Bulgarian Chemical Communications, Volume 51, Special Issue A (pp. 150-155) 2019

Specific ratios in flavonoid profile of fruits

Silvia P. Tsanova-Savova*, Zaharina A. Savova, Tsvetomir I. Dimitrov, Fanny T. Ribarova

Medical University – Sofia, Medical College Yordanka Filaretova, 3 Yordanka Filaretova Str., Sofia 1606, Bulgaria.

Received February 11, 2019; Accepted April 12, 2019

A considerable amount of scientific research is dedicated nowadays to the identification and content of biologically active compounds in plant foods and to the problem of determining their specific activity, which is crucial for human health. The current study aims to present data concerning the content of catechins and flavonols in 19 Bulgarian fruits with the parameters of the statistical variance, and to assess specific flavonoid ratios as more stable indicators of biodiversity. Catechins: (+)-catechin and (-)-epicatechin; and flavonols: myricetin, quercetin, and kaempferol, were analyzed with the HPLC method with UV and fluorescent detection. Flavonoids composition in Bulgarian fruits is presented for the first time together with the assessment of their variability, thus making them comparable with the available flavonoid composition databases, in terms of mean value, standard error or standard deviation. The large variability of data on the flavonoids in fruits has made us to look for more stable indicators of the flavonoid spectrum, namely to evaluate (-)-epicatechin/(+)-catechin and flavonols/catechins ratios. The results show that the (-)-epicatechin/(+)-catechin ratio could be applied as a stable parameter for the estimation of flavonoid composition and biodiversity ($R^2 > 0.8$ in apple Red delicious, blackberry, blueberry, white grape, pear, plum, and sour cherry). The ratio between flavonols and catechin classes is considerably a more instable indicator of biodiversity and further analyses are needed in this respect.

Keywords: flavonoids, flavonols, catechins, ratios, fruits

INTRODUCTION

A considerable amount of scientific research is dedicated nowadays to the identification and content of biologically active compounds in plant foods and to the problem of determining their specific activity, which is crucial for human health [1-4].

An impressive number of biologically active compounds have been identified and data and evidence about their biological role have been gathered. Particular interest is paid to the flavonoids, with over 6000 representatives identified. The flavonoids are secondary plant metabolites with proven physiological effects such as antioxidative activity, anti-inflammatory and immunomodulatory activity, cancer risk reduction, and antibacterial activity [4-8]. All these properties highlight the necessity for increasing the knowledge about their content in different food sources.

The main biological characteristic of the flavonoids, which is crucial for their inclusion in different preventive diets and healthy nutrition regimes, is their antioxidative activity. The hypothesis for the harmful oxidative action of free radicals, conceived by Harman in 1956, is considered as the start of the antioxidant research [9]. Ample studies have been dedicated to the identification of antioxidants in food, which increase the antioxidative protection of the body. In this context, flavonoids are a research target group. Currently, a lot of data may be found in the scientific literature on contents of antioxidants of multiple plant foods [10-15].

Cross-referencing the data concerning the content of a given active compound (flavonoid representative) in different sources indicates the wide range of variation in its contents reported. It is necessary to represent the data of flavonoids along with the parameters of the statistical variance and to search for new indicators enabling the verification of the composition data.

Unfortunately there are a certain difficulties in compiling data from different sources, not only because of the great variance of the different representatives of the various classes of the flavonoid family, but also because of the lack of a uniform approach towards their representation. For example, our results concerning the flavonoid spectrum were presented as minimum, mean, and maximum values without the parameters of the statistic variance [13, 14]. This fact argues for the search of new indicators with a narrower band of variation.

The current study aims to present results of determinations of catechins and flavonols content in Bulgarian fruits with the parameters of the statistical variance, and to assess specific ratios: (-)-epicatechin/(+)-catechin and flavonols/catechins, as more stable indicators of biodiversity.

