A C T A   A L I M E N T A R I A   P O L O N I C A
Vol. XVII (XLI) No. 1   1991

JADWIGA WILSKA-JESZKA
JADWIGA ŁOŚ
MARIA PAWLAK

FRUITS AS BIOFLAVANOIDS SOURCES

Institute of Technical Biochemistry, Technical University, Łódź

Key words: flavanols, polyphenols, fruits, biological activity

Flavanols and total polyphenols contents in selected fruits were analysed. It was found that apple, cherry, strawberry and briar rose are rich in biologically active mono and oligomeric forms of flavanols. On the other hand, the contents of these compounds in bilberry and chokeberry, fruits which are in anthocyanins, are very low.

Fruits are a rich source of biologically active polyphenols, including derivatives of flavone and flavan. Differences in chemical structure and spatial configuration in these two groups of compounds are reflected in their different physico-chemical and biological properties.

The flavone derivatives have flat molecular structure. They are poorly soluble in water, and are not easily absorbed from the digestive tract. Some of them e.g. quercetin, are even mutagenic [3].

The derivatives of flavan have molecules of diverse spatial structure. They are highly soluble in water and are easily absorbed from the digestive tract. Particularly interesting among them are flavan-3-oles, otherwise known as catechins, and flavan-3,4-dioles, or leucoanthocyanidins, as well as oligomers thereof. These compounds display a diverse biological activity, and are described by some authors as bioflavanoids. The latest research show them to be very important in processes responsible for the correct structure and functioning of capillary blood vessels, preventing their brittleness and excessive permeability. The role of bioflavanoids in these processes was elucidated by Masquelier [18, 19], who found that the most active of these compounds are catechin and epicatechin dimers called procyanidins. Masquelier suggests that procyanidins may prevent arteriosclerosis and other diseases of the circulatory system.

Flavanol mono- and oligomers in vegetables, fruits and products of them, considerably affect organoleptic properties such as colour, taste, structure and consistence [5, 17]. The technological processes involved in processing fruits and vegetables may have an adverse effect on some of the sensory qualities, and also decrease the biological value of the final products, and these phenomena are connected among other things with transformations of flavanols [2, 6, 12, 25, 26, 29].

In the 1950s and '60s these compounds were studied primarily in the context of their effect on organoleptic properties of foodstuffs, with research focusing mostly on leucoanthocyanidins, catechins and tannins. Noteworthy are the works of Hermann [10], Swain and Hillis [28], Ribereau-Gayon [27] and Lewak [16].

In the '70s and '80s, as methods of instrumental analysis improved, the interest in flavanoids, and particularly in proanthocyanidins, grew sharply. Extensive information about this may be found in Haslam [9] and Thompson [30].

Procyanidins in fruits and fruit products were studied by Bourzeix, Oszmiański, Jerumanis and Lea, among others. Bourzeix [4] analysed the content of flavanols in various parts of grapes (pulp, skin, seeds, peduncle) finding that a particularly rich source of catechins and procyanidins are the seeds. Oszmiański and Sożyński [21, 23] determined that the content of polyphenols, including also flavanols, in apples and apple products depends to a great extent on the variety and ripeness of fruits.

Oszmiański and Bourzeix [22] found briar-rose to be a rich source of catechins and group-B procyanidins. Lea [14, 15] studied the dependence between the presence of flavanols and organoleptic properties of ciders. Jerumanis et al. [11] investigated the contents and transformations of catechins and proantocyanidins in malt, hops and beer.

The aim of our research was to determine the contents of catechins and low molecular proanthocyanidinsin selected fruits.

EXPERIMENTAL

MATERIAL

The following fruits were used in the determinations: strawberry *(Fragaria chiloensis)*, cherry *(Prunus cerasus)*, goosberry *(Grossularia Mill.)*, black currant *(Ribes nigrum)*, red currant *(Ribes rubrum)*, apples *(Pyrus malus)* of the Jonathan and Bankroft varieties, bilberry *(Vaccinium myrtillus)*, chokeberry *(Aronia melanocarpa)*, briar rose *(Rosa rugosa and Rosa canina varieties)*, rowan *(Sorbus aucuparia)*, and green Chinese tea *(Thea sinensis L.)*. Separation and weight determinations of flavanol mono- and oligomers according to Masquelier [19].

