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Evaluation of Antioxidant Properties of Commercial Pomegranate Juices

Liudmila P. Nilova, Svetlana M. Malyutenkova, Polina S. Lebedeva

Peter the Great St.Petersburg Polytechnic University

CORRESPONDENCE:
Liudmila P. Nilova
Adress: 195251, Russia, St.Petersburg, Polytechnicheskaya, 29
E-mail: nilova_l_p@mail.ru

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ABSTRACT

Background. Consumers consider pomegranate juice one of the most beneficial for health. The limited production of pomegranates in Russia cannot meet the needs of Russian consumers with juices of its own production. Pomegranate juices are produced from concentrates or pasteurized direct-squeezed juices imported from different countries, there is no information about their antioxidant properties.

Purpose. Study of the content of the main biologically active substances and antioxidant activity of pomegranate juice of various brands on the consumer market, and their influence on the formation of antioxidant properties.

Materials and Methods. The objects of research were freshly pressed juice from pomegranate fruits (control)and samples of commercial pomegranate juices produced using different technologies: "Grande", "Benature", "Grante" — direct-squeezed juice; "Swell", "O'кей", "Rich", "Gold Brand" — reconstituted pomegranate juice; "Nar" — reconstituted juice with the addition of direct-squeezed juice. In juices, the total content of phenolic compounds, anthocyanin, flavonoids, tannins and vitamin C was determined. The antioxidant activity of juices was determined by two methods: FRAP and coulometrictitration based on the measurement results of which the antioxidant index was calculated.

Results. Juices of industrial production differed significantly in the amount of biologically active substances, the spread of values in their content between juices was 40–50 %, they contained significantly less total anthocyanin and total flavonoids than freshly-pressed juice. Total tannins prevailed in the composition of phenolic compounds of all juices (40–75 %). The antioxidant activity of juices varied depending on the method of determination. The FRAP test showed the highest values of antioxidant activity in the reconstituted pomegranate juice "Rich", and the coulometric titration showed the highest values in the direct-squeezed pomegranate juice "Benature". Juices had the highest antioxidant index Rich>Benature>Grante, the values of which were more than 85 %. The antioxidant properties of pomegranate juices depend on the total amount of phenolic compounds (R^2 > 0.772) and total tannins (R^2 > 0.538).

Conclusions. The formation of antioxidant properties of pomegranate juices depends on the amount of total phenolic compounds and total tannins. The results of the evaluation of the antioxidant properties of commercial pomegranate juices can be used in the development of functional beverages.

KEYWORDS

pomegranate juice, brands, total phenolic compounds, total flavonoids, total anthocyanins, total tannins, vitamin C, antioxidant activity

Оценка антиоксидантных свойств коммерческих гранатовых соков

Л. П. Нилова, С. М. Малютенкова, П. С. Лебедева

Санкт-Петербургский политехнический университет

КОРРЕСПОНДЕНЦИЯ:
Нилова, Л.П.
Адрес: Санкт-Петербург, Политехническая улица, д. 29, 195251, Россия
E-mail: nilova_l_p@mail.ru

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АННОТАЦИЯ

Введение. Гранатовый сок среди потребителей считается одним из самых полезных для здоровья. Ограниченное производство гранатов в России не может обеспечить потребности российских потребителей соками собственного производства. Гранатовые соки производят из концентратов или пастеризованных соков прямого отжима, импортируемых из разных стран, информация об их антиоксидантных свойствах отсутствует.

Цель. Исследовать содержания основных биологически активных веществ и антиоксидантной активности гранатового сока различных брендов, реализуемых на потребительском рынке, и их влияние на формирование антиоксидантных свойств.

Материалы и методы. Объектами исследований являлись свежеотжатый сок из плодов граната (контроль) и образцы коммерческих гранатовых соков, выработанные по разным технологиям: прямого отжима «Grande»; «Benature»; «Grante»; восстановленного гранатового сока «Swell»; «ОКей»; «Rich»; «GoldBrand»; восстановленного сока с добавлением сока прямого отжима «Nar». В соках определяли общее содержание фенольных соединений, антоцианов, флавоноидов, танинов и витамин С. Антиоксидантная активность соков была определена двумя методами: FRAP и кулонометрическим титрованием, по результатам измерений которых, был рассчитан антиоксиданный индекс.

