



CHEMICAL COMPOSITION OF SOME AUTOCHTHONOUS FRUIT SPECIES FROM MACEDONIA

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Abstract

The scope of this paper is determination of the content of soluble dry matters, total sugar, total acids, invert sugar and citric, malic, tartaric and lactic acid in some autochthonous fruit species: pomegranate (Zumnarija, Bejnarija, Valandovska kisela, Valandovska kiselo-slatka, Hidjas, Kisela, Lifanka, Ropkavec), apple (Ubavo cvetka, Shareno blago, Prespanka, Tetovka, Karapasha, Kozharka, Bela Tetovka) and cherry (Ohridska brza, Ohridska rana, Ohridska crna, Dolga shishka, Dalbazlija, Ohridska bela). Comparative studies were performed in relation to the standard varieties (Karamustafa-pomegranate, Idared-apple and Bigaro burlat-cherry).

Pomegranate fruits have the highest content of soluble dry matters (15.6%), total sugars (149 g/L) and total acids (20.1 g/L). The highest content of invert sugar was observed in pomegranate (113 g/L which means 76.3% of total sugar). In apple and cherry fruits, malic acid dominates (above 5 g/L), while in pomegranate fruits, citric and malic acids are almost equally represented. Tartaric acid is present only in apple fruits (0.383 g/L). Lactic acid was not found in all fruit species.

A strong positive correlation was found between soluble dry matter and total sugar with coefficient of determination $R^2 = 60\%$. A moderate positive correlation exists between the contents of total and invert sugar with coefficient of determination $R^2 = 20\%$, between total acids and invert sugar with coefficient of determination $R^2 = 22\%$ and between the content of malic and citric acid with coefficient of determination $R^2 = 30\%$. A strong negative correlation was found between the content of total acid and malic acid with coefficient of determination $R^2 = 37\%$ and very strong negative correlation between malic acid and invert sugar with coefficient of determination $R^2 = 77\%$.

Key words: pomegranate, apple, cherry, soluble dry matters, sugars, organic acids

INTRODUCTION

Fruit taste is an important trait majorly controlled by organic acids and together with aromatic volatile compounds and soluble sugars, they strongly facilitate overall organoleptic quality and fruit stability. The chemical composition of fruits depends on several factors as: genotype, climatic and pedological conditions, the way of growing the fruit trees, the applied agrotechnical measures, the degree of maturity and the way of keeping and storing the fruits.

Organic acids and soluble sugars play important roles, in fruit flavour and nutrition, thus, can affect fruit flavour and indirectly exert advantageous and disadvantageous effects on commercial fruit quality. Sugars are an important ingredient in fruits. Fresh fruits usually contain 2-15% sugars (glucose, fructose and sucrose) which are the significantly important for nutrition. Invert sugar (a mixture of equal parts of fructose and glucose) is the most prevalent. These are easy digestible sugars

for the body, necessary for the normal function of the muscles, they refresh the body, have a calming effect on the brain, remove fatigue and increase the level of serotonin (Selamovska & Miskoska-Milevska, 2021). According Li et al. (2020) sucrose is the most abundant sugar in peach juice (72.5% of the total sugar content). In blueberry, sweet cherry juice, glucose and fructose are the predominant sugar (Li et al., 2020; Sokol-Letowska et al., 2020), in sour cherry fruits glucose is dominant sugar (Gúndođdu & Bilge, 2012.). The taste in pomegranate fruits is governed mainly by the presence of sugars (glucose and fructose) (Mayuoni-Kirshinbaum & Porat, 2014). Apple, peach, apricot and banana contain almost equal amount of glucose, fructose and sucrose.

