Brief communication

ASCORBIC ACID QUANTIFICATION IN MELON SAMPLES – THE IMPORTANCE OF THE EXTRACTION MEDIUM FOR HPLC ANALYSIS

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Summary. Two high-performance liquid chromatography (HPLC) methods for ascorbic acid (AA) quantification were compared in three cultivars of melon (*Cucumis melo* L.) fruits. Both procedures were analogous except for the extraction medium used: 6 % *meta*-phosphoric acid and 3 % citric acid. The general conclusion is that 3 % citric acid is a better extraction medium compared to 6 % *meta*-phosphoric acid and it can be used for ascorbic acid quantification analyses in melon samples.

INTRODUCTION

Fresh fruits and vegetables are significant sources of dietary vitamin C (Wills et al., 1984). A dose of 100 – 200 mg daily has been highly recommended since stress in modern life is known to increase the requirements for this vitamin (Lee and Kader, 2000). It is defined as the generic term for all compounds exhibiting biological activity of L-ascorbic acid (AA). This is the biologically active form, but L-dehydroascorbic acid (DHA), an oxidation product, also exhibits biological activity.

There are a large amount of analytical methods for ascorbic acid quantification based mostly on AA antioxidant characteristics (Farajzadeh and Nagizadeh, 2003). The most common method is the oxidative titration with 2,6-dichlorophenol-indophenol (Official Methods of Analysis, 1980). This is a rapid method, but its use is diffi-

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cult in colored solutions, due to interference of other oxidizing agents. Furthermore, DHA can not be estimated (Wimalasiri and Wills, 1983). The high-performance liquid chromatography (HPLC) technique deserves an increasing interest mainly due to its rapidity, selectivity and specificity proprieties (Sood et al., 1976; Bui-Ngugen, 1980; Rose and Nahrwold, 1981; Haddad and Lau, 1984; Romero-Rodrigues et al., 1992). It allows a rapid and simultaneous estimation of AA and DHA in some foods and other biological materials (Wills et al., 1984).

Auto-oxidation of AA by air oxygen is greatly decreased by an acidic medium (Romero-Rodrigues et al., 1992) which is required to stabilize AA (Wimalasiri and Wills, 1983). Trichloroacetic, metaphosphoric, oxalic or acetic acids are commonly used as extraction media with further purposes like better AA extraction and protein precipitation (Romero-Rodrigues et al., 1992).

In this study, we compared two different methods for ascorbic acid quantification. The first method is that of AOAC (Official Methods of Analysis, 1980), commonly used (Smith, 1986; Romero-Rodrigues et al., 1992; Bradbury and Singh, 1986) which requires 6 % *meta*-phosphoric acid as the extraction medium. The second method is the Wimalasiri and Wills method in which 3 % (w/v) citric acid, is used as a medium for AA extraction (Wimalasiri and Wills, 1983).

Both methods have been applied on three cultivars of melon fruit (*Cucumis melo* L.): Brazilian *Branco*, Spanish *Pele de Sapo* and winter *Tendral* melons, as the cultivars with huge acceptance (Albuquerque, 2004).

MATERIALS AND METHODS

Preparation of samples

Fully ripened fruits of three melon cultivars (*Tendral*, *Pele de Sapo* and *Branco)* were analyzed. Samples of 20 g from 10 fruits were homogenized with 30 ml of extraction medium and centrifuged at 15000 *g* for 25 min at 4 ºC. The slurry was filtered through filter paper Whatman No 4 and then, through a membrane Millipore (0.45 μm). The extraction medium was as follows: 6 % *meta*-phosphoric acid (Merck) for method 1 and 3 % (w/v) citric acid (Merck) for method 2.

HPLC analysis

An aliquot (20 μl) was injected for AA measurement using a Beckman System Gold. The HPLC system consisted of a 126 Beckman pump and a diode array detector (254 nm) operated by a Gold 8.10 software. An Aminex HPX-87H (BioRad) column was used. A flow rate of 0.4 ml min^{-1} was applied at room temperature with 5 mM H_2SO_4 (pH 2.3) as a mobile phase. Three replicates of each sample were injected. An internal standard of AA (Sigma-Aldrich) was used.

Citric acid quantification

Acid extraction was carried out s described by Hudina and Stampar (2000). Samples of 20 g from 10 fruits were dissolved in 100 ml distillated water and centrifuged at 15000 *g* for 15 min at 4 ºC. Filtration was carried out using Whatman No 4 filters and Millipore (0.45µm) membarnes. Citric acid was identified and quantified by HPLC using a Beckman Gold 168 diode-array detector. An Aminex HPX 87H (BioRad) column was used. A flow rate of 0.5 ml min^{-1} was applied at room temperature to a mobile phase of 5 mM H_2SO_4. An internal standard of citric acid (Merck) was used.

