levels  
348 with this assay. Garchery et al. (2013) adapted the dosage with DPD to take into  
349 account these non-negligible absorbance interferences related to the  $\text{Fe}^{3+}$  reduction in  
350  $\text{Fe}^{2+}$  by compounds other than the AsA contained in the extracts. In order to consider it,  
351 it must be measured in each extract after oxidation in the presence of ascorbate oxidase,  
352 then deducted from the absorbances with and without DTT to calculate the  
353 concentrations of T-AsA and AsA, respectively. To verify the existence of this  
354 interfering absorbance, vitamin C was extracted with the "new method" from 202 TS of  
355 lyophilized powders stored at  $-80^\circ\text{C}$  (41 green, 133 mature, and 28 leaves). The  
356 analytical extracts were assayed according to Garchery et al. (2013) to highlight the  
357 interfering absorbance specific to each one. From the same microplate, the contents of  
358 T-AsA and AsA were calculated both by deducting and without deducting this  
359 interfering absorbance. Figure 6 represents the comparison of these results for T-AsA  
360 showing a low interference absorbance in fruits, whether mature or not ( $< 10\%$  in the  
361 range of T-AsA values measured (110 to  $780\text{ mg } 100\text{ g}^{-1}\text{ DM}$ )). For the leaves, the error  
362 is much more significant because if we refer to the regression line, it is similar to a

363 systematic bias that generates an important overestimation of vitamin C (~85 mg 100  
364 g<sup>-1</sup> DM), regardless of the real concentration of vitamin C (range 130 to 400 mg 100 g<sup>-1</sup>  
365 DM). According to Ueda et al. (2013), this additional absorbance would result in  
366 particular from a significant amount of iron in the matrix or from the presence of  
367 absorbent pigments at the dosing wavelength in the extract. The tomato plants that  
368 supplied our samples received an iron dietary intake that covered their nutritional needs.  
369 When assaying the AsA with DPD (in reality we are dosing the dipyridyl-Fe<sup>2+</sup>  
370 complex), one mole of Fe corresponds to one mole of AsA. In fact, when measuring an  
371 absorbance equivalent to 85 mg of ASA, this corresponds to the measurement of  
372 approximately 27 mg of Fe<sup>2+</sup>. Knowing that the amounts of total iron found in tomato  
373 leaves can reach 30 mg 100 g<sup>-1</sup> DM (Moreno et al., 2005), it is possible that the non-  
374 chelated Fe<sup>2+</sup> present in the leaves alone might explain the essence of the observed  
375 interfering absorbance. Tomato leaves sometimes contain anthocyanins (Larbat et al.,  
376 2014), pigments absorbing at 550 nm, but the samples we used did not contain them.  
377 We have verified in 24 samples that deducting this residual absorbance had no impact  
378 on the reliability of the "new method", which remains excellently accurate, in  
379 comparison with the reference methods of extraction on fresh powder (data not shown).  
380 Therefore, unless an error of 10% is considered negligible (fruits), it seems important to  
381 take into account the interference absorbance.

382

383 By developing and validating a method for extracting vitamin C from the lyophilized  
384 powder which is both reliable and applicable to fruit and tomato leaves, we have freed  
385 ourselves from the constraints of handling fresh powder (working under permanent cold  
386 chain with liquid nitrogen and cold bank). The "new method" proved to be precise,

387 accurate, and linear at the same time over a wide range of concentration of vitamin C  
388 and no matrix effect was observed.

389 The stability of the lyophilized sample contributes to the robustness of the “new  
390 method” by bringing much more flexibility in its handling and implementation  
391 compared to others. It makes it possible to weigh the TS at room temperature and to  
392 dissociate the preparation steps easily (sampling/weighing of TS and extraction)  
393 regardless of the tissue. The lyophilized powder is much less sensitive to oxidation and  
394 temperature than the fresh powder. As a result, even in the case of breakdown of a  
395 freezer or power failure for 24 hours, T-AsA and AsA would still be reliably  
396 quantifiable in lyophilized powder. Under such conditions (however plausible), fresh  
397 powder would lose its integrity by heating up, causing the oxidation and degradation of  
398 vitamin C very quickly, making it impossible to analyze. We have not highlighted the  
399 need to recommend specific freeze-drying conditions, which reinforces the robustness  
400 of the "new method", and lyophilized tomato powders require less space than fresh  
401 powders for freezer storage.

402 Very easy to implement with the use of a vibratory oscillation mill for simultaneous  
403 extractions, this "new method" is about two times faster than the reference methods on  
404 fresh powder, making it easy to analyze at least 48 samples per day.