In fresh fruits, organic acids primarily include malic, citric, quinic and tartaric acid, in smaller quantities succinic, formic, salicylic, oxalic, benzoic and other acids. In pome fruits and stone fruits, malic acids predominate, berry fruits and citrus fruits mostly contain citric acid, less malic acid. Citric acid and quinic acid are the main organic acids in kiwifruit (Nishyama et al., 2008) and blueberry (Zhang et al., 2020). The presence of shikimic acid has been detected in gooseberries. In strawberry and raspberry there is the presence of salicylic acid (0.0011-0.0028 g/L) and small amounts of formic acid, in blackberries isocitric acid is detected, in unripe blackcurrant fruits there is succinic acid (Selamovska & Miskoska-Milevska, 2021). Tartaric acid is detected only in grape juice, malic acid is the predominant organic acid in apple (82.9% of the total acid content) and sweet cherry juice (98.2% of the total acid content), citric acid is the predominant organic acid in strawberry (75.3%) and blueberry juice (80.2% of the total acid content) (Li et al., 2020). Malic acid contributes to the pleasant sour taste of the fruit. It has a significant role in biochemical processes, controls blood sugar levels, high blood pressure and cholesterol, acts against allergies, chronic fatigue, problems with digestive tract, various inflammations, infections etc. Citric acid is natural antioxidant.

Citric and tartaric acids are naturally occurring weak organic acids. They play an

important role in product characteristics like taste and aroma. Their presence determines the tartness and flavour of fruits as well as fruit beverages. Both acids are used extensively as food acidulants. Together with fumaric and phosphoric acids, citric and tartaric acids are used in the food industry to enhance beverage flavour. Some studies indicate that citric acid and tartaric acid can have unwanted effects on human health, high concentrations of citric acid can affect tooth enamel, reduced enamel's hardness by 84% (Ren et al., 2009), high doses of tartaric acid can give rise to acute kidney injury, gastrointestinal symptoms and cardiovascular collapse (Naqvi, 2017; Rusyniak et al., 2012), therefore, regulatory organizations, such as Commission regulation of EU (2011), set up a maximum concentration of citric acid at 5.0 g/L (in fruit nectar) and 3.0 g/L (in fruit juice) and for tartaric acid is set at quantum satis, an amount not higher than necessary to achieve the purpose in accordance with good manufacturing practice.

The research is a part of the scientific project "Antioxidant activity of fruits of some indigenous varieties of fruits species, vegetables and grapes" where the content of specific biologically active substances (antioxidants) in fruits and vegetables are detected for each autochthonous variety and population of fruit species (pomegranate, apple and cherry), grapes and vegetables (tomato and pepper). As expected, confirmation of the quality and high content of biologically active substances in the fruits of our autochthonous varieties of fruit species, grown in agro-ecological conditions of R. N. Macedonia, was obtained. This research will show that the quality does not deviate from the world-famous international, introduced species and varieties and even surpass them. According to the values obtained from the examination of the parameters, most of the autochthonous varieties show higher values compared to the proposed standard varieties, which indicates the fact that the autochthonous varieties are characterized by high antioxidant activity of the fruits and have a strong positive impact on the human organism.

MATERIAL AND METHODS

a) Fruit samples

Eight autochthonous varieties of pomegranate (Zumnarija, Bejnarija, Valandovska kisela, Valandovska kiselo-slatka, Hidjas, Kisela, Lifanka, Ropkavec), seven autochthonous varieties of apple (Ubavo cvetka, Shareno blago, Prespanka, Tetovka, Karapasha, Kozharka, Bela Tetovka), and six autochthonous varieties of cherry (Ohridska brza, Ohridska rana, Ohridska crna, Dolga shishka, Dalbazlija, Ohridska bela) were taken as material for analysis. All fruits are from the harvest year 2021, collected in harvest maturity. Pomegranate fruits are from Valandovo region, cherry fruit from Ohrid and apple fruit from Resen region. Comparative studies were performed in relation to the standard varieties (karamustafa-pomegranate, idared-apple and burlat-cherry).

b) Chemical analyses

The laboratory analyses were performed in the oenological laboratory at the Institute of Agriculture in Skopje. The content of soluble dry matters (%), total sugar (g/L), total acids (g/L), invert sugar (g/L) and some organic acids as citric (g/L), malic (g/L), tartaric (g/L) and lactic (g/L) were examined. The content of soluble dry matters and total sugars was determined refractometrically. The determination of total acids was performed potentiometrically, using

titration with NaOH (0.25 N) to pH=7, according to the ISO 750:1998. Organic acids and the content of invert sugar were determined spectrophotometrically by appropriate enzymatic tests (Poyrazoğlu et al., 2002).

c) Statistical analyses

Correlation analysis (r.) between determined variables was applied using XLSTAT 2014 software. Data matrix has been introduced using descriptive statistical analysis: minimum, maximum, mean value and standard deviation.