* To whom all correspondence should be sent:
E-mail:s.tsanova-savova@mc.mu-sofia.bg

EXPERIMENTAL

This study presents data concerning the flavonoid profile of 19 fruit items. Contents of catechins: (+)-catechin and (-)-epicatechin, were determined after extraction with 80% methanol/water (v/v) and HPLC determination on Alltima reverse phase column (Alltima (100 × 4.6 mm i.d., 3 μm) C18, (Alltima Associates, Inc.). An isocratic elution with 9% acetonitrile in 2% acetic acid, flow rate equal to 1 mL/min, and temperature of 30º C, were applied in fluorescence detection at λ_{EX} = 280 nm and λ_{EM} = 315 nm, and an external standard was applied, as described in detail in our previous study [13]. Contents of flavonols: myricetin, quercetin, and kaempferol, were determined after acid hydrolysis with 1.2 mol/L HCl in 50% methanol by refluxing 0.500 g of the lyophilized analytical sample at 90ºC for 2 h. The chromatographic separation was performed on Alltima column (100 × 4.6 mm i.d., 3 μm) C18. Isocratic elution with 28% acetonitrile in 2% acetic acid was applied, at 0.9 mL/min flow rate and a temperature of 22ºC. A UV detection at λ = 365 nm was carried out. Quantitative determination was performed by the method of the external standard (morin) and parameters of the analytical method were presented in our previous study [14].

RESULTS AND DISCUSSION

The results of determinations of (+)-catechin and (-)-epicatechin, myricetin, quercetin, and kaempferol content of Bulgarian fruits, with their statistical parameters for flavonoids' variability are presented in Table 1. The results are expressed as mg/kg fresh weight.

Variability of flavonoids composition data in fruits

For the assessment of flavonoids composition variability in fruits, we have expressed the results not only as mean values and range (minimum and maximum values), but we have calculated their standard deviation (SD), standard error (SE), and relative standard deviation (RSD%). In the available databases of flavonoid composition, the variability of results is expressed as: SE, minimum (min) and maximum (max) value in US Database [16]; and as: SD, min and max in Phenol-Explorer [17]. For the first time, data concerning flavonoids composition in Bulgarian fruits are presented with the assessment of their variability, thus making them comparable with the flavonoid composition available in databases, in terms of mean value, standard error or standard deviation.

The highest SD calculated for (+)-catechin data variability was found for black grapes (49.80 mg/kg) and white grapes (40.75 mg/kg). The calculation of SE for the same fruit items (n=7) reduces the values to 18.82 mg/kg and to 15.40 mg/kg, respectively. For (-)-epicatechin, the SD did not exceed 39.17 mg/kg (black grapes) and had a minimum value of 0.32 mg/kg (peaches). Correspondingly, the maximum value of the SE computed for (-)-epicatechin data dispersion was again observed in black grape and the minimum value in peach. For quercetin, the highest data variability was determined in plums (SD=16.10 mg/kg; SE=7.20 mg/kg), while the lower one was observed in pear with peel (SD=3.07 mg/kg; SE=1.37 mg/kg). Our results obtained for SD and SE of the flavonoids composition data in the studied fruits are in line with other literature findings [16, 17].

RSD was calculated to enable the comparison of a different set of data of individual flavonoids in fruits. Due to the variation of the parameters studied, currently we could hardly correctly classify the different fruits by their variation magnitude, because it differed greatly between fruits. The review of the data shows that the lowest RSD% of data for (+)-catechin was observed in apple Red delicious with peel (22.93%), followed by peeled apple Red delicious (23.83%) and blueberries (26.82%). The highest variability of the data for (+)-catechin was measured in peeled pear (RSD=112.27%). The variability of our results obtained for (-)-epicatechin, expressed as RSD %, varied between 12.87% in apple Golden delicious with peel and 173.21 % in figs. Myricetin was found only in blueberry and grapes. We can note that myricetin content in blueberries reached a very high mean value (43.1 mg/kg) and relatively low dispersion of results (RSD=25.82%). Among fruits studied, quercetin content reached the highest value in blueberry (99.2 mg/kg), with the lowest variability observed (RSD=5.39%). We could see that data variability for quercetin was generally lower than that determined for (+)-catechin and (-)-epicatechin. However, RSD exceeding 50% was found for quercetin in pear with peel, plum, sour cherry, strawberry, and fig. Kaempferol was detected in 3 fruit items – cornel (*Cornus mas*), white and black grapes. It is interesting to point out that in all the three cases, RSD exceeded 100%. We could explain this large dispersion of data with a very small absolute value of kaempferol in fruits and zero values in the data set for those fruit items.