405 There are other testing methods for vitamin C in the literature. For example, after the  
406 oxidation of AsA to DHA with ascorbate oxidase and derivatization with 1,2-*o*-  
407 phenylenediamine, T-AsA is quantifiable by HPLC–fluorimetry (George et al., 2011).  
408 AsA and T-AsA (after the reduction of DHA to AsA) are also quantifiable by HPLC–  
409 UV (Phillips et al., 2016), or by spectrophotometry by measuring the difference in  
410 absorbance before and after the addition of ascorbate oxidase (Queval and Noctor,

411 2007). Since vitamin C is also extracted in an acid medium when using all these dosing  
412 techniques, it is very likely that the "new method" is compatible with their use.

413 The TS of lyophilized powder are of identical mass (direct weighing), unlike the TS of  
414 fresh powder introduced in approximate quantities into the microtubes. The exact mass  
415 of fresh powder is determined only after the extraction step, by weighing the total mass  
416 of the microtube and deducting the masses of the empty tube and extraction solvent,  
417 which increases the risk of error.

418 With fresh powder, the quantification of the T-AsA and AsA contents is closely related  
419 to the  $DM_{(\%)}$  of each sample because this parameter occurs twice in the calculation (see  
420 equations § 2.4). Firstly, to calculate the aqueous volume of each TS (assimilated by the  
421 difference between the fresh and dry masses) and adding it to the volume of solvent (0.6  
422 mL of TCA at 6%) in order to obtain the total extraction volume, thus differing from  
423 one sample to another. Secondly, to express the vitamin C content of a fresh sample in  
424 relation to its dry mass, a necessary calculation to compare results from both lyophilized  
425 and fresh powders. The influence of  $DM_{(\%)}$  is such that an underestimation of only 0.5%  
426 DM can result in an overestimation of the vitamin C content of almost 10% in fresh  
427 powder. This could also contribute to the differences in concentrations of T-AsA and  
428 AsA (-20%) mentioned by Stevens et al. (2006) between the measurements on fresh  
429 powders and on lyophilized powders. For all these reasons, the quantification of the  
430 vitamin C content seems more reliable with lyophilized powder because in this case, the  
431  $DM_{(\%)}$  is always equal to 100 and the calculation no longer depends on this parameter  
432 (see equation 2.4).

433 The "new method" prevents the phenomenon of "dilution" of the extract related to the  
434 water content of fresh powders and enables the quantification of lower contents of  
435 vitamin C. Lyophilized powder ensures reproducible vitamin C extraction conditions

436 because the parameters are constant for all samples: mass and  $DM_{(\%)}$  of the TS, volume  
437 of extract, and then its concentration of extraction solvent. The extraction conditions in  
438 fresh powders are not as reproducible because the mass and  $DM_{(\%)}$  of the TS are  
439 different for each sample, which induces non-constant extraction volumes and so non-  
440 constant extraction solvent concentrations.

441 We also confirm the existence of an interfering absorbance due to compounds other  
442 than AsA in extracts that varies according to the samples, which we recommend taking  
443 into account, especially regarding the leaves.

444 Finally, the genericity of the "new method" is still to be verified, namely its application  
445 to other vegetable matrices, a work that we have begun to do with apple and peach trees.

446

#### 447 **Acknowledgments**

448 The authors thank Dr. Lescourret and Dr. Vercambre for the assistance in the statistical  
449 treatments.

450

451 **References**

- 452 Aurand, R., Faurobert, M., Hanafi, M., Mazerolles, G., Tisiot, R., Rosso, L., Navez, B.,  
453 Bertin, N. (2015). Integrative Approach of Tomato Fruit Texture Using  
454 Multiblock Analysis. *Acta Horti*, 1099, 827-834.
- 455 Benard, C., Gautier, H., Bourgaud, F., Grasselly, D., Navez, B., Caris-Veyrat, C.,  
456 Weiss, M., Genard, M. (2009). Effects of Low Nitrogen Supply on Tomato  
457 (*Solanum lycopersicum*) Fruit Yield and Quality with Special Emphasis on  
458 Sugars, Acids, Ascorbate, Carotenoids, and Phenolic Compounds. *Journal of*  
459 *Agricultural and Food Chemistry*, 57, 4112–4123.
- 460 Burkey, K. O., Eason, G., Fiscus, E. L. (2003). Factors That Affect Leaf Extracellular  
461 Ascorbic Acid Content and Redox Status. *Physiologia. Plantarum*, 117(1), 51–  
462 57.
- 463 Camarena, V., Wang, G. F. (2016). The Epigenetic Role of Vitamin C in Health and  
464 Disease. *Cellular and Molecular Life Sciences*, 73(8), 1645–1658.
- 465 Chang, C. H., Lin, H. Y., Chang, C. Y., Liu, Y. C. (2006). Comparisons on the  
466 Antioxidant Properties of Fresh, Freeze-Dried and Hot-Air-Dried Tomatoes.  
467 *Journal of Food Engineering*, 77, 478–485.
- 468 El Airaj, H., Gest, N., Truffault, V., Garchery, C., Riqueau, G., Gouble, B., Page, D.,  
469 Stevens, R. (2013). Decreased Monodehydroascorbate Reductase Activity  
470 Reduces Tolerance to Cold Storage in Tomato and Affects Fruit Antioxidant  
471 Levels. *Postharvest Biology and Technology*, 86, 502–510.
- 472 Garchery, C., Gest, N., Do, P. T., Alhag Dow, M., Baldet, P., Menard, G., Rothan, C.,  
473 Massot, C., Gautier, H., Aarouf, J., Fernie, A. R., Stevens R. (2013). A  
474 Diminution in Ascorbate Oxidase Activity Affects Carbon Allocation and