Correlation coefficient (r.) description (LaMorte, 2021):

+1.0	Perfect positive
+0.8 to 1.0	Very strong positive
+0.6 to 0.8	Strong positive
+0.4 to 0.6	Moderate positive
+0.2 to 0.4	Weak positive
0.0 to +0.2	Very weak positive or no association
0.0 to -0.2	Very weak negative or no association
-0.2 to -0.4	Weak negative
-0.4 to -0.6	Moderate negative
-0.6 to -0.8	Strong negative
-0.8 to -1.0	Very strong negative
-1.0	Perfect negative

RESULTS AND DISCUSSION

The obtained results from the analysis are shown in Tab. 1. The examined fruit species have approximately the same values of the content of soluble dry matters. From the analysed fruit species, pomegranate has the highest content of soluble dry matters (15.6%), total sugar (149 g/L), invert sugar (113 g/L) and total acids (20.1 g/L). Compared to the standard varieties, Zumnaria, Lifanka and Ropkavec variety (pomegranate) and all varieties of apple and cherries have a higher content of soluble dry matter.

All varieties of apples, Zumnarija and Lifanka pomegranate variety and Ohridska

brza, Ohridska crna, Dalbazlija and Ohridska bela cherry variety, contain more sugar than the standard variety. Zumnarija (pomegranate), Ubavo cvetka (apple) and Ohridska crna (cherry) have the highest content of soluble dry matter and total sugar. The highest content of invert sugar (sum of glucose and fructose) has pomegranate fruits (113 g/L which means 76.3% from the total sugars), it has almost equal representation of glucose and fructose. The content of invert sugar is lower in apple and cherry (61.4 g/L means 54.9% from total sugars and 64.3 g/L which means 44.7% from total sugars, respectively).

Table 1. Results for the content of soluble dry matters (%), total sugar (g/L), total acid (g/L), invert sugar (g/L) and some organic acids: citric (g/L), malic (g/L), tartaric (g/L) in some autochthonous fruit species.

Fruit kind	Variety	Soluble dry matters /%	Total sugars /g/L	Total acids /g/L	Citric acid /g/L	Malic acid /g/L	Tartaric acid /g/L	Sum of glucose and fructose /g/L
Pomegranate	Zumnarija	19.0	194	7.00	0.898	0.800	Tr	112
	Bejnarija	15.4	134	4.10	0.974	1.891	Tr	110
	Valandovska kisela	11.2	131	35.2	0.983	0.182	Tr	102
	Valandovska kiselo-slatka	14.7	149	7.30	1.028	0.653	Tr	118
	Higjas	14.8	125	43.3	1.486	0.577	Tr	104
	Kisela	15.0	129	52.6	0.425	0.031	Tr	116
	Lifanka	18.2	169	23.3	0.687	0.920	Tr	99.4
	Ropkavec	16.5	150	4.00	1.103	0.865	Tr	129
	Karamustafa	15.6	159	4.20	0.497	0.817	Tr	129
Apple	Ubavo cvetka	20.3	151	5.80	0.971	5.21	0.187	71.8
	Shareno blago	13.2	106	6.10	0.950	6.00	0.336	61.7
	Prespanka	16.2	125	1.65	0.932	5.230	0.487	55.9
	Tetovka	12.0	104	3.00	0.947	3.951	0.381	58.6
	Karapasha	14.0	106	2.40	0.961	4.206	0.444	62.3
	Kozarka	13.2	114	7.00	0.973	6.122	0.285	57.4
	Bela tetovka	15.2	123	7.00	0.936	5.553	0.692	65.5
	Aidared	8.00	63.5	7.30	0.971	3.931	0.250	58.3
Cherry	Ohridska brza	16.8	130	4.60	1.81	5.58	Tr	57.50
	Ohridska rana	12.9	104	5.20	1.12	6.77	Tr	50.47
	Ohridska crna	18.2	156	4.60	1.84	5.98	Tr	73.22
	Dolga shishka	14.5	123	8.10	1.85	7.18	Tr	52.32
	Dalbazlija	16.5	193	5.60	1.13	6.37	Tr	81.57
	Ohridska bela	16.0	172	7.90	2.58	6.46	Tr	76.77
	Burlat	11.9	125	4.90	1.69	6.78	Tr	58.56

