

Quercetin content and ratios to total flavonols and total flavonoids in Bulgarian fruits and vegetables

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Flavonoids as bioactive compounds in vegetable foods have been the subject of numerous research projects. Quercetin, with its powerful antioxidant activity, has also been and is currently in the focus of studies on plant species identification, and on its role in healthy nutrition. The current literature sources provide diverse information on its content in particular plant species but there are almost no data on its ratios to other flavonoids representatives. The aim of this survey was to provide information about quercetin analysis and content as a major flavonols representative and quercetin ratios to total flavonols, expressed as a sum of myricetin, quercetin and kaempferol and to total flavonoids in Bulgarian fruits and vegetables. The survey covered 17 fruit and 13 vegetable samples, complying with the current sampling requirements, with a view to food composition assessment. Quercetin and other flavonols analysis by High Performance Liquid Chromatography (HPLC) method; Total flavonoid content was determined by the aluminum chloride colorimetric assay. Evidence is presented on quercetin content in fruits and vegetables and on its ratio to total flavonols and total flavonoids content. The results demonstrate that quercetin is most frequently the major flavonol representative in the majority of the analyzed samples. There is, though, an interesting exception, presented by the representatives of the *Cruciferae* family - broccoli and Brussels sprouts - where the quercetin ratio to total flavonoids is very high, reaching up to almost 50%. In fruits, quercetin in strawberries is only 44.0% of the total flavonols, thus demonstrating once again the need for a complex approach in the analysis of the data for flavonoids content. The supplementation of the flavonoids composition and content data with evidence about their ratios will enable more correct identification of the biodiversity and will allow to compensate, though to only a certain extent, the effect of the biological variation on the accuracy of the analysis, and will enrich the information needed to build up a data base for flavonoids in foods.

Key words: flavonoids, quercetin, flavonols, fruits, vegetables

INTRODUCTION

The Food Composition Tables are an indispensable part of the food information system that, together with the criteria for the biological role of the food and its nutrients, is involved in the building up and establishment of the food policy. Food, as a basic environmental compartment sets the need of knowledge on its composition to enable its nutritional value and safety. Currently there are huge data arrays containing information about macro- and micronutrients and on the bioactive compounds content in foods as well. An example in this aspect is supplied by the US Department of Agriculture that has developed data bases for phytonutrients, carotenoids, flavonoids, isoflavonoids, anthocyanines in addition to the major food composition data base [1]. The present survey was focused on the content of the flavonoid quercetin in foods, because of its confirmed bioactivity in the prevention of oxidative stress in the organism [2, 3], as well as against the development of a number of degenerative diseases [4-6]. Quercetin attracted our attention as it is the

most comprehensively studied flavonoid of the flavonols group and all data bases for this class of polyphenolic compounds contain any information on it.

Criteria for judging the quality of food composition data and databases have long been established. In 2002 those criteria have been formalized by Holden et al. [7] assessing food data quality. Whether generating new analytical data or assessing existing data, quality criteria are fundamentally related to the following stages: the number of food samples collected, the number of samples prepared for analysis, the number of discrete samples analyzed, the number of analytical replicates, the number that represents the best value and the variability and the quality of analytical procedure used [8].

In this relation, in spite of the variety of data on flavonoids content in foods, they either do not comply with the requirements for a database or the biovariety of the selected plant species is very large, or no relationships have been searched for between the particular representatives in the flavonoids groups. Striving for stability of the results for flavonoids content in plant species used for food purposes, we support that it is appropriate

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to analyze not only the real numerical value of the quantity of the individual representatives of flavonoids classes per unit of plant tissue but also to establish the ratio between the compounds themselves, building up the content of each individual plant species. The aim of this survey was to provide information for analysis and content of quercetin as a major flavonols representative and quercetin ratios to total flavonols expressed as a sum of myricetin, quercetin and kaempferol and to total flavonoids in Bulgarian fruits and vegetables.

MATERIALS AND METHODS

Sampling plan

This survey covered the analysis of 17 fruit and 13 vegetable samples. Each analyzed individual sample of fresh fruits and vegetables was an aggregate sample of three *single* samples purchased at three different premises in one and the same day. The amount of the purchased single samples was as follows: not less than 0.5 kg for berries (raspberries, blueberries, blackberries), not less than 1 kg for the other fruits and vegetables and three pieces for vegetables that are sold either in pieces (cabbage) or bunches (leeks) (BNS ISO 874: 1996) [9]. A sampling protocol was elaborated for each single sample, describing its origin. The single samples were aggregated in a common sample (*aggregate sample*). After a careful check fruits and vegetables with infringed integrity and freshness were pulled out of the aggregate sample. A *subsample* was made of the aggregate sample, through random selection of fruits and vegetables, that was lyophilized. It was weighed before and after the sublimation drying with the task to determine its dry fraction that was necessary for the precise calculation of the results. When preparing the subsample all non-edible parts of the fruits and vegetables were removed. The lyophilized subsample was stored in hermetically vacuum sealed packs at temperature of 4°C until the time of the analysis. Before the analysis the lyophilized subsample was ground, sieved through a sieve with pore size 0.5 mm and homogenized. Part of the lyophilized subsample was taken, representing the analytical sample [10].