Specific ratios for flavonoids composition

The large variability of data concerning contents of flavonoids in fruits has made us to look for more stable indicators of the flavonoid spectrum, namely to evaluate (-)-epicatechin/(+)-catechin and flavonols/catechins ratios (see Table 2). Catechins represent the sum of (+)-catechin and (-)-

epicatechin and flavonols are expressed as the sum of myricetin, quercetin, and kaempferol, all determined by HPLC analysis, as described above. Furthermore, our assumption was that, along with the increase in (-)-epicatechin level, the amount of

Table 1. Flavonoids content of fruits and statistical parameters of the variability

Fruit	n	Parameters	(+)-Catechin	(-)-Epicatechin	Myricetin	Quercetin	Kaempferol
			mg/kg f.w.				
Apple, Golden delicious, whit peel	7	Mean	5.2	36.6	nd	14.0	nd
		SD	2.74	4.70		4.36	
		SE	1.03	1.78		1.65	
		RSD %	52.62	12.87		31.19	
Apple, Red delicious, whit peel	7	Mean	14.9	73.9	nd	15.9	nd
		SD	3.42	10.24		5.66	
		SE	1.29	3.87		2.14	
		RSD %	22.93	13.86		35.52	
Apple, Golden delicious, peeled	7	Mean	4.2	30.0	nd	nd	nd
		SD	2.94	6.07			
		SE	1.11	2.29			
		RSD %	70.12	20.23			
Apple, Red delicious, peeled	7	Mean	7.7	34.0	nd	nd	nd
		SD	1.84	10.21			
		SE	0.69	3.86			
		RSD %	23.83	30.05			
Apricot	5	Mean	61.2	83.4	nd	34.0	nd
		SD	38.70	15.91		8.28	
		SE	17.31	7.11		3.70	
		RSD %	63.22	19.06		24.32	
Blackberry	3	Mean	19.0	37.9	nd	27.0	nd
		SD	8.30	14.24		4.09	
		SE	4.79	8.22		2.36	
		RSD %	43.69	37.57		15.11	
Blueberry	3	Mean	21.0	43.1	43.1	99.2	nd
		SD	5.63	11.12	8.91	5.35	
		SE	3.25	6.42	5.14	3.09	
		RSD %	26.82	25.82	20.65	5.39	
Cherry	5	Mean	15.7	45.9	nd	25.2	nd
		SD	4.34	31.49		7.48	
		SE	1.94	14.08		3.35	
		RSD %	27.66	68.58		29.70	
Cornel	3	Mean	nd	nd	nd	24.9	4.7
		SD				7.32	6.10
		SE				4.22	3.52
		RSD %				29.42	129.89
Fig	3	Mean	4.3	1.4	nd	8.7	-
		SD	1.88	2.48		5.58	
		SE	1.08	1.43		3.22	
		RSD %	43.32	173.21		64.39	
Grapes, black	7	Mean	108.3	87.0	3.5	24.2	1.1
		SD	49.80	39.17	1.66	5.80	2.09
		SE	18.82	14.80	0.63	2.19	0.79
		RSD %	46.00	45.00	47.16	23.98	187.64
Grapes, white	7	Mean	58.9	27.8	1.4	15.6	2.6
		SD	40.75	20.35	0.85	5.55	3.01
		SE	15.40	7.69	0.32	2.10	1.14
		RSD %	69.23	73.21	59.77	35.54	114.98
Peach	5	Mean	12.3	0.3	nd	nd	nd
		SD	8.29	0.32			

		SE	3.71	0.14			
		RSD %	67.15	100.00			
Pear, with peel	5	Mean	3.7	47.1	nd	5.9	nd
		SD	3.05	26.82		3.07	
		SE	1.36	12.00		1.37	
		RSD %	81.91	56.97		51.86	
Pear, peeled	5	Mean	0.8	9.7	nd	nd	nd
		SD	0.85	4.48			
		SE	0.38	2.00			
		RSD %	112.27	46.18			
Plum	5	Mean	33.1	4.2	nd	23.4	nd
		SD	13.28	3.26		16.10	
		SE	5.94	1.46		7.20	
		RSD %	40.16	78.27		68.86	
Raspberry	5	Mean	13.6	36.7	nd	16.3	nd
		SD	8.07	12.56		3.19	
		SE	3.61	5.62		1.43	
		RSD %	59.54	34.24		19.58	
Sour cherry	5	Mean	3.0	6.9	nd	10.8	nd
		SD	1.10	1.69		8.23	
		SE	0.49	0.76		3.68	
		RSD %	37.21	24.51		75.94	
Strawberry	5	Mean.	31.1	1.8	nd	10.2	nd
		SD	21.85	2.81		5.29	
		SE	9.77	1.26		2.36	
		RSD %	70.18	154.26		52.02	

n – number of samples, nd - non detected, SD – Standard deviation, SE – standard error, RSD – relative standard deviation

(+)-catechin increases as well, hence we have calculated also the coefficient of correlation (R^2) of a linear regression between contents of these two individual catechins per each fruit item, and presented respective data in Table 2.

