Biljana Cvetković[1)]
Zvonko Nježić[1)]
Vladimir Filipović[2)]
Biljana Ćurčić[2)]
Milica Nićetin[2)]
Jasmina Gubić[1)]
Ljubinko Lević[2)]

1) University of Novi Sad, Research Institute for Food Technology in Novi Sad, Serbia (biljana.cvetkovic@fins.uns.ac.rs)
2) University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

COMPARATION OF EXTRACTION METHODS FOR HPLC DETERMINATION OF L-ASCORBIC ACID IN VEGETABLES

Abstract: *L-Ascorbic acid (AA) is the main biologically active form of vitamin C. A lot of methods based on the reversible redox reaction of AA oxidation/DHA reduction were developed in the past. The aim of this paper is to compare three different extractants for L-ascorbic acid HPLC determination. Vegetables (red, yellow and green pepper) were purchased from local market. One part of the vegetables was diluted with distilled water, second with potassium hydrogen phosphate, and third diluted with 3% meta-phosphoric acid (MPA) in 8% acetic acid. The HPLC measurements of each sample were repeated three times. Results were statistically evaluated related to sample basis*
Keywords: *L-ascorbic acid, extraction, method, vegetables*

1. INTRODUCTION

Ascorbic acid (vitamin C) is a natural antioxidant mainly present in fruits and vegetables. Its use as an additive in fruit juices, jams, dairy products, etc. is allowed by the European Commission. It is well known for its important role in biochemical processes, such as collagen formation, iron absorption and its involvement in neurotransmission and in immune responses. The biologically-active isomer of ascorbic acid (vitamin C, AA) is L-ascorbic acid, but there is still some discussion about the activity of L-dehydroascorbic acid [1] [2]. D-ascorbic acid (isoascorbic acid, erythorbicacid, iso-AA) is legally used as an antioxidant food additive, but it has only 5% of the antiscorbutic effect of AA [3]. AA is rapidly oxidized to dehydroascorbic acid (DHA) due to the presence of two hydroxyl groups in its structure. Oxidation reactions can be induced by exposure to increased temperatures, high pH, and light, presence of oxygen or metals and enzymatic action. This reaction is reversible and a principal step-in the antioxidant activity of AA. Further oxidation generates diketogluconic acid (DKG), which has no biological function and the reaction is no longer reversible [2] [4]. Ascorbic acid is ubiquitous constituent of all green plants, with the exception of dormant seeds, and it is not difficult to obtain an adequate supply in the daily diet. Relatively high amounts of ascorbic acid are found in strawberries,, citrus and various vegetables, although the availability of ascorbic acid in these foodstuffs is influenced by numerous factors [5]. Since humans cannot synthesize ascorbate, their main source of the vitamin is dietary fruit and vegetables. Fruits and vegetables are the best sources of this vitamin C. An accurate and specific

determination of the nutrients content of fruits and vegetables is extremely important to understand the relationship of dietary intake and human health. Several techniques have been used for the analysis of ascorbic acid in foodstuffs, including spectrophotometric, potentiometric and spectrofluorimetric, chromatographic methods are preferred because of their advantages of simplicity, short analysis time and sensitivity [6]. Several methods are available for the determination of ascorbic acid in food samples. These analytical methods employ titrimetry [7][8], spectrophotometry [9] and electrochemical methods. To ensure that the subsequent HPLC analysis is effective, it is very important to optimize sample extraction. AA is readily oxidized under alkaline conditions. Metaphosphoric acid may provide efficient AA extraction by preventing oxidation [14] [15] compared to other acids [16].

2. MATERIAL AND METHODS

Vegetables (red, yellow, green pepper) were purchased from local market. Peppers were washed and edible parts were chopped into fine pieces in a commercial blender. Chopped peppers (10 grams) were weighed and put in 100 ml volumetric flask. One part was diluted with distilled water (E1), second with potassium hydrogen phosphate (E2), and third diluted with 3% meta-phosphoric acid (MPA) in 8% acetic acid (E3). The high pressured liquid chromatographic method used for the determination of AA consisted of an isocratic elution procedure with UV-visible detection. The analyses were carried out on a system Agilent 1100, USA with Diode Array Detector (DAD) and injection loop of 20 µl. The chromatographic system was equipped with a GROM_SIL 120 ODS-5 ST (5 µm particle size, 150 x 4 mm, using an isocratic 100 mM ammonium acetate mobile phase at a flow rate of 0.4 ml/min. The temperature of the analytical column was kept at 37^{o}C. Detection wavelength was set at 254 nm; 0.16 AUFS. The standard solutions and extracts were filtered through a 0.45 µm membrane filter (Agilent, Germany) before their injection in the chromatograph. AA peak was identified by comparing its UV-visible spectral characteristics and retention time with commercial standard of AA. Chromatographic determinations of L-Ascorbic acid in three extracts were repeated three times.

