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Original Article

Extraction and quantitative determination of ascorbic acid during different maturity stages of *Rosa canina* L. fruit

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Abstract

Dog rose (*Rosa canina* L.) fruit in different stages of proposed was used as a source of ascorbic acid. Two sample preparation methods for extracting ascorbic acid in dog rose fruit were evaluated. These methods used high performance of liquid chromatography (HPLC) for detecting of ascorbic acid, but differed in the preparation of sample (freezing and mild-temperature-drying procedure). Under optimized conditions, the freezing procedure demonstrated better results. The method was used to compare the amount of ascorbic acid in fully ripe, half-ripe and unripe dog rose samples. The results show that dog rose has the highest amount of ascorbic acid in its fully ripe maturity stage. In addition, the intra-day stability of ascorbic acid in standard solution, fully ripe dog rose extract and fully ripe dog rose intact fruit, was investigated. The results show that ascorbic acid has highest stability in untreated dog rose fruits. As a comparative study, orange sample was also analyzed by the methodology developed in this work. The results show that the amount of ascorbic acid in dog rose fruit (417 mg per 100 g) is about 6 times higher than that in orange sample (76 mg per 100 g).

Keywords: *Rosa canina* L.; Dog rose; Ascorbic acid; Vitamin C; Freezing; Liquid chromatography; Extraction

1. Introduction

Vitamin C belongs to the water-soluble class of vitamins. Ascorbic acid (AA) is an odorless, white solid having the chemical formula C6H8O6. This vitamin is easily oxidized to form dehydroascorbic acid (DHAA), and thus oxidation is readily reversible from DHAA (Groff et al., 1995).

The importance of vitamin C was first discovered in 1747. It is the major water-soluble antioxidant within the body. Humans are one of the few species who lack the enzyme to convert glucose to vitamin C. The vitamin readily donates electrons to break the chain reaction of lipid peroxidation. The water-soluble properties of vitamin C allow for the quenching of free radicals before they reach the cellular membrane. Vitamin C is important in collagen formation, thereby resulting in stabilization of the peptide. Indirectly, AA plays important regulatory roles throughout the entire body due to its involvement in the synthesis of hormones, hormone-releasing factors, and neurotransmitters (Groff et al., 1995; Jacoba, 1999).

There is a wide variety of food containing vitamin C. The general public today generally knows that the best sources of vitamin C are citrus fruits such as orange and their juices. A wide variety of other foods also contain sufficient quantities of vitamin C, such as pineapples, sweet peppers, broccoli, curly kale, cauliflower, black currants, and dog rose. Among these foods, dog rose has the highest amount of vitamin C, so the fruits of these roses were used to prevent scurvy (Jacoba, 1999).

Rosa canina L., known as dog rose, is a truly multipurpose plant that grows wild and can be cultivated in the garden, and it is very much under-estimated and -utilized. Teas made from ‘‘rose hips’’ have mild laxative and

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diuretic tendencies. They help regulate the menstrual cycle, and stem heavy periods. Infusions made of the leaves and petals are soothing to the skin, and can help heal rashes and abrasions. Taken as a tea an infusion of the petals is good for bringing down fevers, aiding the liver and gallbladder, and treating the symptoms of colds and influenza, such as runny noses and sore throats. In addition, the petals are also good for stopping diarrhea.

Several methods have been developed for the estimation of ascorbic acid levels in different samples. These include spectrophotometry (Noroozifar and Khorasani, 2003; Aydogmus et al., 2002; Sena et al., 2000), calorimetry (Antonelli et al., 2002), chemiluminescence (Kato et al., 2005), voltammetry (Ahmed et al., 2005; Ensafi, 2003), enzymatic assays (Shekhovtsova et al., 2006; Danet et al., 2000), and amperometric method (Kumar and Narayanan, 2006). However, there may be some drawbacks in sensitivity, selectivity, stability, or difficulties in sample preparation. Nowadays, high-performance liquid chromatography (HPLC) (Iglesias et al., 2006; Shakya and Navarre, 2006; Lopes et al., 2006; Frenich et al., 2005; Kand'ar et al., 2005; Brause et al., 2003; Yuan and Chen, 1999) and capillary electrophoresis (Law et al., 2005; Wu et al., 2007; Tang, and Wu, 2005; Zinellu et al., 2004; Jin and Jiang, 2002), with various detection methods has been the most used technique for the analysis of ascorbic acid in different samples. The amount of ascorbic acid has been reported in dog rose samples using conventional extraction method (Bozan et al., 1998; Halasova and Jicinska, 1998).

