Comparison of Proline and Arginine Contents of Pomegranate and Grape Juices in Turkey for the Detection of Juice Adulteration

Meyve Sularında Tağşişin Saptanmasında Bir Yöntem Olarak Türkiye'de Üretilen Bazı Nar ve Üzüm Sularındaki Prolin ve Arginin Miktarlarının Karşılaştırılması

Research Article

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ABSTRACT

Pomegranate is a fruit that is very popular at the moment due to its many reported health benefit effects. Consumer demands increased to 100% pomegranate juices. Pomegranate juice is subject to adulteration due to its high price. Grape juices are one of the juices used for the pomegranate adulteration. In this study, the contents of major amino acids of grape, i.e. proline and arginine were compared to corresponding amino acids of pomegranate using a micellar electrokinetic chromatographic analysis method coupled with laser induced fluorescence detection. Arginine was found significantly ($p < 0.001$) higher in grape juices compared to pomegranate juices. This amino acid was proposed a possible grape adulteration marker for pomegranate juices.

Key Words
Pomegranate, grape, adulteration, MEKC-LIF.

ÖZ

Nar, sağlığa olan faydaları ile son zamanlarda popüler hale gelmiş bir meyvedir. Artan tüketici talepleri %100 nar suları yönündedir. Nar suyu, pahalı olması nedeniyle gıda hilelendirmesine maruz kalmaktadır. Üzüm suları, nar suyu hilelendirmesinde kullanılan meyve sularından biridir. Bu çalışmada, üzümün başlıca aminoasitlerinden olan prolin ve arjinin miktarları, bu aminoasitlerin nardaki karşılıkları ile lazer indüklenmiş floresans detektör ile birleştirilmiş misel elektrokinetik kromatografi tekniği kullanılarak karşılaştırılmıştır. Üzüm sularındaki arjinin miktarı, nar sularındakine nazaran anlamlı derecede ($p < 0.001$) yüksek bulunmuştur. Bu aminoasit üzüm ile hilelendirilmiş nar sularının tespitinde muhtemel bir gösterge olarak önerilmiştir.

Anahtar Kelimeler
Nar, üzüm, tağşiş, MEKC-LIF.

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INTRODUCTION

Of recent pomegranate has been gaining increasing interest due to its reported health benefit effects. In vitro and in vivo studies have demonstrated antioxidant, antibacterial, anti-inflammatory, antiviral, and anti-carcinogenic activities of this fruit [1,2]. Recent research has shown that pomegranate extracts selectively inhibit the growth of breast, prostate, colon, and lung cancer cells in culture. Recently, Adhami et al., [3] have reviewed the investigations into the effects of pomegranate fruit on cancer. More recently, the effects of the pomegranate on obesity and the positive effects on fat reduction have been shown [4]. Following its health benefits popularity, the demands of consumers to 100% pomegranate juices have increased and recently many commercial pomegranate juices have appeared in markets. However, pomegranate juice has been the subject of adulteration. Food adulteration is a worldwide problem in terms of food quality and food safety. Foods having high commercial value are targets to be adulterated. With food adulteration, not only consumers are being defrauded, but also economies of regions and countries are adversely affected due to unfair competition. Therefore, in terms of human health and economic points of view, detection of fraudulent foods is important. Finding a specific marker from chemical constituents of food products could be a way to detect the adulteration or authenticity of the food.

Capillary electrophoresis (CE) has been widely used in food analysis due to its short analysis time, high separation efficiency, and low environmental impact [5]. Low running costs of the technique and easily regenerable silica columns enable analyses of different food products with the same system. Accordingly, many food quality control laboratories can afford capillary electrophoresis instruments. Employing capillary electrophoretic methods, specific chemicals of foods can be easily detected.

Fruit juice adulteration occurs generally with diluting a more expensive product with a less expensive juice. Because pomegranate is produced in a restricted region in the world and is harvested in a short time period, it is sold at expensive prices in many countries. Thus, pomegranate juices labeled as 100% are at times adulterated. Pomegranate juices are generally adulterated with the addition of cheap and widely available grape and apple juices.