100-g portions of plant material were extracted three times with 100-ml portions of 75% acetone. After centrifugation, the acetone was removed under reduced pressure. Tannins were salted out from the obtained water extract with NaCL and the precipitate was centrifuged. The supernatant was three times extracted with 100-ml portions of ethyl acetate. The organic phase was concentrated to one tenth volume, and then treated with five volumes of chloroform. The precipitate of flavanol mono- and oligomers was dried and weighed.

ANALYTICAL METHODS

Total polyphenols were determined by the method of Folin-Ciocalteu [24], anthocyanins by the method of Fuleki and Francis [7], proanthocyanidins by the method of Pompei [1], flavanols by the method of Swain-Hillis [28]. The separation and identification of proanthocyanidins was done a by HPLC method using Pye-Unicam LC-3 apparatus with a UV detector at wavelength of 280 nm. A column measuring 25 x 0,4 cm field with Lichrosorb RP-18 was used. The mobile phase was the solvents H_2O—CH_3OH—CH_3COOH in 85:10:5 proportion.

RESULTS AND DISCUSSION

The investigations concerned several berry species, two apple varieties, and also briar rose and rowan fruits. Green tea known to be catechins rich was analysed for comparison. Total polyphenols content, total flavanol mono- and oligomers, and anthocyanins were determined to get an idea about the contents of the principal groups of polyphenol compounds in these fruits. The results are collected in Table 1.

The berries containing the largest amounts of polyphenols were bilberry (8.7% dry mass) and chokeberry (8.5%). These fruits were also the richest sources of anthocyanins which constituted 42 and 24% of polyphenols, respectively. Cherry, black currant, and strawberry contained much less polyphenols (about 3% dry mass) and anthocyanins. Briar rose contained about 4% polyphenols in dry mass of its edible parts. The Jonathan and Bankroft apples, analysed in March, displayed rather low polyphenols contents of 1.7 and 1.5% dry mass, respectively. In green tea the polyphenols

Table 1. Polyphenols in the studied fruits

	Total polyphenols (mg/100g)		Anthocyanins (mg/ 100g)		Proportion of anthocyains among polyphenols %	Mass of flavanol mono- and oligomers precipitates (mg/100g)		Proportion of flavanol mono- and oligomers among polyphenols %
	fruits	dry mass	fruits	dry mass		fruit	dry mass	
Strawberry	225	3055	45	615	20.0	101	1370	44.9
Cherry	460	3377	65	475	14.1	161	1180	35.0
Goosberry	200	1908	—	—	—	66	630	33.0
Black currant	560	3097	140	770	25.0	31	170	5.5
Red currant	210	1613	29	220	13.8	83	635	39.5
Jonathan apple	250	1710	—	—	—	217	1490	86.8
Bankroft apple	205	1530	—	—	—	172	1285	83.9
Gilberry	1000	8696	420	3650	42.0	56	485	5.6
Chokeberry	2080	8490	505	2065	24.3	32	130	1.5
Rowan	505	2148	—	—	—	166	705	32.9
„Rosa rugosa" pulp	980	3837	—	—	—	182	713	18.6
stones	1040	1599	—	—	—	312	479	30.0
„Rosa canina" pulp	1430	4454	—	—	—	475	1480	33.2
Green tea	5250	5899	—	—	—	4150	4665	79.0

content was 5.9% dry mass, i.e. about twice higher than in most of the studied fruits, but in relation to dry mass it was lower than in bilberry and chokeberry.

Total polyphenols content is one of the indices of fruit quality but it tells nothing about the actual identity of polyphenol compounds, and hence about biological value which depends primarily on the presence of flavanols. The content of these compounds isolated using Masquelier's method [19] is given in Table 2.

