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TOTAL PHENOLICS AND TOTAL FLAVONOIDS IN BULGARIAN FRUITS AND VEGETABLES

D. Marinova, F. Ribarova*, M. Atanassova

National Center of Public Health Protection,
Department of Food Chemistry, Sofia 1431, Bulgaria
**E-mail: f.ribarova@nchmen.government.bg*

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ABSTRACT

The increasing interest in powerful biological activity of plant phenolics and flavonoids outlined the necessity of determining their contents in Bulgarian fruits and vegetables.

The study comprised 42 food products – 20 fruit and 22 vegetable species.

The total phenolic content was determined by using the Folin-Ciocalteu assay. The content of total flavonoids was measured also spectrophotometrically by using the aluminum chloride colorimetric assay.

The results of fruits showed the highest total phenolic content in blueberries (670.9 mg gallic acid equivalents (GAE)/100 g), dogwood berries (432.0 mg GAE/100 g) and sour cherry (429.5 mg GAE/100 g). The greatest total flavonoid content was revealed in blueberries (190.3 mg catehin equivalents (CE)/100 g). The lowest total phenolics and total flavonoids were established in peaches (50.9 mg GAE/100 g and 15.0 mg CE/100 g, respectively).

The results of vegetables showed the greatest value of phenolics in green peppers (246.7 mg GAE/100 g) and red peppers (173.2 mg GAE/100 g). Significant difference was found between total phenolic content in red and spring onions at almost equal total flavonoid values.

The present paper shows particular specificity not only in the total phenolic and flavonoid content, but also in their ratio and distribution in the different parts of the studied fruits and vegetables.

Keywords: phenolics, flavonoids, fruits, vegetables.

INTRODUCTION

Phenolics are ubiquitous secondary metabolites in plants. They comprise a large group of biologically active ingredients (above 8000 compounds) – from simple phenol molecules to polymeric structures with molecular mass above 30000 Da [1]. On the basis of the number of phenol subunits, the modern classification forms two basic groups of phenolics – simple phenols and polyphenols. The group of simple phenols contains also the so-called “phenolic acids” or phenols with carboxyl group underlying the specificity of their function. Polyphenols contain at least two phenol rings. Flavonoids, a subject of comprehensive studies in recent years, belong to this group. More than 4000 flavonoids have been identified in different higher and lower plant species [2].

The presence of phenolic compounds in diet was long regarded as a negative feature because they were doubted to decrease the availability of nutrients, leading to lower nutritional value of the product [3]. After the identification of the "French paradox" and revealing that the moderate consumption of red wine (rich in polyphenols) decreases cardiovascular morbidity rate among French people, particular emphasis was put on studying phenolic compounds as food ingredients. A number of data showed that the presence of phenolics in foods is particularly important for their oxidative stability and anti-microbial protection [4, 5].

Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic, as well as ability to modify the gene expression [6, 7]. Numerous epidemiological studies confirm significant relationship between the high dietary intake of flavonoids and the reduction of cardiovascular and carcinogenic risk [8]. The formulation of preventive and healthy nutrition requires information about phenolic and flavonoid composition in plant foods.

Based on numerous evidence on the strong biological activity of phenolic compounds and on the scarcity of data for their content in foods the aim of current study was focused on determination of total phenolic and total flavonoid content in Bulgarian fruits and vegetables.

EXPERIMENTAL

Materials

The study covered 42 food products - 20 fruit and 22 vegetable species. Randomized market sampling was applied. The average sample consisted of representative amounts of three individual samples from respectively different region of origin, in similar stage of biological development and ripeness. Only samples complying with the requirements of good manufacturing practice were considered. The sampling lasted one year according to the seasonality of harvesting for individual species. All samples data are stated in the sampling protocol. After removing the non-edible parts the samples were cut into small pieces and frozen under liquid nitrogen. The samples were freeze-dried after one week. The water content in lyophilized fruits and vegetables was not more than 3.5 %, providing for safety storage conditions. The freeze-dried samples were vaccumized in hermetically sealed packages and stored at 4'IC until the time of analysis. Lyophilization is applied aiming to uniform the storage conditions and submit the products for analysis in similar form.

Reagents: Gallic acid, (+)-catechin and Folin–Ciocalteu's phenol reagent were purchased from Sigma Chem. Co. All other chemicals were of analytical grade.

Procedures

Sample preparation

A ground freeze-dried sample of 0.5 g was weighted and phenolic and flavonoid compounds were extracted with 50 ml 80 % aqueous methanol on an ultrasonic bath for 20 min. An aliquot (2 ml) of the extracts was ultracentrifugated for 5 min at 14000 rpm.

Total phenolic assay

The total phenolic content of fruits and vegetables were determined by using the Folin–Ciocalteu assay [9]. An aliquot (1 ml) of extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 mg/l) was added to 25 ml volumetric flask, containing 9 ml of distilled deionised water (dd H_2O). A reagent blank using dd H_2O was prepared. One millilitre of Folin–Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7 % Na_2CO_3 solution was added to the mixture. The solution was diluted to volume (25 ml) with dd H_2O and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with an UV-Vis Spectrophotometer Lambda 5. Total phenolic content of fruits and vegetables was expressed as mg gallic acid equivalents (GAE)/100 g fresh weight. All samples were analysed in duplicates.