Although to date there are many reports giving the chemical compositions of widely consumed fruit juices, such studies on pomegranate have started only in the last few years. The organic acid and sugar contents, as well as some phenolic compounds in pomegranate have been reported [6-13]. Very recently, we reported amino acid profile of pomegranate juices [14].

In parallel to newly reporting the compounds in pomegranate juices, specific markers are now being suggested for the determination of the adulteration of pomegranate juice by other fruit juices. Zhang et al., [15] have established authenticity specifications for pomegranate juices based on information from existing databases and published literature. Our group has detected apple juice adulteration in commercial pomegranate juices by evaluating a combination of data based on antioxidant capacities, total phenolics, organic acid contents and fructose/glucose ratios of commercial juices [8]. In our recent report, the amino acid profile of pomegranate juices has been compared to apple amino acids and L-Asn has been proposed as a marker for the adulteration of pomegranate juices with apple juices [14]. Very recently, Nuncio-Jáuregui et al., [16] have determined and compared organic acids, sugars, minerals, proline and volatile compounds of pure pomegranate juice and two potential juices for adulteration, namely grape and peach juices. Boggia et al., [17] have proposed a UV screening method together with multivariate analysis to detect the differences induced in the pomegranate juice spectra by the addition of different juices.

The aim of the current study is to compare the major amino acids of grapes with the corresponding amino acids of pomegranate grown in Turkey, to reveal juice adulteration using the results of micellar electrokinetic analysis coupled to laser induced detection of amino acids.

MATERIALS and METHODS

Materials

Fluorescein isothiocyanate isomer I (FITC) was purchased from Fluka (Buchs, Switzerland). D- and L-amino acids, sodium dodecylbenzene sulfonate (SDBS), and β-cyclodextrin (β-CD) were obtained from Sigma-Aldrich (Steinheim, Germany). Disodium tetraborate decahydrate was purchased from Merck (Darmstadt, Germany). The pH of solutions was arranged with HCl and NaOH solutions which were both from Merck (Darmstadt, Germany). All solutions were prepared with water purified by an Elga Purelab Option-7-15 model system (Elga, UK).

Stock solutions of L-Arginine and L-Proline amino acids (10 mM) were prepared in deionized water. FITC was dissolved in acetone at a concentration of 20 mM. Stock amino acid solutions and FITC solutions were stored in the dark at 4°C and prepared freshly when needed.

Grapes (five of them were white, three of them were pink and two of them were red) and pomegranates were bought from different local green grocers. They were freshly squeezed in the laboratory.

Derivatization of Standards

A derivatization solution was prepared by mixing 30 μL of stock solutions of each amino acid and 15 μL of FITC and by diluting to 1 mL by 100 mM borate buffer. The optimal ratio of total amino acid concentration to FITC concentration was found sufficient as 1:1. The mixture vial was capped and allowed to stand in the dark at 40°C for 4 h. This solution was diluted with distilled water in the appropriate proportions prior to their injection into CE instrument.

Derivatization of Samples

All the grape juices were filtered from microfilters. The optimal concentration of FITC was selected as 0.8 mM for 40 μL grape or pomegranate juices which are diluted to 1 mL with 100 mM borate buffer. After keeping in dark at 40°C for 4 h, this solution was diluted with distilled water and injected directly.

Apparatus and Operating Conditions

Separations were performed with an Agilent capillary electrophoresis system (Waldbronn, Germany) equipped with a ZETALIF 2000 laser-induced fluorescence detector (Picometrics, Montlaur, France). The excitation was performed by a 488 nm Ar ion laser. The data processing was carried out with the Agilent ChemStation software.

Injections were made at 50 mbar for 6 s. Samples were separated at 25 kV. The temperature was set at 25 °C. The fused silica capillary used for separation experiments has 50 mm i.d. and was obtained from Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary is 60 cm and the length to the detector is 45 cm.

The new fused silica capillary was conditioned prior to use by rinsing with 1M NaOH for 30 min and with water for 10 min. Between runs 2 min. of flushing with 1 M NaOH and water and then 5 min. of buffer was performed.