3. RESULTS AND DISCUSION

Fig. 1 shows L-ascorbic content in peppers extracted by distilled water, buffer and 3% meta-phosphoric acid in 8% acetic acid. Highest values of L-ascorbic content were obtained in the samples extracted by 3% meta-phosphoric acid in 8 % acetic acid, 159.15, 148.91 and 182.39 for green pepper, yellow pepper and red pepper respectively. Determinated L-ascorbic acid content extracted by distilled water was 106.01 for green pepper, 122.05 for yellow pepper and 142.25 for red pepper. Values obtained by buffer extraction were lower than values of L-ascorbic content for pepper samples extracted by metaphosphoric acid in acetic acid, which is in accordance with some authors[14-16]. On table 1. Statistical parameters of red pepper L-ascorbic acid determination are shown. Coefficient of variation indicates that best homogeneity has buffer extracted red pepper (coefficient of variation is lowest). Higher values of Skewness indicate that curve of distribution is negative asymmetric, which mean that results has more higher values compared to normal distribution. Negative values of Kurtosis (ku) indicate that the curve flattened.

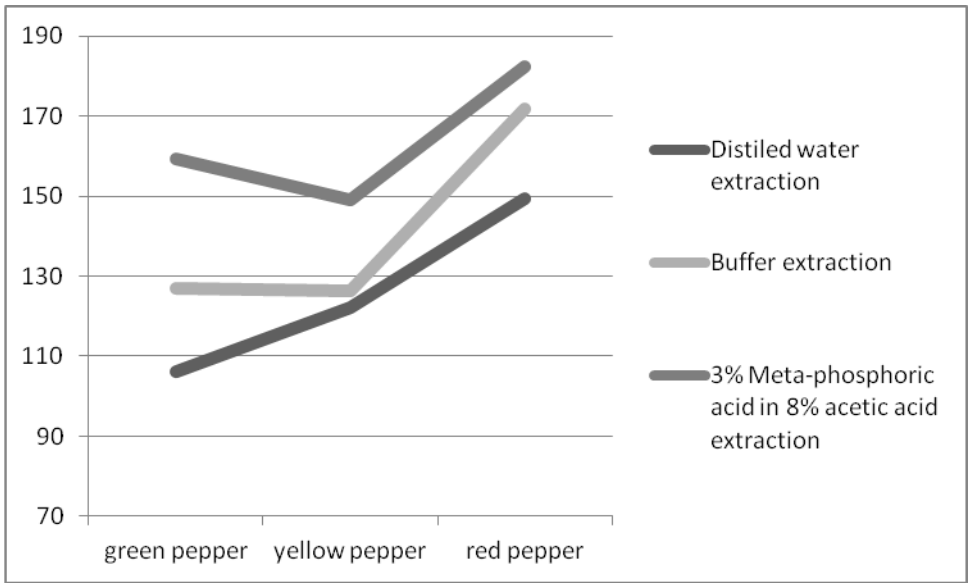

Fig. 1 *L-ascorbic acid content in peppers extracted by distilled water, buffer and 3% meta-phosphoric acid in 8% acetic acid*

Table 1. Statistical parameters of L-ascorbic content determination in red pepper

	Standard deviation	*Coefficient of variation*	*Confidence interval*		*Skewness*	*Kurtosis*
Distilled water extraction	5.14	3.44	145.30	153.20	0.25	-0.89
Buffer KH_2PO_4 extraction	6.20	3.40	177.61	187.14	0.31	-0.91
3% Meta-phosphoric acid in 8% acetic acid extraction	7.07	4.11	166.43	177.30	0.15	-0.73

On table 2. Statistical parameters of yellow pepper L-ascorbic acid determination are shown. Coefficient of variation indicates that best homogeneity has distilled water extracted yellow pepper (coefficient of variation is lowest). Higher values of Skewness indicate that curve of distribution is negatively asymmetric, which mean that results has more higher values compared to normal distribution. Negative values of Kurtosis (ku) indicate that the curve flattened On table 3. Statistical

parameters for determinated L.ascorbic content in green pepper shows that best homogeneity has sample extracted in distilled water (KV=0.95). Results has more higher values compared to normal distribution in case of distilled water exteaction (Skewness 0.13). Lower values of Skewness (-1.25 for meta-phosphoric acid in acetic acid extraction) indicate that distribution is positively asymmertic which mean that there are more lower values compared to normal distribution.

Table 2. Statistical parameters of L-ascorbic content determination in yellow pepper

	Standard deviation	*Coefficient of variation*	*Confidence interval*		*Skewness*	*Kurtosis*
Distilled water extraction	1.15	0.95	121.09	123.02	0.13	-.38
Buffer KH_2PO_4 extraction	2.42	1.92	124.18	128.23	0.03	-1.14
3% Meta-phosphoric acid in 8% acetic acid extraction	2.70	1.81	146.65	151.17	0.53	-1.11

Table 3. Statistical parameters of L-ascorbic content determination in green pepper

	Standard . deviation	*Coefficient of variation*	*Confidence interval*		*Skewness*	*Kurtosis*
Distilled water extraction	6.49	6.13	101.36	110.66	2.51	4.63
Buffer KH_2PO_4 extraction	2.07	1.63	125.39	128.35	0.01	-1.02
3% Meta-phosphoric acid in 8% acetic acid extraction	4.26	2.68	156.10	162.20	-1.25	1.08

5. CONCLUSION

Analysis of L-ascorbic acid content in red, yellow and green pepper shows that there is significant difference between results obtained by different extraction methods. Higher values were obtained in samples extracted in 3% meta-phosphoric acid in 8% acetic acid. Therefore it can be concluded that L-ascorbic acid is best preserved in meta-phosphoric acid which is in accordance with other authors.

This research has also shown that there is the difference in L-asacorbic acid content, depending on the degree of maturity of the peppers.

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