475 Improves Yield in Tomato under Water Deficit. *Plant, Cell & Environment*,  
476 36(1), 159–175.

477 Gautier, H., Diakou-Verdin, V., Benard, C., Reich, M., Buret, M., Bourgaud, F.,  
478 Poessel, J. L., Caris-Veyrat, C., Genard, M. (2008). How Does Tomato Quality  
479 (Sugar, Acid, and Nutritional Quality) Vary with Ripening Stage, Temperature,  
480 and Irradiance? *Journal of Agricultural and Food Chemistry*, 56, 1241–1250.

481 Gautier, H., Massot, C., Stevens, R., Serino, S., Genard, M. (2009). Regulation of  
482 Tomato Fruit Ascorbate Content Is More Highly Dependent on Fruit Irradiance  
483 than Leaf Irradiance. *Annals of Botany*, 103(3), 495–504.

484 George, S., Tourniaire, F., Gautier, H., Goupy, P., Rock, E., Caris-Veyrat, C. (2011).  
485 Changes in the Contents of Carotenoids, Phenolic Compounds and Vitamin C  
486 during Technical Processing and Lyophilisation of Red and Yellow Tomatoes.  
487 *Food Chemistry*, 124, 1603–1611.

488 Gest, N., Garchery, C., Gautier, H., Jimenez, A., Stevens, R. (2013). Light-Dependent  
489 Regulation of Ascorbate in Tomato by a Monodehydroascorbate Reductase  
490 Localized in Peroxisomes and the Cytosol. *Plant Biotechnology Journal*, 11(3),  
491 344–354.

492 ISO 5725 (1994). Application de La Statistique - Exactitude (Justesse et Fidélité) Des  
493 Résultats et Méthodes de Mesure, Partie 4: Méthodes de Base Pour La  
494 Détermination de La Justesse d'une Méthode de Mesure Normalisée. NF ISO  
495 5725-4.

496 Kampfenkel, K., Vanmontagu, M., Inze, D. (1995). Extraction and determination of  
497 ascorbate and dehydroascorbate from plant-tissue. *Analytical Biochemistry*, 225,  
498 165–167.

499 Kennedy J. B., Neville A. M. (1986). *Basic Statistical Methods for Engineers and*  
500 *Scientists*. New York: Harper & Row.

501 Larbat, R., Paris, C., Le Bot, J., Adamowicz, S. (2014). Phenolic characterization and  
502 variability in leaves, stems and roots of Micro-Tom and patio tomatoes, in  
503 response to nitrogen limitation. *Plant Science*, 224, 62–73.

504 Liu, F., Cao, X., Wang, H., Liao, X. (2010). Changes of Tomato Powder Qualities  
505 during Storage. *Powder Technology*, 204(1), 159–166.

506 Massot, C., Bancel, D., Lauri, F. L., Truffault, V., Baldet, P., Stevens, R., Gautier, H.  
507 (2013). High Temperature Inhibits Ascorbate Recycling and Light Stimulation  
508 of the Ascorbate Pool in Tomato despite Increased Expression of Biosynthesis  
509 Genes. *Plos One*, 8.

510 Massot, C., Genard, M., Stevens, R., Gautier, H. (2010). Fluctuations in Sugar Content  
511 Are Not Determinant in Explaining Variations in Vitamin C in Tomato Fruit.  
512 *Plant Physiology and Biochemistry*, 48, 751–757.

513 Massot, C., Stevens, R., Genard, M., Longuenesse, J. J., Gautier, H. (2012). Light  
514 Affects Ascorbate Content and Ascorbate-Related Gene Expression in Tomato  
515 Leaves More than in Fruits. *Planta*, 235, 153–163.

516 Moreno, D. A., Ruiz, J. M., Ahammad, L., Romero, L. (2005) Relationship between  
517 leaf micronutrient concentrations and fruit yield in new tomato cultivars.  
518 *Journal of Horticultural Science & Biotechnology*, 80 (4), 476–480.

519 Moser, M. A., Chun, O. K. (2016). Vitamin C and Heart Health: A Review Based on  
520 Findings from Epidemiologic Studies. *International Journal of Molecular*  
521 *Sciences*, 17(8), 1328.



522 Munyaka, A. W., Makule, E. E., Oey, I., Van Loey, A., Hendrickx, M. (2010). Thermal  
523 Stability of L-Ascorbic Acid and Ascorbic Acid Oxidase in Broccoli (*Brassica*  
524 *oleracea* var. *Italica*). *Journal of Food Science*, 75, C336–C340.