Total flavonoid assay

Total flavonoid content was measured by the aluminum chloride colorimetric assay [10]. An aliquot (1 ml) of extracts or standard solution of catechin (20, 40, 60, 80 and 100 mg/l) was added to 10 ml volumetric flask containing 4 ml of dd H_2O. To the flask was added 0.3 ml 5 % $NaNO_2$. After 5 min, 0.3 ml 10 % $AlCl_3$ was added. At 6th min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with dd H_2O. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content of fruits and vegetables was expressed as mg catechin equivalents (CE)/100 g fresh mass. Samples were analysed in duplicates.

RESULTS AND DISCUSSION

The determined analytical parameters of the total phenolics method was as follows: limit of detection: 0.4 mg GAE/100 g fresh mass; limit of determination: 1.2 mg GAE/100 g fresh mass; recovery: 97% and reproducibility (RSD): 2.7 %. The determined analytical parameters of the total flavonoids method was as follows: limit of detection: 0.6 mg CE/100 g fresh mass; limit of determination: 1.8 mg CE/100 g fresh mass; recovery: 96 % and reproducibility (RSD): 3.7 %. These results proved the viability of the both used methods to determine phenolic and flavonoid compounds in fruit and vegetable extracts.

The results for total phenolic and total flavonoid content and the ratio total flavonoids/phenolics in the studied fruits are presented in Table 1. The data clearly outline the richest phenolics sources – blueberries (670.9 mg GAE/100 g), dogwood berries (432.0 mg GAE/100 g) and sour cherry (429.5 mg GAE/100 g). Blueberries have also the highest flavonoid content (190.3 mg CE/100 g) with ratio of 0.32. This result enables us to support the proverb popular in northern countries "When there are blueberries, we shall not need a doctor". In dogwood berries the total flavonoids have a smaller share of total phenolics, while in sour cherries the ratio is 0.32, even slightly greater than in blueberries. The discussion of this difference would have a higher degree of uncertainty, due to the small number of tests. It is clear that phenolic acids prevail in dogwood berries, which explains their sour astringent taste.

The second group with high total phenolic content comprises blackberries (355.3 mg GAE/

Table 1. Content of total phenolics and total flavonoids in fruits.

Fruit	Latin name	Total phenolics mg GAE/100g fresh mass	Total flavonoids mg CE/100g fresh mass	Flavonoids/ Phenolics
Pear (unpeeled)	*Pyris communis*	124.7	69.9	0.56
Pear (peeled)	*Pyris communis*	91.0	48.5	0.53
Apple, yellow (unpeeled)	*Malus pumila*	99.7	34.8	0.35
Apple, yellow (peeled)	*Malus pumila*	75.8	20.9	0.28
Apple, red (unpeeled)	*Malus pumila*	125.4	48.6	0.39
Apple, red (peeled)	*Malus pumila*	104.3	32.7	0.31
Plum	*Prunus domestica*	303.6	136.2	0.45
Apple, green (unpeeled)	*Malus pumila*	118.1	40.4	0.34
Apple, green (peeled)	*Malus pumila*	97.5	17.3	0.18
Peach	*Prunus persica*	50.9	15.0	0.3
Blackberry	*Rubus coesins*	355.3	55.5	0.16
Raspberry	*Rubus ideaus*	178.6	26.6	0.15
Strawberry	*Fragaria vesca*	244.1	69.7	0.29
Sweet cherry	*Prunus avium*	78.8	19.6	0.25
Sour cherry	*Prunus cerasus vulgaris*	429.5	138.6	0.32
Blueberry	*Vaccinium myrtilus*	670.9	190.3	0.28
Fig	*Ficus carica*	59.0	20.2	0.34
White grape	*Vitis vinifera*	184.1	36.5	0.2
Black grape	*Vitis vinifera*	213.3	77.1	0.36
Dogwood berry	*Cornus mas*	432.0	91.4	0.21

Results are presented as mean value of duplicates.

100 g), plums (303.6 mg GAE/100 g) and strawberries (244.1 mg GAE/100 g). In this group the total flavonoids are found in the greatest ratio in plums (0.45). We could suppose that this is due to the rich abundance of anthocyanidines in combination with the other flavonoids. The low ratio (0.16) in blackberries is probably a result of the rich spectrum of phenolic acids. In strawberries the total flavonoids contribute one third of the total phenolics, with the ratio similar to that of blueberries.

Pears analysis provides very interesting results. We found the highest percentage rate of total flavonoids vs. total phenolics compared to all other studied fruits. This ratio is the same for both unpeeled and peeled pears. The unpeeled pears showed significantly greater content of total phenolics and total flavonoids. The results for unpeeled and peeled apples are quite similar. Our data suggest richer presence of phenolics in the skin of the fruits. The comparative assessment between red and yellow apples favors the red ones because of the greater content of phenolics. The differences in total flavonoid content, following the same pattern, are quite smaller.