We have used RSD % for comparison of the variability of (-)-epicatechin/(+)-catechin and flavonols/catechins ratios with the variability of single flavonoid compounds. The results show that in some cases, the RSD% of the (-)-epicatechin/(+)-catechin ratio was actually lower than that of the (+)-catechin and (-)-epicatechin alone (see Table 1), for instance in apple Red delicious with peel, pear, sour cherry, blackberry, blueberry, and black and white grapes. In other cases, RSD% was higher than that of the individual catechins (peach). In the vast majority of cases, the dispersion of (-)-epicatechin/(+)-catechin ratio fell between the values for RSD% for the individual (+)-catechin and (-)-epicatechin.

The results of determination of the correlation coefficient of a linear regression (R^2) between (-)-epicatechin and (-)-catechin show that there were diverse correlations. In some cases, a strong correlation (R^2>0.8) was observed, like for blackberries (0.837), apple Red delicious (0.866), grapes white (0.900), pear with peel (0.976), plums (0.955), and sour cherry (0.934). However, no correlation (R^2<0.2) was found in peaches (0.146), peeled pears (0.085), and strawberries (0.009). These results suggest that we could use the (-)-epicatechin/(+)-catechin ratio as a stable indicator for the estimation of the flavonoid spectrum, but more data for some fruit items are needed in order to assess the reliability of the assessment.

In the case of the flavonols/catechins ratio, we observed that only for pear, apricot, peach, and white grapes this ratio was characterized by lower values of RSD % than those for individual flavonoids. In this case, the correlation between flavonols and catechins, assessed as R^2 of a linear regression, was much lower in comparison with that of (-)-epicatechin/(+)-catechin, reaching the maximal value of 0.841 in strawberries, followed by pears with peel (0.837), plums (0.744), apricots (0.797), and figs (0.757). In all other cases, R^2 values were lower than 0.7, indicating weak (0.644 in cherries) or no correlation (0.020 in raspberries) between these two classes of flavonoids. Since the factors affecting flavonoid levels in fruits are diverse and of varying nature - fruit variety, geographical origin, climate, maturity state, the demand for data stability is justifiable, but our calculations do not warrant that we could use flavonols/catechins ratio as a robust indicator.

Table 2. Specific ratios in flavonoid profile of fruits

Fruit	n	(-)-epicatechin/(+)-catechin				Flavonols/Catechins			
		Mean	SD	RSD %	R^2	Mean	SD	RSD %	R^2
Apple, Golden delicious, whit peel	7	8.78	4.08	46.46	0.772	0.34	0.11	32.54	0.001
Apple, Red delicious, whit peel	7	5.04	0.48	9.43	0.866	0.18	0.05	30.82	0.380
Apple, Golden delicious, peeled	7	11.20	7.28	64.99	0.626	-			
Apple, Red delicious, peeled	7	4.51	1.18	26.21	0.385	-			
Apricot	5	1.76	0.91	51.89	0.382	0.24	0.04	15.72	0.797
Blackberry	3	2.05	0.21	10.40	0.991	0.57	0.18	32.18	0.621
Blueberry	3	2.06	0.23	11.36	0.837	2.34	0.73	31.00	0.103
Cherry	5	2.80	1.47	52.53	0.469	0.47	0.15	32.38	0.644
Fig	3	0.24	0.41	173.21	0.591	1.52	0.63	41.67	0.757
Grapes, black	7	0.84	0.32	37.95	0.719	0.18	0.10	53.04	0.014
Grapes, white	7	0.50	0.19	38.40	0.900	0.32	0.18	57.14	0.278
Peach	5	0.03	0.05	178.67	0.146	-			
Pear, with peel	5	15.16	4.04	26.66	0.976	0.12	0.03	28.93	0.837
Pear, peeled	5	7.22	20.9	28.97	0.085	1.05	0.83	79.52	0.841
Plum	5	0.11	0.06	60.09	0.955	0.60	0.24	39.88	0.744
Raspberry	5	3.09	1.10	35.72	0.742	0.38	0.18	47.59	0.020
Sour cherry	5	2.45	0.45	18.23	0.934	1.20	1.11	92.49	0.007
Strawberry	5	0.09	0.12	137.66	0.009	1.05	0.83	79.52	0.841

n – number of samples, SD – standard deviation, R^2 – coefficient of variation of linear regression between (-)-epicatechin n and (+)-catechin; between flavonols and catechins