RESULTS AND DISCUSSION

Since DHA can be easily converted into AA in the human body, it is important to measure both forms of vitamin C (AA and DHA) (Lee and Kader, 2000). In our experiment, only AA was measured since at harvest, DHA represents less than 2 % of total vitamin C (Wills et al., 1984). In one and the same sample AA content was totally distinct using both methods for all cultivars (Fig. 1). In addition, both methods correlated in 96 %.

Extraction with 3 % citric acid was more efficient and if we consider the value obtained as total, extraction with 6 % *meta*-phosphoric acid showed 56.1 %, 35.3 % and 12.7 % efficiency, for *Branco*, *Pele de Sapo* and *Tendral* samples, respectively.

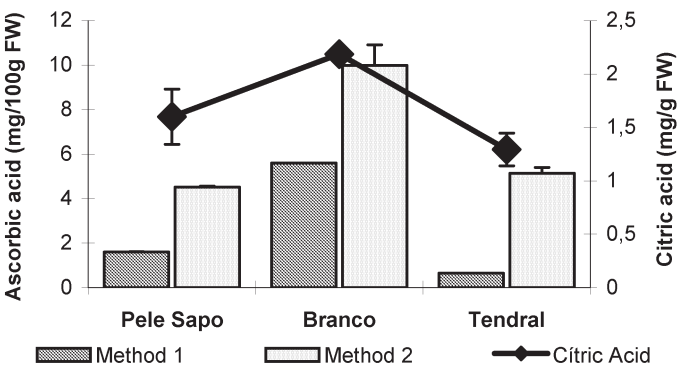

Fig. 1. Ascorbil acid content in three melon cultivars using two methods and citric acid content in different melon cultivars. Each value represents the mean of three replicates ± standard error. Different letters indicate significant differences in a multiple range analysis for 95 % confidence level.

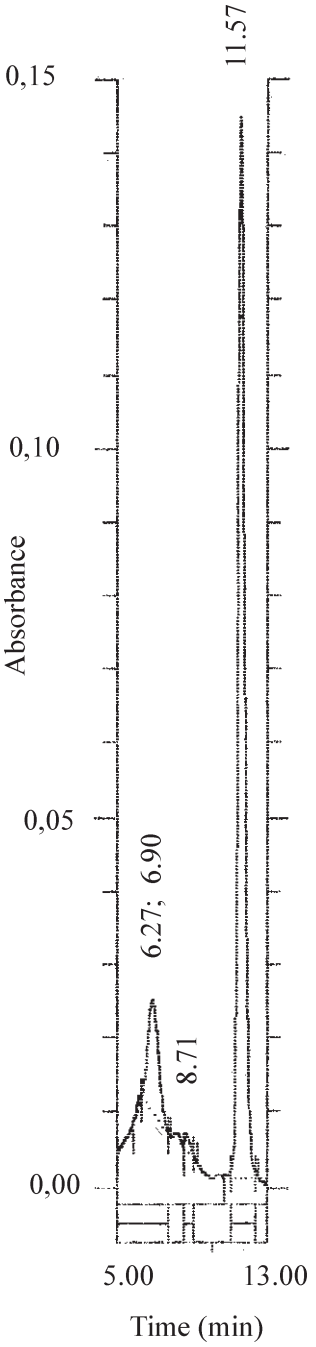

Fig. 2. – Chromatogram of AA in *Branco* melon extract in 3 % citric acid.

These extraction efficiencies might be explained by the different citric acid content in the cultivars tested (Fig. 1). *Branco* melon has higher citric acid concentration, in contrast to *Tendral* melon, the data being significantly different. Our data agree with the above trend. In fact, if ascorbic acid content correlated with the citric acid concentration for each melon cultivar sample, a clear link could be seen ($r^2 = 0.97$). For example, in a *Branco* melon sample, extraction with 6 % *meta*-phosphoric acid resulted in 5.61 mg of AA in 100 g FW while extraction with 3 % citric acid (Fig. 2) resulted in 9.08 mg of AA in 100 g FW.

It has been reported that AA extraction solution containing 6 % *meta*-phosphoric acid can be stored up to 8 h at 4 ºC before HPLC analysis (Smith, 1986) while AA extraction solution with 3 % citric acid remains stable for 3 h at room temperature (Wimalasiri and Wills, 1983). Both samples are therefore stable during the procedures, although the different extraction efficiencies found could not be explained. The ability of some compounds to form ring structures with metal ions (chelation), thus preventing metal ions from reacting with other materials or from acting as a catalyst, might justify our data. Citric acid is the strongest chelating agent of the common food acids and can enhance the effectiveness of AA antioxidant proprieties by providing a synergistic effect (Kuntz, 1993). So, the addition of citric acid provides a more stable environment for the AA.

Vitamin C content in melon cultivars might fluctuate between 6 and 60 mg in 100 g FW (Odet, 1991). The content of this vitamin in fruits and vegetables varies between cultivars and tissues (Lee and Kader, 2000). Our general conclusion based on the results presented is that 3 % citric acid is a better extraction medium when compared to 6 % *meta*-phosphoric acid for AA analyses in melon samples. The application of both procedures with other samples would be of great interest.

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