

UDC 581.192

DETERMINATION OF THE CONTENT OF ORGANIC ACIDS AND SUGARS IN FRUIT JUICE OF AZERBAIJAN WILD POMEGRANATE

A.M.Zeynalova, E.N.Novruzov

(Presented by Academician of ANAS V.M.Alizade)

The quantitative and qualitative determination of organic acids and sugars in the fruit juice of Azerbaijan wild growing pomegranate was carried out by high-performance liquid chromatography. It has been shown that pomegranate juice contains oxalic, tartaric, malic, lactic and citric acids. The dominant organic acid of the juice was citric acid, accounting for approximately 98% of the total organic acids (29.43 g / kg). The contents of oxalic, tartaric, malic and lactic acids were 0.025 g / kg, 0.079 g / kg, 0.525 g / kg, and 0.125 g / kg, respectively. Glucose and fructose were found in the sugar content of wild pomegranate fruit juice. The fructose content (60.3 g / l) exceeded the glucose content (47.3 g / l). The content of reducing sugars was 14.24 g / 100 ml.

Keywords: fruit juice, organic acids, sugars, high performance liquid chromatography

Introduction

The pomegranate (*Punica granatum* L.) is a member of the Lythraceae family and *Punica* L. genus, native to Transcaucasia and Central Asia from Iran and Turkmenistan to Northern India [1, 2]. At present, wild pomegranate is widely distributed in the foothill and lower mountain zones of the Great and Lesser Caucasus, in the Kur-Araz and Samur-Divichinsk lowlands and in the lower mountain belt of the Talysh Plateau, on dry slopes up to 700 m above sea level [3].

The use of wild pomegranate is diverse. Pomegranate has been widely used in folk medicine in many cultures [4]. Recent scientific data confirm the traditional use of pomegranate as a medicinal product and indicate the content of biologically active substances in the tissues of fruits, flowers, bark and leaves of pomegranate that have antimicrobial properties, as well as reducing blood pressure and counteracting serious diseases such as diabetes and cancer [5]. The results of numerous studies allow us to recommend it for use as an antitumor, antibacterial, antidiarrheal, antifungal, anti-ulcer and antioxidant agent [6]. Studies have also been conducted on the effects of various pomegranate-derived products on the prevention and reduction of atherosclerosis in which these pro-

ducts have demonstrated anti-atherogenic effects [7]. The juice of wild pomegranate can serve as a raw material for the production of citric acid [8]

The value of pomegranate juice is due to a number of biologically active substances contained in its composition, such as anthocyanins, flavonoids, tannins, etc. In addition, pomegranate juice contains organic acids, sugars, vitamins, polysaccharides, and important minerals [9].

The purpose of this work was to determine the quality and quantity of organic acids and sugars in the samples of wild pomegranate juice by using the HPLC method.

Materials and methods

Plant material

The object of the study was the fruit of *P. granatum* collected on the territory of Galalti in Siyazan district (40°52'43.91" N - 49°11'18.73" E) during the period of full maturation.

Chemicals and reagents

Organic acids (oxalic, tartaric, malic, ascorbic, acetic, citric, lactic, fumaric and succinic acids) and sugars (arabinose, fructose, galactose, glucose, maltose and sucrose) as well as all solvents for HPLC were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Preparing a juice sample

The pomegranate fruits were separated and the exocarp was removed manually. The arils were separated manually from the mesocarp and endocarp, and the juice was obtained by mechanical press. Sample preparation and determination of organic acids were performed in accordance with GOST 33410-2015, sugars-GOST 32167-2013 (reducing sugars-GOST 5903-89). To measure the mass concentration of all determined organic acids, the sample was diluted 2 and 50 times.

HPLC analysis of organic acids and sugars

Organic acids and sugars were analyzed using Varian LC / MS (US Ser. No. 05755). The injection volume was 10 µl for both samples. Organic acids were separated on a Supelcogel TMC-610H column (internal diameter 30 cm × 7.8 mm, Supelco, Bellefonte, PA, USA), isocratic, at 30 ° C, using 0.1% phosphoric acid as the mobile phase at a flow rate 0.5 ml / min. Acetonitrile / water (85:15, v / v) was used as the mobile phase in the analysis of sugars on a µBondapak-NH2 column (30 cm × 3.9 mm, i.e., Waters, Milford, MA, USA).

Results and discussions

Organic acids and sugars in fruits are important components of their taste and organoleptic qualities. These compounds serve as unambiguous markers for assessing not only sensory traits, but also genotype characteristics [10]. Since citric and malic acids are the main organic acids in fruits, most research is related to their metabolism and accumulation. As a rule, intermediate precursors in organic metabolism are other organic acids or sugars, so organic acids can be synthesized from sugars and

vice versa. The main pathway for synthesis is via the respiratory oxidation, and carboxylation or decarboxylation reactions with specific reactions associated with individual acids [11]. After synthesis, organic acids are transported and compartmented into vacuoles. The final content of organic acid in fruits is determined by the balance of the acid synthesis, degradation or utilization and storage compartmentation in the vacuole [7].

