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COMPARISON OF ASCORBIC AND CITRIC ACID CONTENTS IN *Rosa Canina L.* FRUIT GROWING IN THE CENTRAL ASIAN REGION*

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The evaluation of Rosa canina L. *fruits and their products is depended in part on their organic acid contents. In this study, ascorbic and citric acid contents in* Rosa canina L. *fruits collected from five different regions in Central Asia were investigated. Amounts of acids determined with the HPLC method were found to be 0.048-0.1143 g/100 g for ascorbic acid and 5.9-7.5 g/100 g for citric acid.*

Rosehip is dried, ripe, aggregate fruits of *Rosa canina L.* (Rosaceae), a shrub native in Europe and Western and Central Asia [1, 2].

Ascorbic acid (Vitamin C) is an important constituent ranging from 0.5 to 1.7% in fresh rosehip. In addition to ascorbic acid, it contains a large number of different chemical compounds including pectins, tannins, and organic acids (mainly citric and malic acids) [1-3].

Rosehip is mainly used for supportive therapy in cases of ascorbic acid deficiency. The mild laxative and diuretic action which lies at the basis of its use in folk medicine is supposedly due to the pectin and organic acids content. The drug is used to prepare teas, extracts, purees, marmalades, even soups, all of which are consumed for their ascorbic acid content. The extracts are also incorporated into a number of "natural" vitamin preparations including tablets, capsules, and syrups, in addition to Vitamin C. A number of medical drugs such as "Kholosas," "Dry extract of Rosa," "Carotolinum," and "Oil of Rosa" have been produced from rosehips [1-5].

The aim of this study was to investigate the ascorbic acid and citric acid contents in *Rosa canina L.* samples growing in various regions of Central Asia.

Ascorbic acid and citric acid contents in various *Rosa canina L.* samples were quantitatively determined by the HPLC method.

It is known that considering the instability of ascorbic acid in aqueous solutions, extraction and sample preparation cause problems [6, 7]. The extraction method and stability tests have been the subject of numerous investigations while the stabilizing properties of metaphosphoric acid have been confirmed in several publications [3, 8, 9]. The extraction procedure used in this study and stability tests have already been investigated in our previous study [10].

Extraction with 40 ml of 3% metaphosphoric acid of 1 g of drug for 30 min at ambient temperature was found to be the best extraction condition for good recovery and stability of ascorbic acid. Therefore, the same extraction procedures were applied to all samples. In the HPLC conditions used, ascorbic acid and citric acid were separated with a Zorbax C18 column eluted within 15 min. Liquid chromatograms of standard solutions and an extract are shown in Fig. 1.

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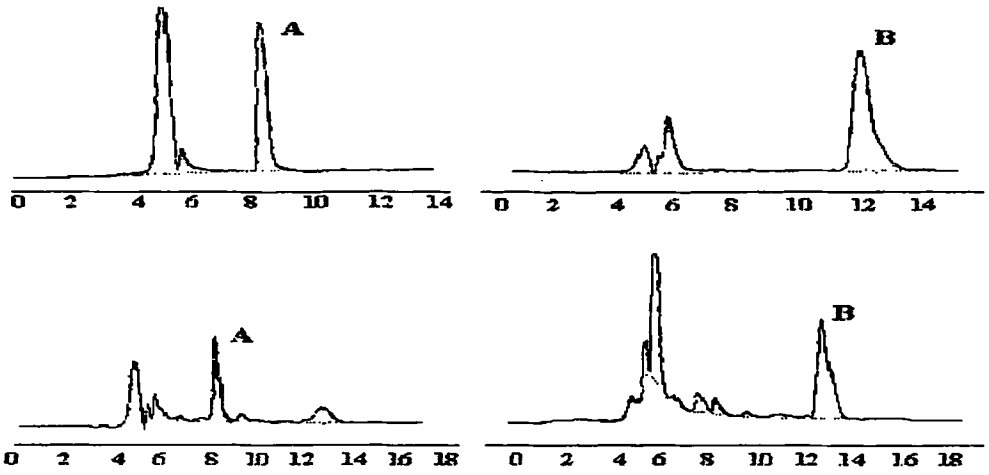

Fig. 1. HPLC chromatograms of ascorbic acid (A), citric acid (B), and an extract (C at 242 nm, D at 210 nm).