Результаты. Соки промышленного производства значительно различались количеством биологически активных веществ, разброс значений которых между соками составлял 40-50%,содержали значительно меньше антоцианов и флавоноидов, чем свежеотжатый сок. В составе фенольных соединений всех соков преобладали танины (40–75%). Антиоксидантная активность соков изменялась в зависимости от метода определения. FRAP-тест показал самые высокие значения антиоксидантной активности у восстановленного гранатового сока «Rich», а кулонометрическое титрование — у гранатового сока прямого отжима «Benature».Наиболее высоким антиоксидантным индексом отличались соки Rich>Benature>Grante, значения которых были больше 85%. Антиоксидантные свойства гранатовых соков зависят от общего количества фенольных соединений (R^2>0,772) и танинов (R^2> 0,538).

Выводы. Формирование антиоксидантных свойств гранатовых соков происходит под влиянием общих фенольных соединений и танинов. Результаты оценки антиоксидантных свойств коммерческих гранатовых соков могут быть использованы при разработке функциональных напитков.

КЛЮЧЕВЫЕ СЛОВА
гранатовый сок, бренды, фенольные соединения, флавоноиды, антоцианы, танины, витамин С, антиоксидантная активность

INTRODUCTION

Among fruit juices, pomegranate juice is in particular demand among consumers due to its high taste properties and established opinion of its health benefits. In many countries of the world, pomegranate fruits and pomegranate juice are recommended for strengthening the immune status and preventing cardiovascular, oncological, degenerative and other diseases (Karomatov et al., 2018; Sahar et al., 2019; Morittu et al., 2020; Pepe et al., 2020; Khalimova, 2022). Pomegranate fruits and juice are classified as "super fruits" rich in antioxidants (Martins et al., 2019; Hegazi et al., 2021). Its antioxidant activity can be up to three times that of red wine and green tea (Gil et al., 2000). The antioxidant properties of pomegranate juice are provided by various groups of phenolic compounds — flavonoids, anthocyanin, condensed and hydrolysable tannins, phenolic acids, which make up the majority of known bioactive compounds. The high variability in the quantitative composition of pomegranate phenolic compounds under the influence of internal and external factors, juice production technology did not allow the European Food Safety Authority (EFSA) to establish a causal relationship between the consumption of pomegranate juice and the submitted claims for its health benefits (Giménez-Bastida et al., 2021).

The composition of biologically active substances of pomegranate fruits

Nowadays, more than 500 compounds have been isolated and identified from pomegranate fruits, including ellagitannins, gallotannins, anthocyanin, flavanol-anthocyanin adducts, flavonoids, phenolic acids, and other phenolic compounds (Yisimayili & Chao, 2022). The amount of the main phenolic compounds in pomegranate fruits varies greatly depending on the botanical variety and growing location (Türkyilmaz & Özkan, 2014; Nuncio-Jáuregui et al., 2015; Legua, et al., 2016; Topalović et al., 2020). Juice wild-growing pomegranates can contain 4387–8461 mg/l of phenolic compounds, and exceed their amount in cultivated varieties by five or more times (Topalović et al., 2020).

The total phenolic content in pomegranate juice of cultivated varieties depends both on the botanical variety and on the place of growth and varies: in Spain — 900–1450 mg/L (Legua et al., 2016; Topalović et al., 2020); in Florida — 365.7–1167.4 mg/L (Shahkoomahally et al., 2021); in North Africa — 2890–4500 mg/L (Fawole et al., 2012). Pomegranate fruits cultivated in Iran (5264–7874 mg/L) and China (3151–7428 mg/L) contain the most phenolic compounds (Zarei et al., 2010; Li et al., 2015; Asadi-Gharneh et al., 2017).

The composition of phenolic compounds is affected by the amount of solar energy received in the process of fruit growth. On the example of pomegranate juice of the "Dentedi Cavallo" variety grown in different regions of Sicily, differences in the content of certain groups of phenolic compounds depending on the cardinal direction were established. The maximum amount of flavonoids and anthocyanin contains juice from pomegranates grown in the south of Sicily, tannins — in the west, hexosides of phenolic acids — in the north. The juice from pomegranates grown on the western side contains the least phenolic compounds (Di Stefanoetal et al., 2020). Botanical varieties of pomegranates of the same name grown in China and Spain differ in the amount of anthocyanins with a predominance in Chinese fruits (Chen et al, 2022).