525 Pearson, J. F., Pullar, J. M., Wilson, R., Spittlehouse, J. K., Vissers, M. C. M.,  
526 Skidmore, P. M. L., Willis, J., Cameron, V. A., Carr, A. C. (2017). Vitamin C  
527 Status Correlates with Markers of Metabolic and Cognitive Health in 50-Year-  
528 Olds: Findings of the CHALICE Cohort Study. *Nutrients*, 9(8), 831.

529 Phillips, K.M., Council-Troche, M., McGinty, R.C., Rasor, A.S., Tarrago-Trani, M.T.  
530 (2016). Stability of vitamin C in fruit and vegetable homogenates stored at  
531 different temperatures. *Journal of Food Composition and Analysis*, 45, 147–162.

532 Queval, G., Noctor, G. (2007). A plate reader method for the measurement of NAD,  
533 NADP, glutathione, and ascorbate in tissue extracts: Application to redox  
534 profiling during *Arabidopsis* rosette development. *Analytical Biochemistry*, 363,  
535 58–69.

536 Ripoll, J., Urban, L., Bertin, N. (2016a). The Potential of the MAGIC TOM Parental  
537 Accessions to Explore the Genetic Variability in Tomato Acclimation to  
538 Repeated Cycles of Water Deficit and Recovery. *Frontiers in Plant Science*, 6.

539 Ripoll, J., Urban, L., Brunel, B., Bertin, N. (2016b). Water Deficit Effects on Tomato  
540 Quality Depend on Fruit Developmental Stage and Genotype. *Journal of Plant*  
541 *Physiology*, 190, 26–35.

542 Sherrer, B. (1984). In G. Morin (Ed.), *Biostatistique*. Québec (pp 528–534).

543 Stevens, R., Buret, M., Garchery, C., Carretero, Y., Causse, M. (2006). Technique for  
544 Rapid, Small-Scale Analysis of Vitamin C Levels in Fruit and Application to a  
545 Tomato Mutant Collection. *Journal of Agricultural and Food Chemistry*, 54,  
546 6159–6165.

547 Tarrago-Trani, M. T., Phillips, K. M., Cotty, M. (2012). Matrix-Specific Method  
548 Validation for Quantitative Analysis of Vitamin C in Diverse Foods. *Journal of*  
549 *Food Composition and Analysis*, 26, 12–25.

550 Truffault, V., Fifel, F., Longuenesse, J. -J., Vercambre, G., Le Quillec, S., & Gautier, H.  
551 (2015). Impact of temperature integration under greenhouse on energy use  
552 efficiency, plant growth and development and tomato fruit quality depending on  
553 cultivar rootstock combination. *Acta Horticulturae*, 1099, 95-100.

554 Truffault, V., Gest, N., Garchery, C., Causse, M., Duboscq, R., Riqueau, G., Sauvage,  
555 C., Gautier, H., Baldet, P., Stevens, R. (2014). Variation in Tomato Fruit  
556 Ascorbate Levels and Consequences of Manipulation of Ascorbate Metabolism  
557 on Drought Stress Tolerance. *Acta Horticulturae*, 1048, 75-84.

558 Truffault, V., Gest, N., Garchery, C., Florian, A., Fernie, A. R., Gautier, H., Stevens, R.  
559 G. (2016). Reduction of MDHAR Activity in Cherry Tomato Suppresses  
560 Growth and Yield and MDHAR Activity Is Correlated with Sugar Levels under  
561 High Light. *Plant, Cell & Environment*, 39, 1279–1292.

562 Ueda, Y., Wu, L. B., Frei, M. A. (2013). Critical Comparison of Two High-Throughput  
563 Ascorbate Analyses Methods for Plant Samples. *Plant Physiology and*  
564 *Biochemistry*, 70, 418–423.

565 Zaoui, S., Gautier, H., Bancel, D., Chaabani, G., Wasli, H., Lachaâl, M., Karray-  
566 Bouraoui, N. (2016). Antioxidant Pool Optimization in *Carthamus tinctorius* L.  
567 Leaves under Different NaCl Levels and Treatment Durations. *Acta*  
568 *Physiologiae Plantarum*, 38(8), 187.

569

570 **Figure Captions**

571

572 **Figure 1.** Procedure for vitamin C micro-extraction on tomato powder: “new method”  
573 on lyophilized fruit and leaf; “reference methodv1” on fresh leaf and “reference method  
574 2” on fresh fruit. (TCA – trichloroacetic acid).

575

576 **Figure 2.** Precision of the “new method”. Double vitamin C analysis in 44 tomato  
577 samples with several days apart between sets (◇ green tomato; ○ mature tomato; △  
578 tomato leaf; T-AsA - total vitamin C; AsA - ascorbic acid).