As can be seen, the contents of flavanol mono- and oligomers in the studied raw materials are much more diversified than the total polyphenols contents and quite unlike them. The fruits containing the largest amounts of polyphenols and anthocyanins — bilberry and chokeberry — contain the least amounts of flavanols (5.6 and 1.5% of total polyphenols contents, respectively). The richest flavanols source is green tea (4.7% in dry mass amounting to 80% of total polyphenols). Worth noting is the high content of flavanol mono- and oligomers (1.3-1.5% dry mass) in apples, strawberry, cherry and briar rose. In apples these biologically active compounds constitute about 85% of total polyphenols.

These results confirm the important role apples in the nutrition. Containing rather small amounts of vitamins, they provide a number of other important components including large amounts of flavanol mono- and oligomers playing important roles in preserving the correct structure and function of capillary blood vessels. Our study do not confirm the suggested high „vitamin P" content in chokeberry [8, 13], which is characterized by very high contents polyphenols but the lowest content of flavanol compounds having a biological activity corresponding to definition of „vitamin P".

The purity and composition of precipitates obtained with Masquelier's method were investigated by determining total polyphenols, flavanols and proanthocyanidins contents by colorimetric methods, and by separating and identifying the compounds by HPLC. The results of colorimetric analyses of flavanol mono-and oligomer precipitates are given in Table 2.

The contents of polyphenols in most of the analysed precipitates ranged from 40 to 70%, only in this obtained from red and black currants and from goosberry were very low (8-25%) which indicate their low purity.

Our results suggest that in addition to flavanols, the precipitates obtain by Masquelier method contain compounds of reducibility lower than that of catechin which was used as standard in polyphenols determination by the Folin-Ciocalteu method. These compounds may be polymerized flavanol forms or even substances which do not enter reactions with this reagent. It seems therefore that in the applied conditions the results based on precipitates mass are to high, with the error being greater when the flavanols content is low.

Table 2. Characteristic of flavanol mono- and oligomer precipitates obtained from fruits by Masquelier's method

	Contents in precipitates %			Calculated content in fruits (mg/100g)		
	total polyphenols	proanthocyanidins	flavanols	total polyphenols	proantocyanidins	flavanols
Strawberry	43.8	15.4	15.0	44.2	15.6	15.2
Cherry	48.8	17.9	27.7	78.6	28.8	44.5
Goosberry	15.2	8.1	8.8	10.0	5.3	5.9
Black currant	25.0	7.4	11.7	7.8	2.3	3.6
Red currant	8.3	2.5	3.6	6.9	2.0	2.9
Chokeberry	50.0	14.6	13.8	16.0	4.7	4.4
Bilberry	55.0	12.6	16.3	30.8	7.1	9.1
Jonathan apple	53.0	30.6	33.0	115.0	66.4	71.5
Bankroft apple	56.9	24.6	21.4	98.0	42.4	36.8
Rowan	60.0	5.2	—	99.6	8.6	—
„Rosa rugosa" pulp	61.0	5.9	—	111.0	10.7	—
stones	70.0	9.4	—	218.4	29.3	—
„Rosa canina"	41.3	11.9	19.1	196.0	56.7	90.5
Green tea	60.0	5.2	41.4	2490.0	215.0	1719.0

The catechins and proanthocyanidins contents in the precipitates were determined with two colour reactions: the Bate-Smith and the Swain-Hillis vanillin ones. These reactions are not selective. The vanillin reaction involves ring A of flavan-3-oles and flavan-3,4-dioles. Positions 6 and 8 of ring A are activated by the presence of OH groups, and may be deactivated by CO groups, and so flavanol molecules do not react with vanillin. Highly polimerized forms of flavanols also do not form complexes with vanillin, since the active positions 6 and 8 are blocked by interflavan bonds [27]. The Bate-Smith reaction on the other hand leaves undetermined the catechins which, heated in the n-butanol-HCL solution, form yellow-brown products with a peak at 450 nm; this however, does not affect absorbance measured at 550 nm [28].