During the ripening of pomegranate fruits, the amount of individual phenolic compounds changes: the content of tannins decreases by 6–27 %, while the content of flavonoids and anthocyanin increases by 3.2–3.5 and 1.5–2 times, respectively (Mphahlele et al., 2014). There is the evidence of more pronounced changes as pomegranate fruits ripen — a decrease in phenolic compounds by 3–7 times due to the hydrolysis of tannins and the content of ellagic acid and its derivatives. It reduces their antioxidant activity: test with ABTS (2.2'-azinobis (3 -ethylbenzihiazoline)-6-sulfonic acid) and DPPH (diphenilpicrylhydrazil) radicals — 1.6–2.0 times, FRAP test (Ferric reducing antioxidant power) — 5 times (Legua et al., 2016; Nuncio-Jáuregui et al., 2015).

The influence of technology on the antioxidant properties of pomegranate juices

The amount of phenolic antioxidants and the antioxidant activity of pomegranate juice also depend on the technology (direct squeezing, reconstituted, cold bottling, alternative technologies) (Tezcan et al., 2009; Türkyilmaz & Özkan, 2014; Conidi et al., 2020; Di Stefano et al., 2020). As a result, commercial varieties of pomegranate juice differ significantly in the content of total phenolic compounds and their individual representatives (Tezcan et al., 2009; Mphahlele et al., 2014; Li et al., 2015; Mphahlele et al., 2016; Morittu et al., 2020; Esposto et al., 2021). This has a direct impact on its antioxidant activity and, accordingly, therapeutic and prophylactic properties.

Pomegranate juice can be obtained by squeezing whole fruits or halves, from bags arils or pomegranate arils plus seeds, which affects the composition of phenolic compounds. When using whole fruits for squeezing juice, it contains less flavonoids compared to juice from half fruits, and there are more a monomeric anthocyanins. The largest amount of phenolic compounds passes into the juice from pomegranate halves due to tannins, the amount of which increases by 1.7 times compared to juice from pomegranate seeds and increases antioxidant activity by 2.2 times (Mphahlele et al., 2016). Compared with juice from pomegranate arils plus seeds, the content of hydrolysable tannins in juice from whole fruits increases by 6.55 times, increasing the antioxidant activity of juices by 21.8% (Muhacir-Güzel et al., 2014). With the increase in the duration of pressing and the increase in pressure, a more intense transition of tannins occurs. Strong positive linear correlations were found between pressing pressure and antioxidant activity (r = 0.973) and total phenolic content (r = 0.979) (Türkyilmaz et al., 2013).

Freshly pressed juice made from pomegranate seeds contains more anthocyanins and significantly less condensed tannins and ellagic acid than commercial juice from concentrates. The amount of hydrolyzable tannins remains almost the same regardless of the method of juice production (Gil et al., 2000).

Further changes in the composition of phenolic compounds in pomegranate juices can occur during their purification and heat treatment. Sedimentation leads to a greater loss of tannins (more than 50%), the amount of anthocyanins decreases by 4–6% (Muhacir-Güzel et al., 2014; Erkan-Koc et al., 2015). When clarifying juices with polysaccharide-based agents (chitosan, xanthan gum), the loss of phenolic compounds is higher than with protein-based agents (albumin, casein, gelatin), reducing the antioxidant activity of juices by 2.4–26.6% (Erkan-Koc et al., 2015). Losses of phenolic antioxidants can be less when membrane technologies are used for clarification of pomegranate juice: for polyphenols, flavonoids and anthocyanins they are no more than 4%. Largely, when using membrane technologies, vitamin C is destroyed — by 32% (Morittu et al., 2020).

Pasteurization of juices leads to further losses of biologically active substances, especially vitamin C — up to 50%, and for some phenolic acids (gallic, chlorogenic), campferol, rutin, catechins exceed 50% (Akyildiza et al., 2020). Anthocyanin losses are 8–14% for non-clarified juices and about 2% for clarified ones (Vegara et al., 2013).