When the sample was diluted 2 times in the juice of wild pomegranate fruits, the following organic acids were detected and quantified: oxalic, tartaric, malic and citric acids, and when diluted 50 times –oxalic, tartaric, malic, lactic and citric acids (Table).

As can be seen from the table, the contents of oxalic, tartaric, malic and lactic acids were 0.025 g / kg, 0.079 g / kg, 0.525 g / kg, and 0.125 g / kg, respectively, in the juice of wild pomegranate fruit. The main organic acid of the juice is citric acid, which makes up approximately 98% of the total amount of organic acids. According to Singleton V. L. and Gartner W. A. [12] changes in the concentration of citric acid are associated with the development and maturation of fruits. Short-term environment factors such as solar radiation do not appear to affect the citric acid level. Change in malic acid concentration is not related to fruit maturation but inversely related to evapotranspiration, and ascorbic acid is positively correlated with solar radiation. It is known that organic acids have a strong bacteriostatic effect [13].

Table

Composition and content of individual organic acids of *P. granatum* fruit juice (50-fold dilution)

Organic acids	Content (g / kg)	Retention time (minutes)
Oxalic acid	0.025	7.213
Tartaric acid	0.079	7.419
Malic acid	0.525	9.067
Lactic acid	0.125	10.867
Citric acid	29.43	16.071

Like many antimicrobial substances, organic acids work in the direction of reducing pH, so the acid suppresses the work of cellular enzymes and affects the transport system of nutrients, which causes metabolic disorders of microorganisms and kills them. Numerous studies have confirmed that acidification of animal feed with various weak organic acids, such as formic, fumaric, lactic and sorbic acids, reduces pathogen colonization and production of toxic metabolites, improves the digestibility of protein, Ca, P, Mg and Zn, increases the efficiency of animal growth and reduces their incidence [14]. The relatively high content of citric acid (29.43 g / kg) in the juice of wild pomegranate fruit allows us to consider it as a valuable raw material for the production and use of citric acid as an antimicrobial agent.

Pomegranate fruit is a rich source of sugars. According to research by Shwartz E. et al. [15] the sugar level in pomegranate juice strongly correlates with the level of total soluble solids ($R^2 = 0.89$, $P < 0.01$). The content of reducing sugars in the juice of Azerbaijan wild pomegranate was 14.24 g / 100 ml. Glucose and fructose were found in the composition of wild pomegranate juice sugars. Wherein, the fructose content (60.3 g/l) exceeded the glucose content (47.3 g/l). Our findings contradict the statements of Melgarejo P. and Artes F. who claim that glucose is the predominant sugar in pomegranate, and the amount of glucose in this fruit is greater than fructose [10]. In addition to the main sugars (glucose and fructose), trace amounts of arabinose, sucrose and maltose were also found in the juice [15].

Conclusions

As a result of the study, the qualitative composition and quantitative content of organic acids and sugars in the juice of wild pomegranate fruit were determined by the method of high-performance liquid chromatography. The qualitative composition of organic acids is represented by the following acids: oxalic, tartaric, malic, lactic and citric acids. It was found that the dominant organic acid of the juice is citric acid, which makes up approximately 98% of

the total amount of organic acids (29.43 g / kg). The contents of oxalic, tartaric, malic and lactic acids were 0.025 g / kg, 0.079 g / kg, 0.525 g / kg, and 0.125 g / kg, respectively. Glucose and fructose were found in the qualitative composition of wild pomegranate juice sugars. The fructose content (60.3 g/l) exceeded the glucose content (47.3 g / l). The content of reducing sugars was 14.24 g/100 ml.