579

580 **Figure 3.**

581 **3a:** Internal standard calibration determination of vitamin C with the “new method” in  
582 three samples (from the 20): green fruit, mature fruit and leaf (◇ GT – green tomato; ○  
583 MT – mature tomato; △ TL – tomato leaf; T-AsA – total vitamin C; AsA – ascorbic  
584 acid).

585 **3b.** Comparison between measurements of vitamin C concentrations with external  
586 (series 1) and internal standards (series 2) in the 20 samples (◇ green tomato; ○ mature  
587 tomato; △ tomato leaf; T-AsA – total vitamin C; AsA – ascorbic acid).

588

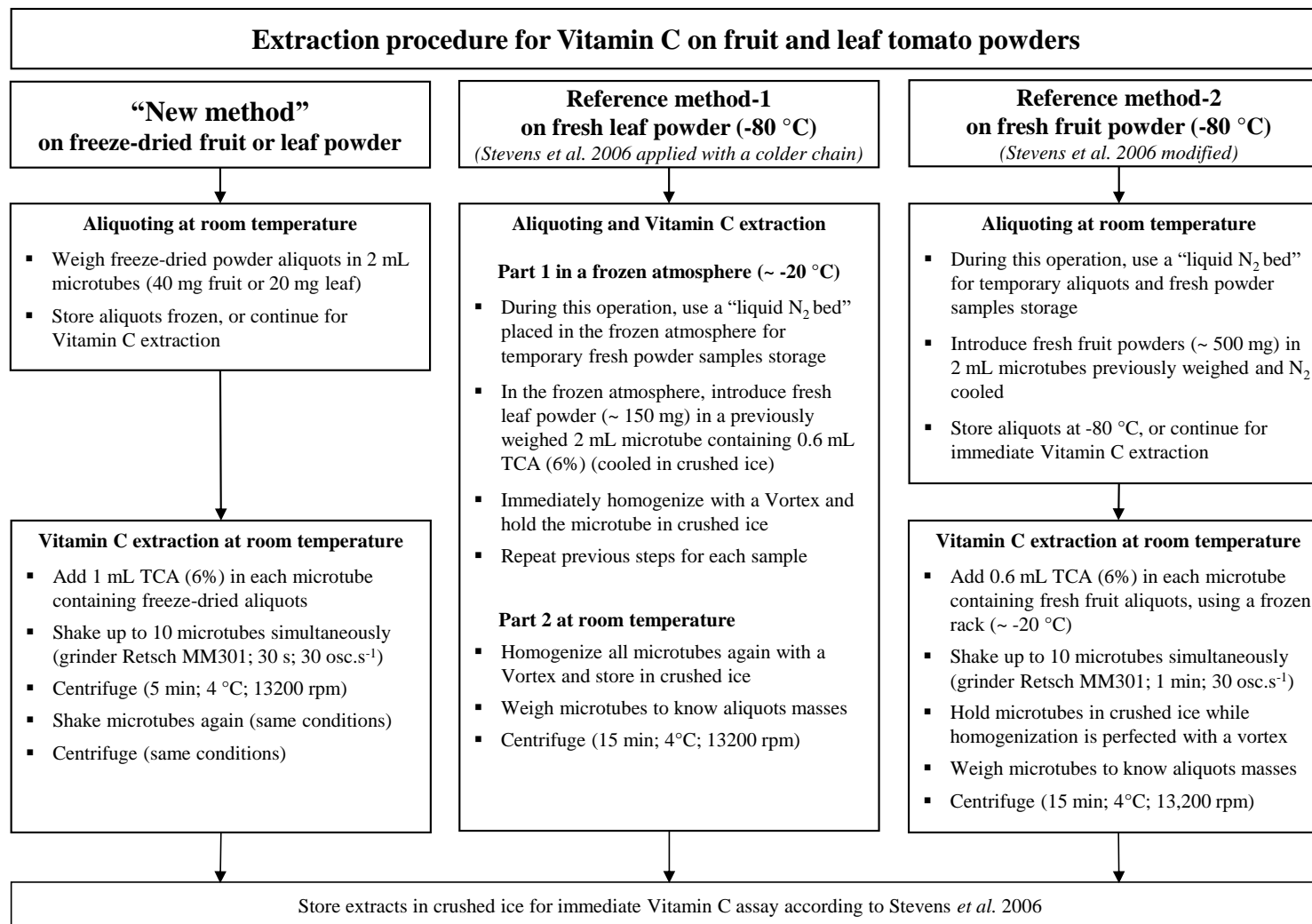
589 **Figure 4.** Accuracy of the “new method”. Comparison between measurements of  
590 vitamin C concentrations in 44 tomato samples analyzed with “reference method 1”  
591 (fresh leaves) or “reference method 2” (fresh fruits) as reference, and the “new method”  
592 (lyophilized fruits and leaves) (◇ green tomato; ○ mature tomato; △ tomato leaf; T-  
593 AsA – total vitamin C; AsA – ascorbic acid).