Changes in the antioxidant properties of pomegranate juices during storage

Antioxidant properties of pomegranate juices depend not only on raw materials and juice technology, but also on the type of packaging and storage conditions before and after bottling. The influence of technology on the change in the content of phenolic antioxidants during storage of juices has been established. In clarified juices, when stored under different conditions for 6 months, the content of total phenolic compounds practically does not change, in contrast to non-clarified ones, in which it drops sharply, especially under the influence of light (Mena et al., 2014). High-temperature pasteurization during further storage of pomegranate juices contributes to a lower loss of total phenolic compounds and vitamin C than long-term low-temperature pasteurization. Anthocyanins, ellagitannins and punicalagin are the first to degrade. For 120 days of storage of pomegranate juices at +5°C before bottling, more than 40% of anthocyanins degrade (Vegara et al., 2013). After bottling into consumer packaging, the degradation of anthocyanins continues, because of which their amount can be only 35–40% of the original content before bottling (Gafizov & Gafizov, 2015). Anthocyanin monoglucosides are the most unstable (Mena et al., 2014).

The influence of the type of consumer packaging on the change in the content of phenolic antioxidants in pomegranate juice is ambiguous. According to Pérez-Vicente et al. (Pérez-Vicente et al., 2004) the type of consumer packaging has a significant impact on the degradation of anthocyanins. When storing pomegranate juice in bottles of transparent and green glass for 160 days at room temperature, the degradation of anthocyanins was 77–78%, and in paperboard carton with polyethylene layers (Minibrik-200) — 95%. At the same time, the degradation of total phenolic compounds was 20%, regardless of the type of consumer packaging of pomegranate juice. An increase in storage temperature and exposure to light do not significantly affect the change in the content of total phenolic compounds and the antioxidant activity determined by the TEAC and FRAP methods of pomegranate juice (Mena et al., 2014).

Freshly pressed, cold-pressed and unpressurized juices are associated by consumers with healthy and natural products (Martins et al., 2019). This is facilitated by studies of the antioxidant properties of commercial pomegranate juices in a number of countries and the publication of the results in the open press (Tezcan et al., 2009; Esposto et al., 2021). In Russia, studies of the antioxidant properties of commercial pomegranate juices have not been conducted. TR TS 022/2011 "Food products in terms of their labeling" do not provide for information on the content of natural antioxidants in juices in the labeling. The situation is complicated by the fact that in Russia the production of pomegranates is limited and cannot meet the needs of Russian consumers with juices of its own production. Pomegranate juices are imported or produced from concentrates or pasteurized direct-squeezes juices after storage. The variability of the content of biologically active substances in pomegranate juices and the lack of information about their content in various types of commercial pomegranate juices does not allow recommending them to the consumer as a healthy drink.

The aim of the research was to determine the content of the main biologically active substances of pomegranate juice of various brands sold on the consumer market and their influence on the formation of antioxidant properties.

MATERIALS AND METHODS

Objects of study

To conduct research in the retail trade of St. Petersburg, samples of pomegranate juice were purchased, made using different technologies (Table 1). All direct-squeezed juices were produced in the second half of September 2022 according to the information in the labeling. Reconstituted juices were selected with the date of production, so that the period of their presence in retail trade until the time of research was comparable to direct-squeezed juices and did not exceed 30 days. Freshly pressed pomegranate juice from Azerbaijan was used as a control. Juice was pressed from pomegranate seeds.

Table 1
Samples of commercial pomegranate juice

Type of juice	Brand	Producer	Country
Pomegranate direct-squeezed juice	Grande	LLC "NAR", city Slantsy, Leningrad region	Russia
	Benature	LLC "Azgranata"	Azerbaijan
	Grante	CJSC "Aznar"	Azerbaijan
Pomegranate reconstituted juice	Swell	PC "Leader", Moscow region, Lyubertsy	Russia
	O'Кей	LLC "NAR", city Slantsy, Leningrad region	Russia
	Rich	JSC "Multon", St. Petersburg	Russia
	Gold Brand	LLC "AZ-SERVICE", Baku, manufactured by LLC "ANTARIO-CITY", Moscow	Russia Azerbaijan
Pomegranate reconstituted juice with direct-squeezed juice	Nar	LLC "NAR", city Slantsy, Leningrad region	Russia

Equipment

Spectrophotometer "UNICO-2800", USA, glass cuvettes with a layer thickness of 1 cm.