In this work dog rose fruit in different maturity stages was used as a source of AA. Two methods were applied for sample preparation and AA content was then determined in all samples using liquid chromatography method. Also stability of AA in different type of samples (standard solution, dog rose extract, and dog rose fruit) was investigated. In addition, the amount of AA in this fruit was compared with its amount in orange fruit using the same sample preparation procedure and chromatographic method.

2. Methods and materials

2.1. Reagents and chemicals

L-ascorbic acid (AA), metaphosphoric acid (MPA), orthophosphoric acid, and acetonitrile (HPLC-Grade) were all purchased from Merck (Darmastdt, Germany). For chromatographic analysis, de-ionized water of 18 $M\Omega\,cm^{-1}$ resistivity purified with a milli-Q system (Millipore, Bedford, USA) was used. Ascorbic acid stock standard solution was prepared in water and stored in a glass-stoppered bottle at 4 °C in the dark.

2.2. Plant materials

Dog rose (*R. canina* L.) fruits were obtained from the Tehran's Botanical Garden (Tehran, Iran) in April of 2006. Ripeness stage of dog rose samples was classified by the color of fruits. So that green, orange, and red colors were characterized as the sign of unripe, half-ripe and full ripe samples, respectively. The orange sample was collected at fully ripeness and used for comparative study.

2.3. Instrumentation

The HPLC system consists of Waters liquid chromatograph (Milford, MA, USA) equipped with a 600E multisolvent delivery system, an in-line degasser, a manual injection with 20 μL loop (Rheodyne 7125), and Waters 2487 dual λ absorbance detector. Empower® software was used for controlling the analytical system and data processing.

2.4. Extraction of ascorbic acid

Sample preparation was performed according to the freezing method or mild-temperature-drying technique. In the first method, dog rose samples were sliced, frozen into liquid nitrogen and in the second method, samples were dried in mild temperature (15–20 °C) and ground to find powder dust. These two methods in combination with extraction processes are described in the following sections.

2.4.1. Freezing procedure

About 100 g of each maturity stages (fully ripe, half-ripe, and unripe) of dog rose and orange samples were separately frozen into liquid nitrogen and stored at −70 °C until the analysis were carried out. Frozen pulverized samples were weighed (1.0 g for orange, 1.0 g for fully ripe, 1.0 g for half-ripe, and 2.0 g for unripe dog rose sample) and mixed with 5 mL of the extractant solution containing 5% of MPA. The mixture was homogenized for 5 min and then it was centrifuged at 2000 rpm for 10 min. All extractions were carried out under reduced light and at 4 °C. All the extraction processes was three times replicated. The extracts were stored at 4 °C less than 1 h before analysis. Dog rose extracts were diluted 5 times with HPLC-grade water and subsequently were injected. The injection of extracts into HPLC system was performed twice.

2.4.2. Mild-temperature-drying procedure

This procedure is a modification of the method done by Bozan et al. (1998). About 100 g of dog rose samples in each maturity stage were separately weighed and dried under mild temperature (15–20 °C) and ground to find powder dust before extraction. Then obtained powder were weighed (1.0 g for orange, 1.0 g for fully ripe, 2.0 g half-ripe, and 2.5 g for unripe dog rose sample) and subsequently extracted with 25 mL of extractant solution, containing 5% MPA, at 10 °C and in the dark. Extraction process was performed using a shaker for 4 h. All extractions were carried out in triplicate and obtained solutions were then filtered and stored at 4 °C before