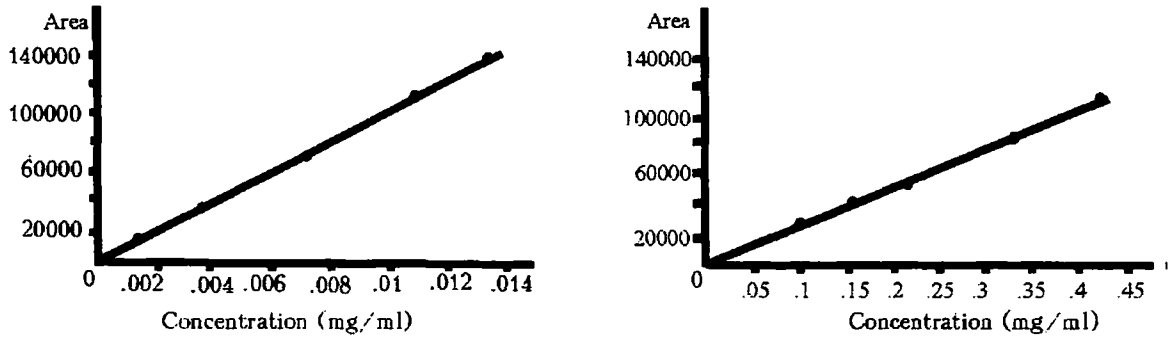

Fig. 2. Calibration curves of ascorbic acid and citric acid.

The peaks with retention times of about 8 min and 12 min correspond to ascorbic and citric acids, respectively. The peak areas of each compound were found to vary linearly with the measured concentrations (Fig. 2).

Regression equations were formulated as y=10610076x-3542 (r=0.9997) for ascorbic acid and y=252656x+98 (r=0.9998) for citric acid, where y is the interpolation unit and x is the weight, in mg per ml of acid.

The amounts of ascorbic acid and citric acid were varied and depend on the collection area of the *Rosa canina L.* fruits investigated, and were found to be between 0.048-0.1143 g/100 g for ascorbic acid and 5.9-7.5 g/100 g for citric acid. The results are given in Table 1.

The highest ascorbic acid content was found in *Rosa canina L.* samples collected from Andijan and citric acid from Bostanlik. Although citric acid content found in the Andijan sample was slightly less than that of Bostanlik sample, Andijan sample can be more suitable for preparing rosehip products due to richness of both ascorbic acid and citric acid contents.

EXPERIMENTAL

Fresh *Rosa canina L.* fruits were collected from five different regions - Tashkent, Samarkand, Andijan, Bostanlik, and Chimkent - in Central Asia, and dried under mild temperature (15-20°C). L-Ascorbic acid and citric acid were obtained from Toprak Ilac (Turkey) and Sigma (USA), respectively. Metaphosphoric acid used in the mobile phase was of Merck quality.

Sample Preparation. 1 g of seedless, dried, and powdered rosehip samples were extracted with 40 ml of 3% metaphosphoric acid for 30 min using a shaker and then filtered. The solution was then brought to a final volume to 50 ml in a volumetric flask using the same solvent used in extraction.

TABLE 1. Ascorbic Acid and Citric Acid Contents in *Rosa canina* Samples

Sample	Ascorbic acid (g/100g)	Citric acid (g/100g)
Tashkent	0.0816	5.90
Samarkand	0.0764	6.63
Andijan	0.1140	7.05
Bostanlik	0.0460	7.50
Chimkent	0.0650	7.00

Standard Stock Solutions and Calibration Curves. Standard stock solutions were prepared by dissolving 13 mg of ascorbic acid and 10 mg of citric acid each in 10 ml of 3% metaphosphoric acid. Stock solutions containing 0.1, 0.3, 0.5, 0.8, and 1 ml of ascorbic acid, and 0.5, 0.7, 1.0, 1.5, and 2.0 ml of citric acid were diluted with 3% metaphosphoric acid in 5 ml volumetric flask. A 10 µl volume of each solution was injected into the column. Peak areas of the chromatograms were plotted against concentrations (mg/ml).

HPLC Assay. The LC system used a Varian Model 2010, equipped with a Rheodyne Model 7125 syringe-loading valve fitted with a 10 µl sample loop. HPLC experiments were conducted using a Zorbax C18 (5 µm particle size, 25 cm x 4.6 mm I.D., Dupont, USA) column with flow rate of 0.5 ml/min at ambient temperature. The mobile phase was 0.5% methaphosphoric acid. A Shimadzu SPD6-AV UV - Vis detector set at 242 nm for ascorbic acid and 210 nm for citric acid was used. Chromatographic data were processed by a Shimadzu CR 4-A Model Chromatographic Integrator.

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