Coulometer "Expert-006-antioxidants", Russia.

Methods

In pomegranate juices, the total content of phenolic compounds, anthocyanins, flavonoids, tannins, vitamin C, and antioxidant activity were determined by two methods — FRAP and coulometric titration.

The total content of phenolic compounds was determined spectrophotometrically using the Folin-Ciocalteu reagent, after the reaction of which with the studied juice, the optical density was measured at the wavelength of 750 nm (Tezcan et al., 2009). The calibration curve was built on gallic acid.

The total content of anthocyanins was determined by pH-differentiated spectrophotometry according to GOST 32709–2014[1]. The mass concentration of anthocyanins in terms of cyanidin-3-glucoside in pomegranate juice was determined based on changes in the absorption of light with the wavelength of 510 nm with a change in the acidity of juice solutions pH from 1 to 4.4.

The total content of flavonoids was determined spectrophotometrically by reaction with aluminum chloride at the wavelength of 420 nm according to GOST R 55312–2012[2]. The calibration curve was built according to the routine.

Total tannins were determined by titration of pomegranate juice with potassium permanganate with the participation of indigo carmine as an indicator according to GOST 19885–74[3].

The determination of vitamin C was carried out by the titrimetric method with a solution of sodium 2.6-dichlorophenolindophenolate according to GOST 24556–89[4].

Determination of antioxidant activity

Antioxidant activity by the FRAP method (Ferric reducing antioxidant power) was determined by the ability of ferric chloride (III) to oxidize antioxidants. In this case, ferric chloride (III) is reduced to ferric chloride (II), the amount of which is determined by the change in color intensity when *O*-phenanthroline is added. The light absorption of the extract was measured at the wavelength of 505 nm against a solution of 96% ethanol on the UNICO-2800 spectrophotometer, USA. Antioxidant activity was determined from the calibration curve and expressed in terms of ascorbic acid[5].

Antioxidant activity was determined by coulometric titration on an Expert-006-antioxidants coulometer. As a titrant, electro-generated bromine was used, the electro-generation of which was carried out at a constant current of 50.0 mA from an aqueous 0.2 M solution of potassium bromide in a 0.1 M solution of sulfuric acid with the determination of the end of titration by potentiometric indication. The time, second, spent on titration of the volume of the aliquot of the studied juice, introduced into the measuring cell, was recalculated into the amount of electricity in coulombs. The electrolytic cell of the coulometer was calibrated with ascorbic acid.

The antioxidant index, percentage, of each juice was calculated as the arithmetic mean of the sum of the ranked scores of antioxidant activity determined by different methods. Ranking of scores from 0 to 1 was performed using the Harington desirability function methodological approach. To do this, the maximum value of the antioxidant activity of juices was assigned 1 point, and then the scores for the remaining juices were calculated as the ratio of the value of their antioxidant activity to the maximum value in the data series for each method separately (Madrigal-Carballo et al., 2009; Nilova et al., 2011).

[1] GOST 32709–2014. (2014). *Juice products. Methods for the determination of anthocyanins*. M.: Standartinform.

[2] GOST 55312–2012. (2012). *Propolis. Methods for determination of flavonoid compounds*. M.: Standartinform.

[3] GOST 19885–74. (2009). *Tea. Methods for determining the content of tannin and caffeine*. M.: Standartinform.

[4] GOST 24556–89. (2011). *Processed products of fruits and vegetables. Methods for determining vitamin C*. M.: Standartinform.

[5] Rogozhin V.V., Rogozhina T.V. (2016) *Workshop on the biochemistry of agricultural products*. S-Petersburg: GIORD.

Research Procedure

One pomegranate juice sample was analyzed daily for all parameters according to the methods outlined above; minimizing sample preparation time between analyzes to prevent oxidation of antioxidants.