CONCLUSIONS

The results of the present study show that the (-)-epicatechin/(+)-catechin ratio could be used as a stable parameter for the estimation of flavonoid composition and biodiversity of the plant species, but further analyses are required in this respect. The ratio between flavonol and catechin classes was considerably a more instable indicator and more data are needed for correct assessment. Due to the vast variability of flavonoid content data in the contemporary scientific literature and because of the considerable biodiversity of the plant kingdom, it is necessary to identify additional parameters for more precise assessment of the flavonoid spectrum in various plant food sources.

REFERENCES

1. S. Rokayya, C.J. Li, Y. Li, C.H. Sun, *Asian Pac. J. Cancer Prev.* **14**, 6657 (2013).
2. B. Radovanovic, J. Mladenovic, A. Radovanovic, R. Pavlovic, V. Nikolic, *J.Food Nutr. Res.*, **3**, 564 (2015).
3. D. Lin, M. Xiao, J. Zhao, Z. Li, B. Xing, X. Li, Q. Zhang, Y. Liu, H. Chen, W. Qin, H. Wu, S. Chen. *Molecules*, 21, 1374, (2016).
4. A. Scarano, M. Chieppa, A. Santino, *Plants (Basel)*, **7**(4), 98 (2018).
5. M. Fragis, A. Murayyan, S. Neethirajan, *Funct. Foods Health Dis*., **8**, 159 (2018).
6. K. Kozics, Z. Valovicova, D. Slamenova, D., *Neoplasma*, **58**(6), 516 (2011).
7. G. Lima, F. Vianello, C. Correa, R. da Silva Campos, M. Borguini, *Food Nutr. Sci.*, **5**(11), 1065 (2014).
8. V. Munhoz, R. Longhoni, J. Souza,J. Zequi, E. Leite Mello, G. Lopes, J. Mello, *Rev. Bras. Farmacogn*., **24**, 575 (2014).
9 D. Harman, *J. Gerentol*., **11**, 198 (1956).
10 J. Gry, L. Black, F. Ericsen, K. Pilegaard, J. Plumb, M. Rhodes, D. Sheehan, M. Kiely, P. Kroon, *Trends Food Sci. Technol*., **18**, 434 (2007).
11 A. Scalbert, C. Andres-Lacueva, M. Arita, P. Kroon, C. Manach, M. Urpi-Sanda, D.Wishart, *J. Agric. Food Chem.*, **59**, 4331 (2011).
12 J. Plumb, J. Lyons, K. Norby, M. Thomas, E. Norby, R. Poms, L. Bucchini, P. Restani, M. Kiely, P. Finglas, *Food Chem*., **193**, 121 (2016).
13 S. Tsanova-Savova, F. Ribarova, M. Gerova, *J. Food Comp. Anal.*, **18**, 691 (2005).
14 S. Tsanova-Savova, F. Ribarova, V. Petkov, *Riv. Ital. Sostanze Gr.*, **94**(3), 175 (2017).
15 M. Carlsen, B. Halvorsen, K. Holte, S. Bohn, S. Dragland, L. Sampson, C. Willey, H. Senoo, Y. Umezono, C. Senada., I. Barikmo, N. Berhe, W.

Willett, K. Phillips, D. Jacobs, R. Blomhoff, *Nutr. J.*, **9**, 3 (2010).
16 S. Bhagwat, D. Haytowitz, USDA Database for the Flavonoid Content of Selected Foods. Release 3.2; U.S. Department of Agriculture Agricultural Research Service: Beltsville, MD, USA (2015).
17 J. Rothwell, J. Pérez-Jiménez, V. Neveu, A. Medina-Ramon, N. M'Hiri, P. Garcia Lobato, C. Manach, K. Knox, R. Eisner, D. Wishart, A. Scalbert. Phenol-Explorer 3.0: a major update of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. Database, 10.1093/database/bat070 (2013).