For most of the studied fruits, the results of both determinations are similar, which indicates a large proportion of proantocyanidins. Green tea was the only material containing several times more flavanols than proanthocyanidins, this being due to the well known fact that the principal polyphenols in tea are catechins and gallocatechins. The flavanol mono- and oligomers content were also studied by the HPLC method. The separation of the available standard substances — (+) catechin, (-) epicatechin and procyanidine B_2 is presented in Fig. 1 The subsequent Figures 2-5 illustrate the obtained separations of flavanols occurring in some of the studied fruits. Worth noting are the similar chromatograms of chokeberry and bilberry.

Table 3 lists the contents of flavanols identified in the chromatograms. The compound dominating in green tea, apples and strawberry is epicatechin; in cherry the predominating compound is catechin. Procyanidin B_2 occurs in substantial quantities only in cherry and apples; the remaining fruits contain only trace amounts of this compound (less than 2mg/100g). The flavanols contents determined by the HPLC and vanillin methods are comparable,

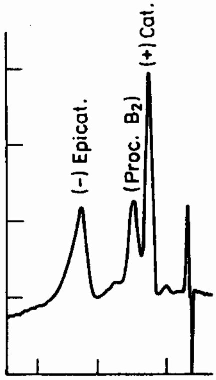

Fig. 1. HPLC chromatogram of standards

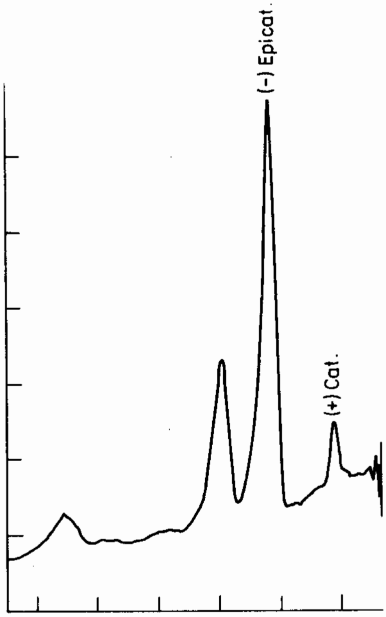

Fig. 2 HPLC chromatogram of green tea flavanols

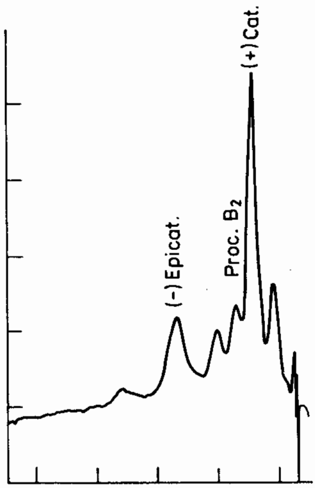

Fig. 3 HPLC chromatogram of cherry flavanols

with the differences being due to various proportions of unidentified flavanol compounds in the studied fruits.

The reported study of bioflavanoids contents in selected fruits is of a preliminary nature. We did not study neither the influence of a variety of the fruits nor the conditions in which they were stored. It seems, however, that the following conclusions are justified.

Table 3. Flavanols content in fruits determined by the HPLC method

	(mg/100 g) (+) — catechin	(mg/100 g) (-) — epicatechin	(mg/100 g) proanthocyanidin B_2	Total flavanols determined by the HPLC method (mg/100 g)	Flavanols determined by the vanillin method (mg/100g)
Strawberry	0.8	7.1	0.4	8.3	15.2
Cherry	11.6	9.2	4.2	25.0	44.5
Black currant	0.9	0.2	1.2	2.3	3.6
Red currant	1.1	—	1.4	2.5	2.9
Jonathan apple	7.4	36.9	23.6	67.9	71.5
Bankroft apple	1.7	22.7	8.5	32.9	37.8
Bilberry	0.4	1.1	trace	1.5	9.1
Chokeberry	0.4	0.8	0.1	1.3	4.4
Green tea	69.2	1247.9	trace	1317.1	1719.0