594

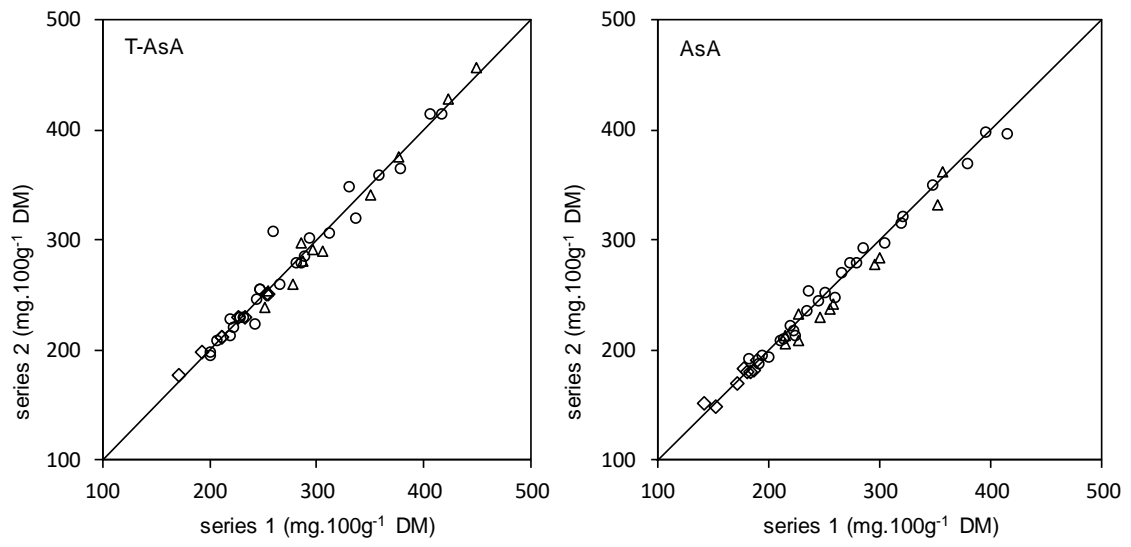
595 **Figure 5.** Robustness of the “new method”. Double vitamin C analysis in 58 mature  
596 tomato samples with lyophilized powder storage at  $-80\text{ }^{\circ}\text{C}$  for 20 months between  
597 series 1 and series 2 ( $\diamond$  green tomato;  $\circ$  mature tomato;  $\triangle$  tomato leaf; T-AsA – total  
598 vitamin C; AsA – ascorbic acid).

599

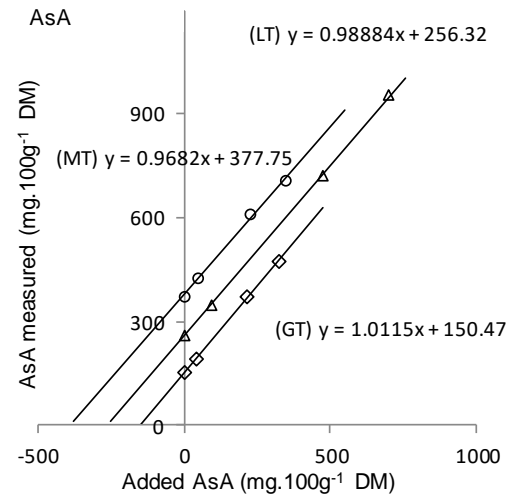
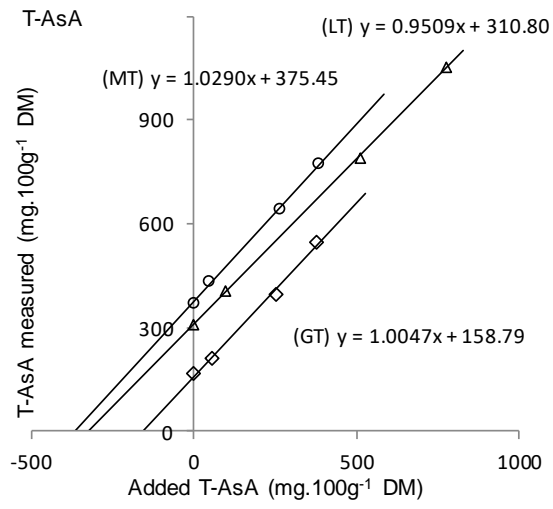
600 **Figure 6.** Influence of ripening stage and tomato organ on interfering absorbance levels.  
601 Comparison between measurements of total vitamin C concentrations in 202 tomato  
602 samples analyzed with the “new method” and assayed without ascorbate oxidase (series  
603 1) and with ascorbate oxidase in parallel (series 2) ( $\diamond$  GT – green tomato;  $\circ$  MT –  
604 mature tomato;  $\triangle$  TL – tomato leaf; T-AsA – total vitamin C).