The analysis of both grapes species – white and black logically revealed higher content of total phenolics and total flavonoids

Table 2. Content of total phenolics and total flavonoids in vegetables.

Vegetable	Latin name	Total phenolics mg GAE /100g fresh mass	Total flavonoids mg CE /100g fresh mass	Flavonoids/ Phenolics
Carrot	*Daucus carota*	96.0	26.7	0.28
Celery (leaves)	*Apium graveolens*	113.0	46.4	0.41
Parsley	*Petroselium sativum*	188.0	27.2	0.14
Okra	*Hibisons eculentus*	153.7	49.1	0.32
Tomato	*S. lycopersicum*	76.9	12.8	0.17
Red pepper	*Capsicum anuum*	173.2	13.7	0.08
Green pepper	*Capsicum anuum*	246.7	27.4	0.11
Salad	*Lactuca sativa*	116.2	76.5	0.66
Lettuce	*Lactuca sativa capitata*	124.5	97.2	0.78
Kohlrabi	*Brassica oleracae var. caulorapa*	44.9	8.9	0.19
Red cabbage	*Brassica oleracae var. botritis*	139.3	23.7	0.17
Brussels sprout	*Brassica oleracae var. Gemmifera*	161.5	33.1	0.20
Broccoli	*Brassica oleracae var. Italica*	101.7	18.8	0.18
Radish	*Raphanus sativus, var. Radicula*	160.0	48.5	0.30
Red onion	*Allium cepa*	154.1	18.7	0.12
Spring onion	*Allium cepa*	120.0	16.0	0.13
Spring onion (leaves)	*Allium cepa*	81.0	11.7	0.14
Spring onion (stem)	*Allium cepa*	36.5	2.5	0.07
Leek (leaves)	*Allium porrum*	35.7	3.9	0.11
Leek (stem)	*Allium porrum*	27.7	2.6	0.09
Green bean	*Phaseolus vulgaris*	35.5	4.1	0.12
Yellow bean	*Phaseolus vulgaris*	55.7	8.2	0.15

Results are presented as mean value of duplicates.

in red grapes, associated with the higher content of anthocyanidines.

Peaches show particularly low content of total phenolics and total flavonoids (50.9 mg GAE/100 g and 15.0 mg CE/100 g, respectively) as well as figs (59.0 mg GAE/100 g and 20.2 mg CE/100 g, respectively) at almost equal ratios – respectively 0.3 and 0.34.

The variation of phenolic compounds content in the fruits depends on many factors. It is known that it decreases in the process of fruits development. Thus, for example, in white-coloured fruits it decreases constantly with the progress of the ripening, while in red-coloured varieties it increases during the last ripening stage due to the maximal accumulation of anthocyanines and flavonols. Although we applied a very strict selection of the samples in equal ripening stage we support the opinion that this criterion outlines data variations to the greatest extent.

The general assessment of the analytical results for fruits definitely shows individual specificity of each studied sample and a rich diverse spectrum of phenolic compounds differing from the flavonoids group.

Table 2 presents the analytical data for total phenolic and total flavonoid content of the studied vegetables. The highest total phenolic content was found in green peppers (246.7 mg GAE/100g), followed by parsley (188.0 mg GAE/100g) and red peppers (173.2 mg GAE/100g). The ratio total flavonoids/ phenolics is comparatively low (about 0.1). The results suggest rich availability of hydroxycinnamic acids (chlorogenic acid, ferulic acid, p-coumaric acid, etc.) presented mainly in esterified form with organic acids, sugars or lipids in the analyzed vegetable species. Comparatively high total phenolic content was found in brussels sprouts (161.5 mg GAE/100 g), radishes (160.0 mg GAE/100 g), red onions (154.1 mg GAE /100 g) and okra (153.7 mg GAE/100 g). Of these vegetables, the highest total flavonoid content is detected in okra (49.1 mg CE/100 g) and radishes (48.5 mg CE/100 g). Parallel high content of both total phenolics and total flavonoids was found in the studied salads and lettuces.

The substantial difference in total phenolic content of red and spring onions should be emphasized (154.1 mg GAE/100 g vs. 120.0 mg GAE/100 g) at very close total flavonoids values (18.7 mg CE/100 g vs. 16.0 mg CE/100 g). Different content was also detected in the different parts of the plant. The green part of the spring onion has more total phenolics compared to the white part, while the total flavonoids are more concentrated in the green part.

The analytical data for leeks follow the same pattern concerning the stem and the leaves. The results for yellow and green beans are also similar. It is clear that the change in the colouring of the plant is a process associated with redistribution of phenolics and flavonoids.

The results from the analysis of vegetables show specific total phenolic and flavonoid content and specific distribution in the particular representatives, especially for peppers and onions that are traditional for Bulgarian diet.

CONCLUSIONS

The presented data for total phenolic and total flavonoid content are a basis for assessment of the preventive role of fruits and vegetables against free radicals effect and will enrich the national food composition database.

They are a necessary step towards in-depth studies on the spectrum of multiple phenolics and flavonoids representatives.

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