Data analysis

The studies were carried out in triplicate of two samples of each juice. The results were expressed as mean ± standard deviation. The assessment of the reliability of the results was carried out using the Student's t-test. The difference was considered significant at a probability of 95%. Pearson correlation coefficient was used to determine the effect of each biologically active substance on antioxidant activity. Statistical processing of experimental data was carried out using the Microsoft Excel 2010 software package.

RESULTS

The content of biologically active substances in commercial pomegranate juices

The studied samples of pomegranate juice significantly differed in the quantitative composition of biologically active substances (Table 2). Freshly pressed pomegranate juice contained by an order more total anthocyanins and total flavonoids than industrially manufactured juices, and the amount of vitamin C in it could differ by more than 3 times ("Grande", "O'Кей", "Gold Brand"). The amount of total phenolic compounds and total tannins in all juices was of the same order of magnitude. Despite this, the spread of values for all biologically active substances, even in the series of commercially produced juices, could reach 40–50%, although there were no statistically significant differences in the data series for some juices. Thus, statistically significant differences were established in the content of: total phenolic compounds between freshly pressed juice and juices "Swell" and "Nar"; total flavonoids between "Benature", "O'Кей" and "Nar" juices; total tannins between freshly pressed juice and "Swell" juice and between "Rich" and "Nar" juice.

In the composition of total phenolic compounds of all studied juice samples, total tannins prevailed, ranging from 40 to 75%. Their number was not affected by the method of making juices. In direct-squeezed juices, the amount of total tannins was in the range of 166.3–353.3 mg / 100 ml, and in reconstituted juices — 103.9–328.6 mg / 100 ml. The method of making juice did not affect the content of other biologically active substances — total anthocyanins, total flavonoids and vitamin C. For example, direct-squeezed juices contained less total

Table 2
The content of biologically active substances, mg / 100 ml, in various samples of pomegranate juice

Juice Samples	Total phenolic compounds	Total anthocyanins	Total flavonoids	Total tannins	Vitamin C
Freshly-pressed	411.2 ± 8.2	10.59 ± 0.05	25.22 ± 0.60	205.5 ±10.6	40.12 ±1.2
Direct-squeezed juice brands:					
Grande	280.5 ± 6.8	0.64 ± 0.01	7.68 ± 0.20	166.3± 8.3	12.8± 0.2
Benature	494.8 ± 6.2	0.68 ± 0.02	6.26 ± 0.10	290.9±7.5	31.3 ±0.7
Grante	468.0 ± 7.6	0.50 ± 0.02	13.90 ± 0.65	353.3±5.6	24.4± 0.4
Reconstituted juice brands:					
Swell	426.1 ± 8.0	1.27 ± 0.04	16.0 ± 0.20	187.0±9.0	29.4±0.3
O'Кей	304.7 ± 6.0	1.00 ± 0.04	6.21 ± 0.25	124.7±3.5	9.6± 0.5
Rich	511.8 ± 9.0	0.31 ± 0.01	16.5 ±0.20	328.6 ±9.6	20.4 ± 0.6
Gold Brand	222.3 ± 5.5	0.45 ± 0.02	4.93 ± 0.10	103.9±3.5	12.0 ± 0.4
Reconstituted juice with direct-squeezed juice					
Nar	418.0 ± 7.0	0.89 ± 0.03	6.45 ± 0.10	311.7±9.0	19.2± 0.9

anthocyanins than reconstituted "Swell" and "O'Кей" juices, but more than reconstituted juices "Rich" and "Gold Brand".

Antioxidant activity of commercial pomegranate juices

The antioxidant activity of juices, as well as the amount of biologically active substances, did not depend on the method of juice production and changed in a series of values obtained by the FRAP method: Rich > Benature > Grante > Swell > freshly pressed juice > O'Кей > Nar > Grande > Gold Brand (Table 3). When using coulometric titration to determine the antioxidant activity of juices, the antioxidant series changed somewhat: Benature > Swell > Grante > Rich > >Nar > freshly pressed juice > O'Кей > Grande > Gold Brand. For an objective assessment of the antioxidant properties of juices, the antioxidant index was calculated.