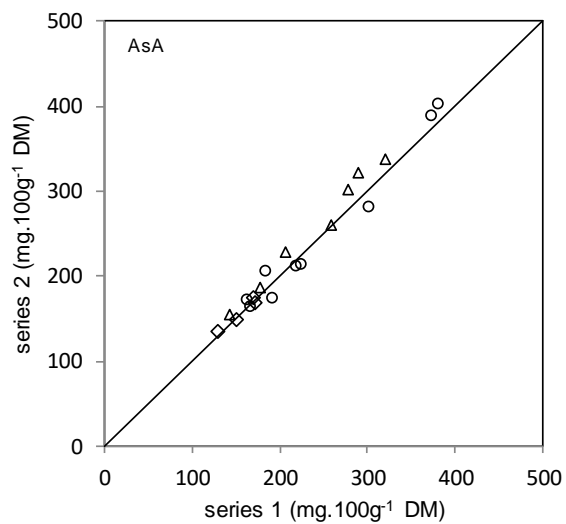
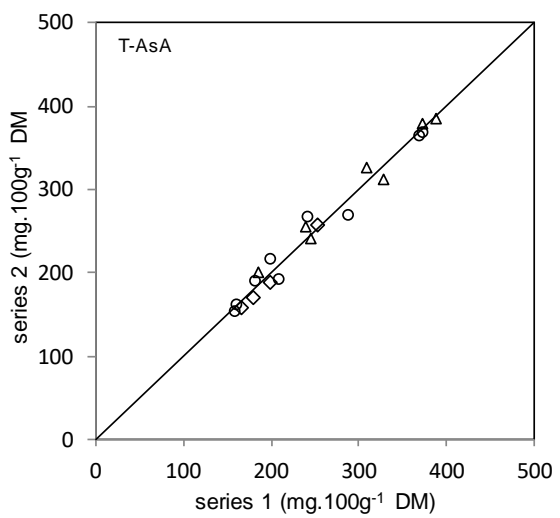
**Figure 1.**