A strong positive correlation was found between soluble dry matters and total sugars. The coefficient of determination $R^2=60\%$ dependence of the variations in the content of soluble dry matters with the content of total sugars (Tab. 2). A moderate positive correlation was observed between total sugars and the

content of sum of glucose and fructose with coefficient of determination $R^2 = 20\%$, as well as between the content of total acids and the content of sum of glucose and fructose with coefficient of determination $R^2= 22\%$. Summery statistic is given in Tab. 3.

Table 2. Matrix of correlation (Pearson correlation).

Variables	Soluble dry matters /%	Total sugars /g/L	Total acids /g/L	Citric acid /g/L	Malic acid /g/L	Tartaric acid /g/L	Sum of glucose and fructose /g/L
Sol. dry maters /%	1	0.7768	-0.0662	0.1008	-0.0189	0.0514	0.2459
Total sugars /g/L	0.7768	1	0.0218	0.1763	-0.1314	0.0551	0.4477
Total acids /g/L	-0.0662	0.0218	1	-0.2224	-0.6101	-0.0213	0.4638
Citric acid /g/L	0.1008	0.1763	-0.2224	1	0.5446	-0.0143	-0.3611
Malic acid /g/L	-0.0189	-0.1314	-0.6101	0.5446	1	0.0168	-0.8776
Tartaric acid /g/L	0.0514	0.0551	-0.0213	-0.0143	0.0168	1	-0.0088
Glucose and fructose/g/L	0.2459	0.4477	0.4638	-0.3611	-0.8776	-0.0088	1

Values in bold are different from 0 with a significance level $\alpha=0.05$

Table 3. Summary statistics.

Variable	Observations	Minimum	Maximum	Mean	Standard deviation
Soluble dry matters /%	29	8.00	20.3	14.96	2.47
Total sugars /g/L	29	63.5	194	135	27.9
Total acids /g/L	29	1.65	52.6	10.8	12.4
Citric acid /g/L	29	0.42	2.58	1.17	0.45
Malic acid /g/L	29	0.03	7.18	3.98	2.40
Tartaric acid /g/L	29	0.18	0.69	0.38	0.07
Glucose and fructose/g/L	29	50.4	129	80.5	25.0

The content of these parameters depends on the type, variety, climatic conditions, degree of maturity etc. The determined results match the available literature data. Self-sprouting pomegranate varieties contain 9.5-13.8% dry matters (Markovski et al., 2017). Apples contain 6.6-15.5% total sugars and cherries contain 4.7-11.5% (Niketic-Aleksic according to Seelamovska & Miskoska-Milevska, 2021), while self-sprouting pomegranate varieties contain 110-152 g/L total sugar (Markovski et al., 2017). Cherry cultivars contents 119.94-215.90 g/kg glucose, 25.24-61.44 g/kg fructose and 1.73-7.60 g/kg sucrose (Gündoğdu & Bilge, 2012). The sum of sugars (glucose, fructose, sucrose and sorbitol) in sweet cherry fruit is ranged from 125 to 265 g/kg fresh weight (FW). In different cherry cultivars glucose content is range between 61.8-123 g/kg, fructose content between 47.6-101 g/kg and sucrose content between 3.57-12.5 g/kg (Usenik et al., 2008). Total sugars in pomegranate are ranged between 139-160 g/L (average 148 g/L) (Poyrazoğlu et al., 2002). The taste in pomegranate fruits is governed content mainly by the presence of sugars (glucose and fructose) and organic acids (Mayuoni-Kirshinbaum & Porat, 2014). According Sokol-Letowska et al. (2020) in sour cherries the main sugars are glucose (2.81-5.68 g/100gFW) and fructose (2.74-4.88 g/100g FW).