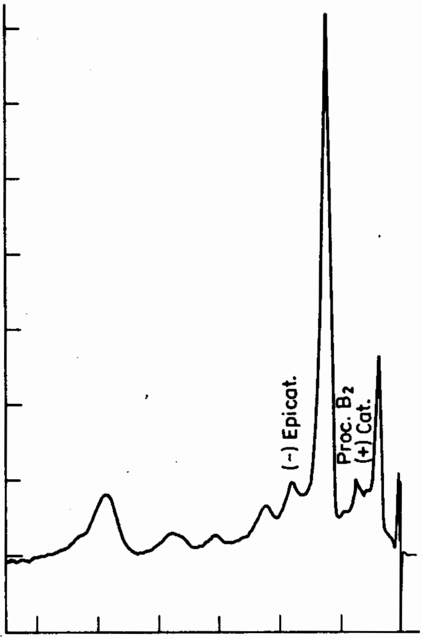

Fig. 4. HPLC chromatogram of chokeberry flavanols

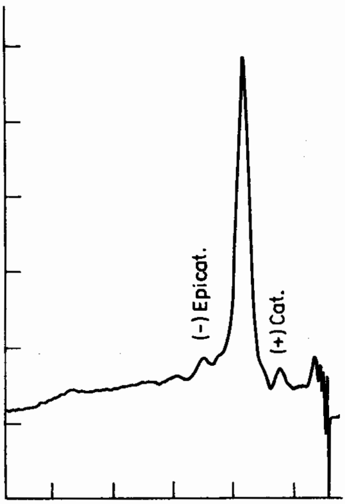

Fig. 5 HPLC chromatogram of bilberry flavanols

CONCLUSIONS

1. Of all the investigated fruits, apples are the richest source of flavanols mono- and oligomers. This confirms the important role of apples in nutrition.

2. Relatively rich sources of flavanols are also briar rose, especially dog rose (Rosa canina), as well as cherry and strawberry.

3. Bilberry, chokeberry and black currant contain large amounts of polyphenols and anthocyanins, but low amounts of flavanols.

4. Masquelier's metod of flavanol mono- and oligomers total content determination is insufficiently selective in the case fruits, and additional studies of the precipitates composition are necessary.

LITERATURE

1. Amerine M. A., Ough C. S.: Wine and must analysis. A. Wielly-Intersci. Publ., New York 1974, 72.
2. Beveridge T., Harrison J. E.: J. Food Sci., 1984, **49**, 1335.
3. Bjeldanes L. F., Chang G. W.: Science 1977, 197, 577.
4. Bourzeix M., Clarens M.: Bull. Liaison, Grope Polyphenols 1986, **13**, 404.
5. Buren van J. K., Senn G., Neukom H.: J. Food Sci., 1966, **31**, 964.
6. Cornwell C. J., Wrolstad R. E.: J. Food Sci., 1981, **46**, 515.
7. Fuleki T., Francis F. J.: J. Food Sci., 1963, **33**, 78.
8. Grochowski W.: Jadalne owoce leśne, PWRiL, Warszawa 1986, 182.
9. Haslam E.: Phytochem., 1977, **16**, 1625.
10. Hermann K.: Z. Lebensm. Unters. Forsch., 1973, **151**, 41; 1974, **154**, 6; 1975, **159**, 31, 85, 341.
11. Jerumanis J. et all.: Bull. Liaison, Groupe Polyphenols 1984, 108.
12. Johnson G., Donnelly B. J., Johnson D. K.: J. Food Sci., 1968, **33**, 254.
13. Kalemba D., Góra J., Kurowska.: Przem. Owoc. Warzywny i Ferment., 1985, **29**, 1051.
14. Lea A. G. H.: Ann. Nutr. Alim., 1978, **32**, 1051.
15. Lea A. G. H.: J. Food Agric., 1978, 29, 471, 478, 484, 493.
16. Lewak S., Radomińska A.: Roczniki Chemii: 1964, **38**, 1773; 1965, **39**, 1839.
17. Lewicki P.: Przem. Spoż, 1965, **19** (5), 276.
18. Masquelier J.: Symp. „Alimentation et consommation du vin", Werona 1982.
19. Masquelier J., Michaud J., Bronnun-Hansen K.: Bull. Liaison, Groupe Polyphenols 1980, **9**, 35.
20. Masquelier J. et all.: Bull. Liaison. Groupe Polyphenols 1980, **9**, 429.
21. Oszmiański J.: Zeszyty Naukowe A. R., Wrocław Techn. Żywn., 1986, **163**, 31.
22. Oszmiański J., Bourzeix M.: Bull. Liaison, Groupe Polyphenols 1986, **13**, 488.
23. Oszmiański J., Sożyński J.: Acta Aliment. Polonica 1986, XII, 11.
24. Peri C., Pompei G.: Am. J. Enol. Viticult., 1971, **22**, (2), 55.
25. Pilando S. L., Wrolstad R. E., Heatherbell D. A.: J. Food Sci., 1985, **50**, 1121.
26. Polakowa M. M., Smirnowa G. G.: Kons. i Owoszcz. Prom., 1984, **7**, 20.
27. Ribereau-Gayon P., Stonestreet E.: Chim. Anal., 1966, **48**, 188.
28. Swain T., Hillis H. E.: J. Sci. Food Agric., 1959, **10**, 63, 135.