Separation Conditions and Analytical Parameters

Amino acid analyses of grape and pomegranate fruit juices were performed by a MECK-LIF analysis method which was optimized according to our previous study [14]. The optimal separation electrolyte was selected as 5 mM SDBS, 10 mM β-cyclodextrin, and 50 mM borate at pH: 9.5. SDBS was used in borate buffer for micelle formation and the use of SDBS instead of commonly used surfactant sodium dodecyl sulfate (SDS) enhanced the fluorescent intensities of FITC-derivatized amino acids. The addition of β-cyclodextrin to the separation buffer significantly decreases migration times of amino acids and also enables to check possible D- enantiomer species. The pH of borate buffer was selected as 9.5 mainly because of the maximum fluorescence intensity of FITC at this pH value [17].

Linear concentration range is between 0.03-0.75 μM for L-arginine and between 0.03-0.90 μM for L-proline respectively. The precisions of corrected peak areas as RSD% are 1.62% and 2.68% for arginine and proline respectively. The limit of detection was calculated as 3 times of the

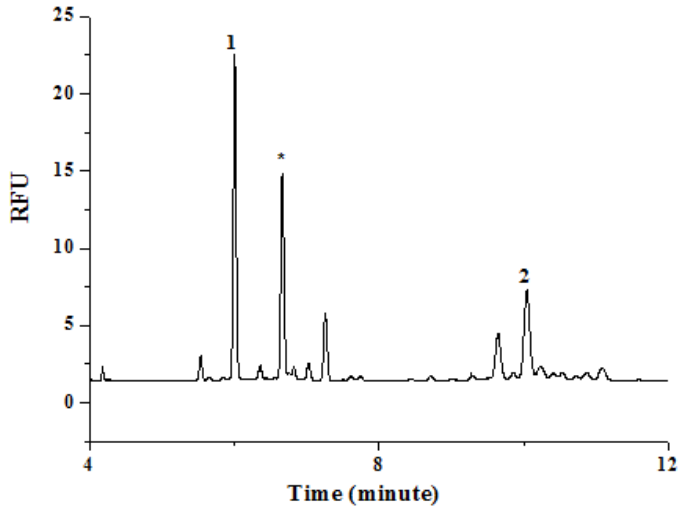

Figure 1. Electropherogram of a 1:500 diluted white squeezed grape juice sample. Buffer: 5 mM SDBS, 10 mM β-cyclodextrin, and 50 mM borate at pH: 9.5; injection: 50 mbar, 6 s. voltage: 25 kV; detection: excitation at 488 nm, emission at 520 nm; 1: L-Arginine, 2: L-Proline, *: peak from FITC.

average noise taken for three different baseline areas and found to be 1.09 and 0.848 nM for arginine and proline respectively.

Data Analysis

The data were reported as mean ± standard deviation. The results were analyzed using the IBM SPSS 23.0 statistical software program for windows. To compare the significant differences of the mean values at $p < 0.05$ and $p < 0.001$, one way analysis (ANOVA) and the Duncan's new multiple range test were applied to the result.

RESULTS and DISCUSSION

MEKC-LIF analysis of grape juices revealed that major amino acids of grape juices are proline and arginine. A representative electropherogram of a grape juice is given in Figure 1. This result is consistent with the results previously reported research for grape juices by chromatographic analysis [18-20]. Consequently, proline and arginine contents of grape and pomegranate juices were analyzed in the same conditions by the MEKC technique.

L-Arginine and L-Proline contents of grape and pomegranate juices are given in Table 1. As seen from Table 1, the proline contents of 10 grape juices investigated were found as between 153 and 426 mg/L and the proline contents of 6 pomegranate juices were between 202 and 872 mg/L. The concentration ranges for arginine in grape and pomegranate juices were 1103-1879 mg/L and 72-202 mg/L, respectively. There is a significant difference in proline contents between grape varieties and also between grape and pomegranate varieties ($p < 0.05$).

It does not seem that there is any significant difference in arginine contents between grape varieties ($p > 0.05$). However the arginine contents of grape juices are significantly higher than those of pomegranate juices ($p < 0.001$).