REFERENCES

1. Holland D., Hatib K., Bar-Yaakov I. (2008) The pomegranate: New interest in an ancient fruit. *Chronica Horticulturae* 48: 12-25
2. Levin G.M. (2006) Pomegranate (1 st Edn). Third Millennium Publishing. East Libra Drive Tempe. AZ, 1-129
3. Asadov K.S., Asadov A.K. (2001) Dikorastushchie plodovye rasteniya Azerbajdzhana. Baku, "Azerbajdzhan Milli Ensiklopediyasy" 252 p. (In Russian)
4. Li Y., Guo C., Yang J., et al. (2006) Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chem.* 96: 254–260
5. Aviram M., Rosenblat M., Gaitini D., et al. (2004) Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. *Clin. Nutr.* 23: 423–433
6. Valadares M.C., Pereira E.R.T., Benfica P.L., et al. (2010) Assessment of mutagenic and antimutagenic effects of *Punicagranatum* in mice. *Braz. J. Pharm. Sci.*, 46(1): 121-127
7. Brune A., Gonzalez P., Goren R., Zehavi U., et al. (1998) Citrate uptake into tonoplast vesicles from acid lime (*Citrus aurantifolia*) juice cells. *Journal of Membrane Biology* 166: 197-203
8. Aliev R.K., Damirov I.A. (1949) Ispol'zovanie plodov dikogo granata dlya polucheniya medicinskoj limonnoj kisloty i limonnokislogo natra. *Doklad AN Az SSR* 5(9): 16-18. (In Russian)
9. Gokoglu N., Topuz O. K., Yerlikaya P. (2009) LWT – Food Science and Technology 42: 113–118
10. Melgarejo P., Artes F. (2000) Organic acids and sugar composition of pomegranate juice. *Eur. Food Res. Technol.* 4: 30–31
11. Ulrich R. (1970) Organic Acids In: A.C. Hulme (ed.). *The Biochemistry of Fruits and their Products*. Academic Press, London, 89-118
12. Singleton V.L., Gortner W.A. (1965) Chemical and physical development of the pineapple fruit II. Carbohydrate and acid constituents. *J Food Sci.* 30: 19-23
13. Ricke S.C. (2003) Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poult Sci.* 82(4):632–639

14. Gunal M., Yayli G., Kaya O., et al. (2006) The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. *Int J Poult Sci.* 5(2):149–155

15. Shwartz E., Glazer I., Bar-Ya'akov I., et al. (2009). Changes in chemical constituents during the maturation and ripening of two commercially important pomegranate accessions. *FoodChem.* 11: 965–973

Institute of Botany of ANAS
aydan.zeynalova.az@gmail.com

AZƏRBAYCANDA YAYILMIŞ YABANI NAR MEYVƏSİNİN ŞİRƏSİNDƏ ÜZVİ TURŞULARIN VƏ ŞƏKƏRLƏRİN MİQDARININ TƏYİNİ

A.M.Zeynalova, E.N.Novruzov

Yüksəkeffektli maye xromatoqrafiya metodu ilə Azərbaycanada bitən yabanı nar meyvəsinin şirəsində üzvi turşular və şəkərlərin miqdarı və keyfiyyət tərkibi tədqiq edilmişdir. Nar meyvəsinin şirəsinin tərkibində quzuqulağı, şərab, alma, limon və süd turşuları aşkar edilmişdir. Şirənin üzvi turşu cəmində limon turşusu əsas komponent olub, ümumi cəmin 98%-ni (29,43 q/kq), quzuqulağı turşusu 0,025 q/kq, alma turşusu 0,525 q/kq, çaxır turşusu 0,079 q/kq, süd turşusu 0,125 q/kq təşkil edir. Yabanı nar şirəsinin tərkibində qlükoza və fruktoza müəyyən edilibdir. Fruktozanın miqdarı (60,3 q/L) qlükozanın miqdarından çoxdur. Reduksiyaedici şəkərlərin miqdarı 14,24 q/100 ml təşkil edir.

Açar sözlər: meyvə şirəsi, üzvi turşu, şəkər, yüksəkeffektli maye xromatoqrafiya

ОПРЕДЕЛЕНИЕ СОДЕРЖАНИЯ ОРГАНИЧЕСКИХ КИСЛОТ И САХАРОВ В СОКЕ ПЛОДОВ ДИКОРАСТУЩЕГО ГРАНАТА, ПРОИЗРАСТАЮЩЕГО В АЗЕРБАЙДЖАНЕ

А.М.Зейналова, Э.Н.Новрузов

Методом высокоэффективной жидкостной хроматографии (ВЭЖХ) проведено количественное и качественное определение органических кислот и сахаров в соке плодов дикорастущего граната, произрастающего в Азербайджане. Показано, что сок плодов граната содержит в составе щавелевую, винную, яблочную, молочную и лимонную кислоты. Доминирующей органической кислотой сока является лимонная кислота, составляющая приблизительно 98% от общего количества органических кислот (29.43 г/кг). Содержание щавелевой кислоты составляло 0.025 г/кг, содержание винной кислоты – 0.079 г / кг, содержание яблочной кислоты – 0.525 г / кг, содержание молочной кислоты – 0.125 г / кг. В составе сахаров сока дикого граната были обнаружены глюкоза и фруктоза. Содержание фруктозы (60.3 г/л) превышало содержание глюкозы (47.3 г/л). Содержание редуцирующих сахаров составило 14,24 г/100 мл.

Ключевые слова: сок плодов, органические кислоты, сахара, высокоэффективная жидкостная хроматография