Rich>Benature>Grante juices had the highest antioxidant index, the values of which were more than 85%. The antioxidant index in the range of 50–85% had juices: Swell>Nar>freshly pressed juice>O'Кей. The direct-squeezed juice "Grande" and the reconstituted juice "Gold Brand" had an antioxidant index less than 50% and amounted to 48.7 and 30.45%, respectively.

Effect of biologically active substances on the antioxidant activity of commercial pomegranate juices.

Establishing the influence of the studied biologically active substances on the antioxidant activity of pomegranate juices is presented in Table 4.

Table 3
Indicators of antioxidant activity of pomegranate juice

Juice samples	FRAP, mg / 100 ml	Coulometric titration, mg / 100 ml	Antioxidant index, %
Freshly pressed	49.10 ± 1.20	69.89 ± 0.80	63.55
Direct-squeezed juice brands			
Grande	41.92 ± 1.00	46.98 ± 0.64	48.70
Benature	62.84 ± 1.50	112.91 ± 1.30	91.81
Grante	60.87 ± 1.25	106.31 ± 1.36	87.58
Reconstituted juice brands:			
Swell	52.94 ± 0.90	110.68 ± 1.43	84.23
O'Кей	46.55 ± 1.10	48.34 ± 0.90	52.38
Rich	75.15 ± 1.83	97.76 ± 1.15	93.29
Gold Brand	34.63 ± 0.92	16.74 ± 0.65	30.45
Reconstituted juice with direct-squeezed juice			
Nar	43.62 ± 1.00	83.00 ± 0.86	65.78

Table 4
Correlation coefficients (R2) between some biologically active substances and indicators of antioxidant activity of the studied samples of pomegranate juice ($p < 0.05$)

Indicators	Total phenolic compounds	Total anthocyanins	Total flavonoids	Total tannins	Vitamin C
FRAP	0.772	0.002	0.152	0.538	0.152
Coulometric titration	0.879	0.163	0.131	0.610	0.387
Antioxidant Index	0.934	0.002	0.153	0.649	0.324

Total phenolic compounds (R2 > 0.772) and total tannins (R2 > 0.538) demonstrate the most marked characteristic between the amount of biologically active substances and the antioxidant activity of juices. The low content of total anthocyanins and flavonoids in commercial pomegranate juices produced using different technologies does not allow us to determine their effect on antioxidant activity.

DISCUSSION OF THE RESULTS

A significant spread of values in the content of biologically active substances in the studied pomegranate juices is not accidental. Basically, their bottling was carried out in Russia from direct-squeezed juices or concentrates obtained from different sources, which increases the uncertainty of the influence of botanical varieties of pomegranate, the region of growth and the degree of ripeness of fruits, technology and the duration of transportation and storage of semi-finished products before bottling. Commercial varieties of pomegranate juice can contain total phenolic compounds from 1379 to 10086 mg/L (Mphahlele et al., 2016; Morittu et al., 2020; Esposto et al., 2021).