As far as we know, the only chromatographic method on amino acids of pomegranate was recently reported by our group [14]. Since proline is chemically different from the other 20 amino acids, some spectrophotometric methods exist for proline analysis. In the literature spectrophotometric analysis result of proline contents for pomegranate juices were reported by three groups by now [15,16,21]. Zhang et al., [15] suggested that proline contents above 25 mg/L are indicative of adulteration of pomegranate juice with grape juice, while Halilova and Yıldız [21] and Nuncio-Jáuregui et al., [16] reported much higher proline contents in pomegranate juices. Halilova and Yıldız [21] compared proline contents of pomegranates for consecutive two years and pointed to the change in proline

Table 1. L-Arginine, L-Proline, and the ratio between L-Arginine and L-Proline of grape and pomegranate juices.

	L-Arginine (mg L^{-1} ± SD)*	L-Proline (mg L^{-1} ± SD)*	L-Arginine/L-Proline
Grape Juice			
W1	1327 ± 21^{j}	306 ± 15^{g}	4.34^{h}
W2	1690 ± 42^{m}	426 ± 22^{k}	3.97^{g}
W3	1621 ± 38^{k}	268 ± 12^{f}	6.05^{m}
W4	1268 ± 25^{h}	236 ± 11^{e}	5.37^{k}
W5	1732 ± 54^{n}	376 ± 31^{j}	4.61^{i}
P1	1879 ± 48^{o}	338 ± 19^{g}	5.56^{l}
P2	1193 ± 36^{g}	306 ± 13^{g}	3.90^{f}
B1	1631 ± 35^{l}	153 ± 9^{a}	10.7^{o}
B2	1103 ± 25^{f}	219 ± 9^{d}	5.04^{j}
B3	1315 ± 18^{i}	193 ± 8^{b}	6.81^{n}
Pomegranate Juice			
POM1	80 ± 7^{b}	421 ± 15k	0.19^{b}
POM2	202 ± 12^{e}	738 ± 22l	0.27^{d}
POM3	93 ± 8^{d}	371 ± 12j	0.25^{cd}
POM4	81 ± 9^{bc}	872 ± 37m	0.09^{a}
POM5	72 ± 7^{a}	202 ± 28c	0.36^{e}
POM6	82 ± 7^{bc}	368 ± 11i	0.22^{bc}

*Means ± standard deviations. Different letters in the same lines are significantly different at the 5% level ($p < 0.05$).
W: white grape, P: pink grape, B: black grape, POM: pomegranate.

content by climate. The average proline content of three pomegranate cultivars were reported as 30 mg/L in year 2007 and 93 mg/L in year 2008 by authors.

Nuncio-Jáuregui et al., [16] reported proline content in pomegranate juice as 251 mg/L and concluded that proline contents higher than 250-300 mg/L are indicative of addition of grape juices. Though proline contents of fruits change according to climate, maturation state, and nitrogen fertilization [22,23] the used analysis method discrepancy of reported proline values are quiet high. On the other hand, some reported ranges in proline content of grape varieties are also broad. Recently Long et al., [20] have reported the proline content of the analyzed grape juices as ranging from 194.7 to 3281.9 mg/L by HPLC analysis for 10 well-known grape species used in wine production. Thus, the setting of proline limits should not be a trustable marker for grape juice adulteration for pomegranate juices. The best way might be the analysis of amino acid contents of both fruits from the same region with the same analysis methods. According to our findings in the present study, pomegranate juice contains proline levels close to those of grape juice, so that proline cannot be used as a trustable marker for the adulteration of pomegranate juice with grape juice. As a result of simultaneous chromatographic analysis of proline and arginine in pomegranate and grape juices in the present study, arginine is proposed as a marker for the adulteration of pomegranate juices with grape juices.

In conclusion, this work shows that the combination of MEKC-LIF analysis of FITC derivatized amino acids, arginine and proline, and principal component analysis technique is

an adequate tool to distinguish pomegranate and grape juices. Arginine was found significantly higher in grape juices compared to pomegranate juices ($p < 0.001$) and this amino acid was proposed a possible grape adulteration marker for pomegranate juices.

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