The autochthonous varieties of pomegranate have the highest content of total organic acids (about 20%). In pomegranate fruits, citric and malic acid are almost equally represented, with a slight predominance of citric acid, whose content is most pronounced in the Valandovska kiselo-slatka, Higjas and Ropkavec (more than 1.0 g/L). Compared to the standard variety, almost all pomegranate varieties have a higher number of total acids

(with the exception of Ropkavec and Bejnaija).

Apple and cherry fruits contain fewer total acids (about 5%). They are dominated by malic acid (above 5 g/L). Cherry fruits on average contain a higher citric acid content (1.72 g/L) than pomegranate and apple (0.898 g/L and 0.955 g/L, respectively). All autochthonous varieties of apples have a lower content of total acids compared to the standard variety, while autochthonous varieties of cherries, with exception of Ohridska crna and Ohridska brza, have a higher content of total acids than the standard variety. The highest content of citric acid was found in the fruits of the Ohridska bela variety (2.58 g/L).

A very small amount of tartaric acid was found in apple fruits, an average of 0.383 g/L, while in pomegranates and cherries, tartaric acid was not found. The presence of lactic acid was not found in all fruit species.

A statistically strong negative correlation was observed between the contents of total acids and malic acid, as well as weak negative correlation dependence with the content of citric acid. This means that if, for example, the content of total acids in the tested fruits increases, the part of citric and malic acids in the total acids decreases, so the increase of total acids is due to some other untested acids. The coefficient of determination R^2 shows a weak dependence of 37% between the contents of total acids and malic acid and only 5% between the content of total acids and citric acids. A moderate positive correlation was found between the content of malic and citric acid with coefficient of determination $R^2=30\%$. The very strong negative correlation between the content of malic acid and the sum of glucose and fructose in the fruits is particularly emphasized. The coefficient of determination R^2

shows as much as 77% dependence between the content of malic and the sum of glucose and fructose (Tab. 2).

Ma et al. (2018) noticed a strong positive correlation was detected between fruit total organic acid with malic acid and citric acid content. In contrast to malic acid, citric acid was predominantly detected in partial wild apples, while extremely low to undetectable concentrations of citric acid were observed in cultivated apple fruits.

The results obtained from the examination of the content of total acids, as well as some organic acids, match the available literature data. Total titratable acidity in pomegranate is ranged between 4.58-17.30 g/L (average 9.82 g/L), citric acid is predominant acid with a range of 0.33-8.96 g/L, malic acid is the second most abundant with a range of 0.56-6.86 g/L and tartaric acid respectively 0.28-2.83 g/L (Poyrazoğlu et al., 2002). Self-sprouting pomegranate varieties contain 4.9-34.7 g/L total acids (Markovski et al., 2017). According Mayuoni-Kirshinbaum and Porat (2014) pomegranate fruits content primarily citric and malic acids. The aroma evolves from the presence of dozens of volatiles, including alcohols, aldehydes, ketones and terpenes which provide a mixture of various "green", "woody", "earthy", "fruity", "floral", "sweet" and "musty" notes.

The sum of organic acids (malic, citric, shikimic and fumaric) according Usenik et al.

(2008) in sweet cherry ranged from 3.67 to 8.66 g/kg FW. According Sokol-Letowska et al. (2020) the content of total acids in sour cherries is 1294.4-2300.5 mg/100g FW. The main organic acids in sour cherries are malic and malonic acids. Apples contain 0.4-0.8% total acids, while cherries 0.3-0.6% (Niketic-Aleksic, cit. Selamovska & Miskoska-Milevska, 2021). Malic acid is the primary acid detected in apple (Ma et al., 2015, 2019), cherry (Gundogdu and Bilge, 2012; Serra et al., 2011), sweet cherry (Hayaloglu & Demir, 2015; Usenik et al., 2008), sour cherry (Sokol-Letowska et al., 2020), pear (Wu et al., 2022). The level of citric and tartaric acids in fruits nectars ranged from 1.26 to 4.42g/L⁻¹ and 0.68 to 0.86 g/L⁻¹ and in fruits juices ranged from 3.03 to 7.67 g/L⁻¹ and 3.09 to 4.68 g/L⁻¹. The level of tartaric acid in fruit juices is higher than in fruit nectars. In general, the level of both acids is higher in fruit juices than in fruit nectars (Inić et al., 2020).