Total tannins, the amount of which can reach up to 71–85 % (Esposto et al., 2021), dominate the composition of phenolic compounds. Depending on the degree of maturity of pomegranates and the technology for making juices, the content of total tannins in them can vary widely from 1 to 376 mg/100 ml (Türkyılmaz et al., 2013; Li et al., 2015; Khomich et al., 2019). Freshly pressedjuice from pomegranate seeds can contain more than 300 mg/100 ml of tannins (Erkan-Koc et al., 2015). In juice from unripe pomegranate fruits, the amount of total tannins is 4–27 % more than from fruits of full ripeness. Fluctuations depending on the botanical variety of immature pomegranates are 88.7 % (Mphahlele et al., 2014). The use of whole or halved pomegranates for high-pressure pressing increases the amount of total tannins in pomegranate juice (Türkyılmaz et al., 2013; Mphahlele et al., 2014; Mphahlele et al., 2016). Therefore, it is difficult to establish the cause of the scatter in the values of the amount of total tannins in the studied juices. The studied commercial juices differed significantly in total anthocyanin content compared to freshly pressed juice. The total amount of anthocyanins in them ranged from 0.31–1.27 mg / 100 ml, and in freshly pressed juice, it was 10.59 mg / 100 ml. These results are consistent with published scientific studies. The amount of total anthocyanins in freshly pressed pomegranate juice is usually in the range of 10.9–128.4 mg/100 ml. Their content is increased in the juice from mature red pomegranate seeds grown on the sunny side (Vegara et al., 2013; Conidi, et al., 2020; Di Stefano et al., 2020; Yuan et al., 2022). In freshly pressed juice from pomegranate seeds growing in Azerbaijan, the content of total anthocyanins is 13.93–15.35 mg/100 ml (Hafizov & Hafizov, 2021), which is close to the values we obtained in freshly pressed juice from pomegranate seeds. Commercial varieties of pomegranate juice sold in Russia contain total anthocyanins from 0.2 to 2.24 mg/100 ml (Khomich et al., 2019). Differences in total anthocyanin content between freshly pressed and commercial pomegranate juices may be due to the technological stages of industrial production. Pasteurization at +90 °C reduces the total amount of anthocyanins in clarified pomegranate juice by 5 % and in non-clarified pomegranate juice by 35–40 % (Akyıldız et al., 2020). Commercial juices from concentrates can contain total anthocyanins in the range of 0.8–2.9 mg/L (Esposto et al., 2021). When storing juices before bottling, further degradation of anthocyanins occurs, reducing their amount by 2 times at a temperature of +5°C in 120 days and by 12 times at room temperature in 45 days. At the end of the shelf life before bottling, the anthocyanin content of pomegranate juice can be 1–2 mg/100 ml (Vegara et al., 2013).

Differences in total flavonoid and vitamin C content between freshly pressed juice and commercial types of pomegranate juice were not as significant compared to total anthocyanins. The amount of total flavonoids in pomegranate juices can range from 4.5–142.2 mg/100 ml, increasing in juices from mature pomegranates by 3–4 times (Morittu et al., 2020; Di Stefano et al., 2020). In freshly pressed juice from 10 botanical varieties of pomegranate grown in 4 different regions of China, the amount of total flavonoids ranged from 4.5 to 33.5 mg/100 ml (Li et al., 2015). Commercial juices sold in Russia contain about 10 mg/100 ml of total flavonoids (Khomich et al., 2019).

The content of vitamin C in pomegranate juice can range from 9.0 to 50.0 mg / 100 ml (Mphahlele et al., 2014; Mphahlele et al., 2016; Morittu et al., 2020). Vitamin C is destroyed during pasteurization, the loss of which can reach up to 50 % when pasteurized at +90°C (Akyıldız et al., 2020). Increasing the duration of pasteurization at 95°C from 2 to 3 minutes reduces the vitamin C content of pomegranate juice by almost 2 times (Bhagat & Chakraborty, 2022). The different content of biologically active substances in pomegranate juice samples influenced the formation of their antioxidant activity, in which tannins played the leading role. Tannins and, first, punicalagins, provide 87 % of the antioxidant activity of pomegranate juice obtained in an industrial way (Gil et al., 2000). The role of tannins in the formation of the antioxidant activity of pomegranate juice was confirmed by studies of the composition of phenolic compounds and antioxidant activity (ABTS, DPPH, FRAP) of ripe and unripe pomegranates of 9 botanical varieties (Nuncio-Jáuregui et al., 2015). The antioxidant activity of juice from whole fruits is due mainly to hydrolysable tannins (r = 0.994), and from pomegranate seeds — to condensable tannins (r = 0.956) (Muhacir-Güzel et al., 2014).

CONCLUSIONS

The studies have shown that commercial pomegranate juices presented on the Russian consumer market contain phenolic antioxidants and vitamin C in different amounts, the spread of values between juices being 40–50 %. The composition of phenolic antioxidants of all juices was dominated by total tannins (40–75 %), which influenced the formation of their antioxidant properties (R2=0.649).

Indicators of antioxidant activity of pomegranate juices differ depending on the method of determination, changing the order of significance of juices. The antioxidant index calculated on their basis made it possible to single out three brands of commercial pomegranate juices — Rich>Benature>Grante, with values above 85 %, which can be recommended to consumers as a drink for preventive nutrition.

The obtained results of the quantitative composition of biologically active substances and the antioxidant activity of pomegranate juices will be of interest to researchers in the development of functional drinks, medical workers to recommend juices to patients for a healthy diet.

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