**Figure 2.**

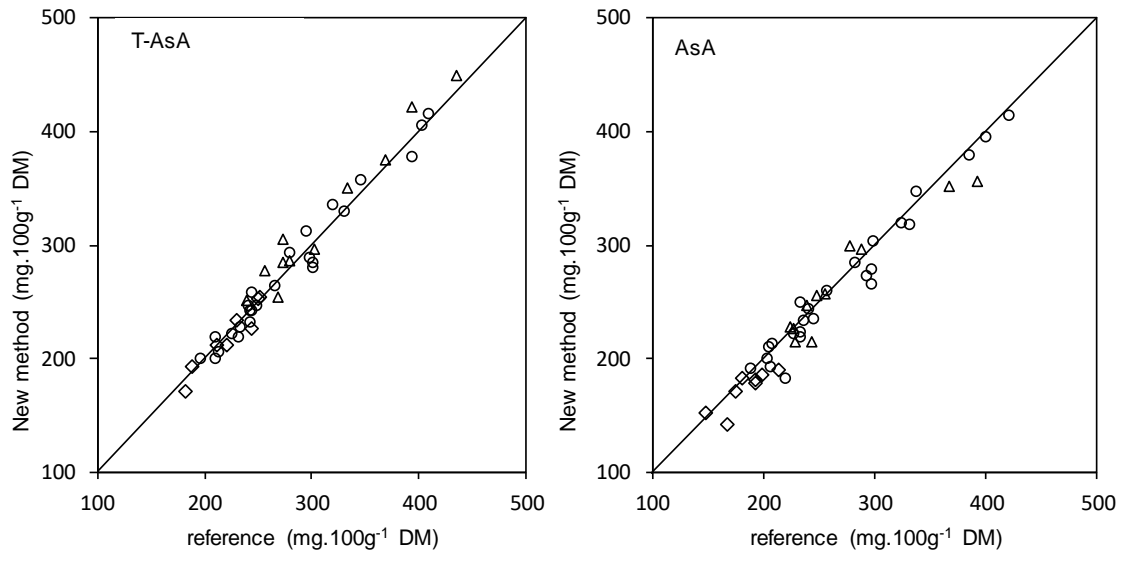


**Figure 3a.**

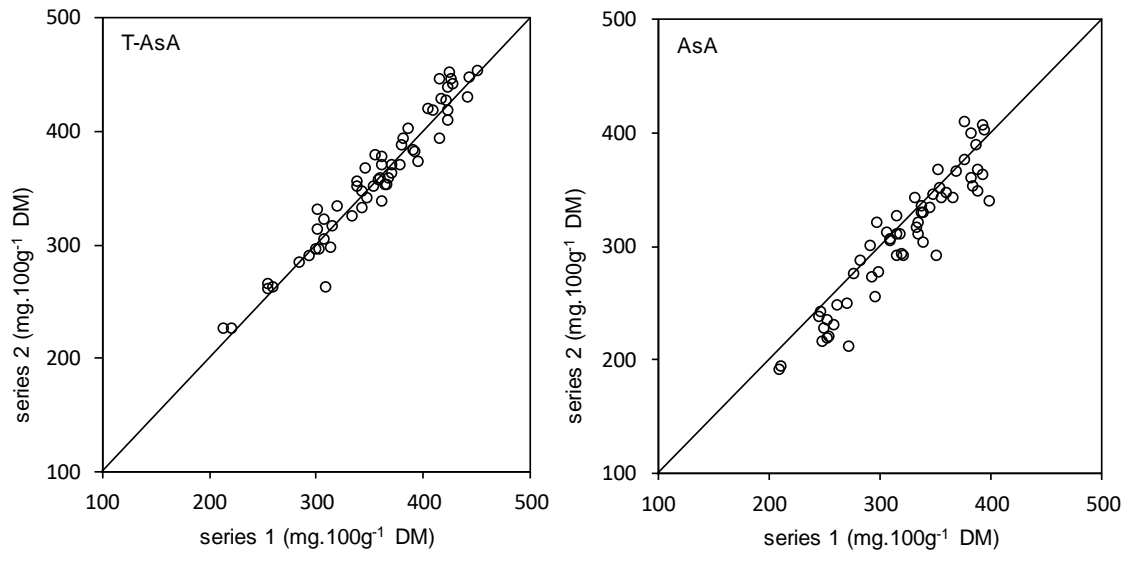


**Figure 3b.**

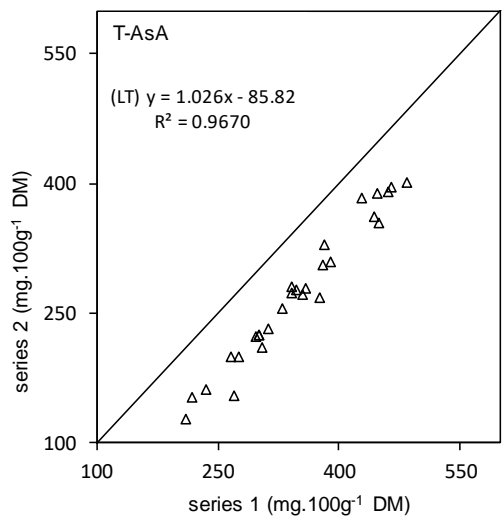
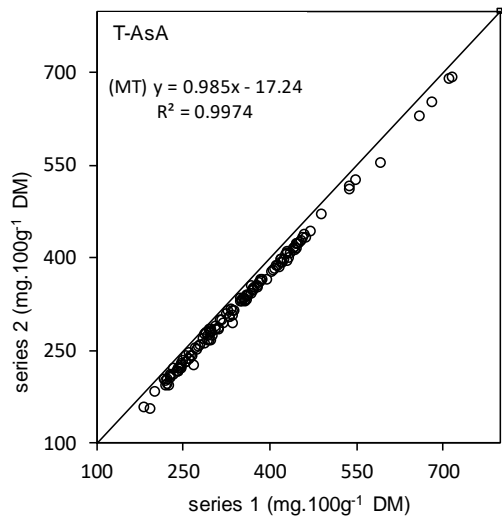
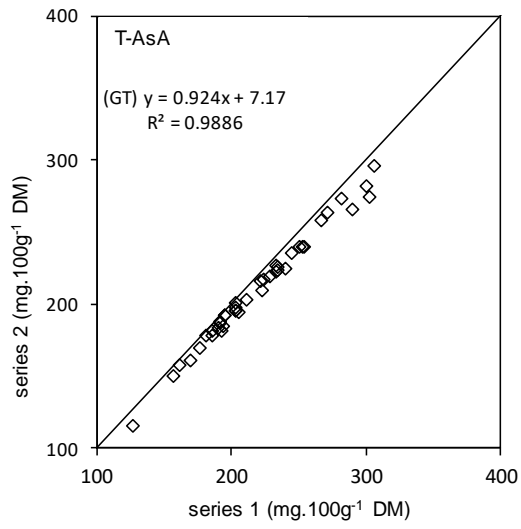




**Figure 4.**



**Figure 5.**



**Figure 6.**

**Table 1.** Summary of statistical analysis results

Figure	Vitamin C	No.	<i>p</i> -value Wilcoxon's test	$\rho$ Spearman's test	Reproducibility test (mg 100g <sup>-1</sup> )	RRMSE	
						Series 1	Series 2
2	T-AsA	44	0.4484	0.9790	21.63	0.0393	0.0394
2	AsA	44	0.0409	0.9862	18.61	0.0376	0.0381
3b	T-AsA	20	0.9273	0.9774			
3b	AsA	20	0.0484	0.9714			
4	T-AsA	44	0.2531	0.9758			
4	AsA	44	0.0154	0.9711			

# Graphical Abstract

“New method” for Vitamin C extraction on lyophilized tomato powder (fruit and leaf)