The results of this study indicate that the level of citric acid (0.898-1.719 g/L) and tartaric acid (0.38 g/L only in apple fruits) in fruit available on the market is below the concentration allowed by EU regulations and do not pose a threat to human health, confirming good manufacturing practices (GMP). The monitoring of the level of citric and tartaric acids in fruit beverages is needed in order to protect human health.

CONCLUDING REMARKS

According to the data obtained from determination of chemical composition of the fruits of autochthonous varieties of fruit species, the following conclusions can be extracted.

Pomegranate fruits have the highest content of soluble dry matters, total sugar, invert sugar and total acids. In the fruits of apple and cherry varieties, malic acid dominates, while in pomegranate varieties, the contents of citric and malic acid are almost equally represented. A very low content of tartaric acid is observed only in apple fruits. The presence of lactic acid was not found in the fruits of all fruit species.

A strong positive correlation was found between soluble dry matters and total sugars. A moderate positive correlation was established between the content of total sugars and the

sum of glucose and fructose in fruits, between the content of total acids and the sum of glucose and fructose, as well as between the content of malic and citric acid. Strong negative correlation exists between the content of total acids and malic acid, and very strong correlation between the content of malic acid and the sum of glucose and fructose.

According to the results obtained from the investigated parameters, most of the autochthonous apple, pomegranate and cherry varieties compared to the proposed standard varieties show higher values, which indicates the fact that the autochthonous varieties are characterized by the quality that does not deviate from the world-famous international introduced species and varieties.

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ХЕМИСКИ СОСТАВ НА НЕКОИ АВТОХТОНИ ОВОШНИ ВИДОВИ ОД МАКЕДОНИЈА

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Резиме

Целта на научниот труд е определување на содржината на растворливи суви материи, вкупни шеќери, вкупни киселини, инвертен шеќер и лимонска, јаболкова, винска и млечна киселина во некои автохтони овошни видови: калинка (зумнарија, бејнарија, валандовска кисела, валандовска кисело-слатка, хиџас, кисела, лифанка, ропкавец), јаболко (убаво цветка, шарено благо, преспанка, тетовка, карапаша, кожарка, бела тетовка) и цреша (охридска брза, охридска рана, охридска црна, долга шишка, далбазлија, охридска бела). Извршена е споредба со стандардните сорти *карамустафа* - калинка, *ајдаред* - јаболко и *бигаро бурлат* - цреша.

Плодовите од калинка имаат највисока содржина на растворливи суви материи (15.6%), вкупни шеќери (149 g/L) и вкупни киселини (20.1 g/L). Содржината на инвертен шеќер е најголема во плодовите од калинка (113 g/L односно 76.3% од вкупните шеќери). Во плодовите од јаболко и цреша доминира јаболковата киселина (над 5 g/L), додека во калинката речиси подеднакво се застапени лимонската и јаболковата киселина. Присуство на винска киселина има само во плодовите од јаболко (0.383 g/L). Нема присуство на млечна киселина во сите овошни видови.

Констатирана е силна позитивна корелација меѓу растворливите суви материи и вкупните шеќери (коефициент на детерминација $R^2=60\%$). Умерена позитивна корелација постои меѓу вкупниот и инвертниот шеќер со коефициент на детерминација $R^2=20\%$, меѓу вкупните киселини и инвертниот шеќер со коефициент на детерминација $R^2=22\%$ и меѓу содржината на јаболковата и лимонската киселина со коефициент на детерминација $R^2=30\%$. Висока негативна корелација постои меѓу содржината на вкупните киселини и јаболковата киселина со коефициент на детерминација $R^2=37\%$ и многу висока негативна корелација меѓу јаболковата киселина и инвертниот шеќер со коефициент на детерминација $R^2=77\%$.

Клучни зборови: *калинка, јаболко, цреша, растворливи суви материи, шеќери, органски киселини.*