29. Šapiro D. K., Čekalinskaja I. I, Dawnar T. W.: Refer. Żurnał Chimija 1977, Abstr. 24R, 136.
30. Thompson R. S. et all.: J. Chem. Soc. Perkin Trans. I, 1972, 1387.

Manuscript received: April 1989
Authors adrress: 90-924 Łódź, Stefanowskiego 4/10

J. Wilska-Jeszka, J. Łoś, M. Pawlak

OWOCE JAKO ŹRÓDŁO FLAWANOIDÓW

Instytut Biochemii Technicznej, Politechnika, Łódź

Streszczenie

Przeprowadzono badania zawartości niskocząsteczkowych flawanoli w wybranych gatunkach owoców oraz w zielonej herbacie stanowiącej bogate źródło tych związków. Do oznaczania sumy monomerów i oligomerów flawanolowych zastosowano metodę Masquelier, polegającą na wydzieleniu tych związków w postaci osadów i oznaczeniu ich metodą wagową. W badanych surowcach oraz w wydzielonych z nich osadach mono- i oligomerów flawanolowych oznaczono kolorymetrycznie polifenolowe ogółem, flawanole i procyjanidyny. Analiza osadów otrzymanych metodą Masquelier wykazała, że charakteryzują się one bardzo zróżnicowaną zawartością polifenoli (od 15% do 70%) oraz niską zawartością flawanoli i procyjanidyn — poniżej 30% (tab. 2). Dowodzi to małej selektywności tej metody w odniesieniu do owoców. Konieczne jest więc prowadzenie uzupełniających analiz składu tak otrzymanych osadów.

Wyniki przeprowadzonych badań wykazały, że do owoców szczególnie bogatych w aktywne biologicznie mono- i oligomery flawanolowe można zaliczyć jabłka, dziką różę, a także wiśnie i truskawki. Natomiast owoce czarnej jagody, aronii i czarnej porzeczki, szczególnie bogate w polifenole i antocyjany, wykazały bardzo niską zawartość związków flawanolowych (tab. 1 i 2).

Zastosowanie techniki HPLC pozwoliło oznaczyć w badanych surowcach zawartość (+)-katechiny, (—)-epikatechiny i procyjanidyny B_2. Z owoców najbogatsze w te związki okazały się jabłka, jednak w zielonej herbacie zawartość katechin była kilkadziesiąt razy wyższa niż w jabłkach (tab. 3). W większości badanych owoców dominującą formą flawanoli była (-)-epikatechina.