Methods for determination of flavonols in fruits and vegetables

Extraction and hydrolysis

The lyophilized subsamples were ground to fine powder. The analytical sample was weighed in a 200 ml Erlenmeyer flask with ground glass joints and water, solution of tertiary butylhydroquinone

(TBHQ) (2 mg/ml MeOH), and hydrochloric acid (10 M) were added to it as follows (Table 1):

Table 1 Sample preparation for flavones and flavonols analysis

Sample	Weight (g)	TBHQ (ml)	H ₂ O (ml)	HCl (ml)
Lyophilizate	0.500 – 1.500	25	19	6

Each analytical sample was completed with 500 µl internal standard morin in a way that the final morin concentration in the sample would reach 2.5 µg/ml.

The extraction and hydrolysis of the sample in this survey was performed at 1.2 M HCl in 50% MeOH in a water bath at 90°C for 2 h under a reverse condenser. After the hydrolysis period had expired, the sample was allowed to cool down for about 5 min, and after that 1 ml solution of ascorbic acid was added to it (1 mg/ml). The sample was transferred to a 100 ml graduated flask and the marked volume was made up by adding methanol. The sample was subjected to an ultrasound bath for 3 min and, after that, if necessary, the volume was again adjusted to the mark. The extract was homogenized and an aliquot part of it was ultracentrifuged for 5 min at 14000 rpm. The supernatant was filtered through a membrane filter (HV-Millipore) with pore diameter 0.45 µm.

High performance liquid chromatographic analysis

The separation was performed by an Alltima column (100 × 4.6 mm i.d., 3 µm) C18, Alltima Associates, Inc., connected to a pre column packed with the same filling. The elution was isocratic with c 28 % acetonitrile in 2% acetic acid (Eluent I). The flow rate was 0.9 ml/min, with working pressure 11.5 – 12 MPa.

The amount of flavonols and flavones in the samples was determined by the method of the internal standard. For this purpose a linear correlation equation of the relationship between the ratio of the signals of the standard solutions to the internal standard and the concentration of the determinable compounds in the calibration standard solutions was constructed. The results were listed in mg/100 g fresh weight.

Since by the present HPLC analysis only 3 individual flavonols may be determined, we have decided to refer the quercetin content, as their main representative, to total flavonoids load in fruits and vegetables.

Total flavonoids assay

Total flavonoid content was determined by the aluminum chloride assay [11]. All samples were analyzed in duplicates. In brief an aliquot of 1 ml of

extracts or standard solution of catechin (20-100 mg/l) was added to 10 ml volumetric flask containing 4 ml H₂O, and after that 0.3 ml 5% NaNO₂ was added. After 5 min, 0.3 ml 10% AlCl₃ was added and at 6th min, 2 ml 1M NaOH was added and the total volume was made up to 10 ml with H₂O. The absorbance of the solution was measured against the reagent blank at 510 nm. Total flavonoids content was expressed as mg catechin equivalents CE/100 g fresh mass [12].

RESULTS AND DISCUSSION

The results for quercetin and total flavonols content (expressed as a sum of myricetin, quercetin and kaempferol) in Bulgarian fruits, determined by HPLC method were presented in Table 2 in mg/100 g fresh weight. All results complied with the requirements for food data representativeness as they were a mean value of duplicate analyses of a pool of 3 market samples. In addition, each value was an average result of at least three aggregated (pool) samples, which meant at least 9 individual samples. In this relation, the presented data were among the most precise data, available in the literature sources, concerning the sampling plan [13, 14].

The table also contains the quercetin ratios to total flavonols (sum of myricetin, quercetin and kaempferol) determined by HPLC method, and to total flavonoids determined by a colorimetric method, expressed in percents (%). The results shown in Table 2 revealed that the data obtained by HPLC analytical methods were normally lower than those provided by non-specific colorimetric spectrophotometry and, in some cases, for example with sour cherries, only 1% of the total flavonoids were on the account of the flavonol quercetin. This could be explained by the fact that the total flavonoids assay reported also other classes of phenolic compounds which, in fruits, were most frequently anthocyanins, catechins and their forms associated with gallic acid.

In all cases it should be taken into account that it was possible that the colorimetric method for analysis of total flavonoids and other phenolic compounds, and even some tannins would provide positive results. That was the cause for the inclusion in data bases for flavonoid content in foods only of results from chromatographic quantitative analysis.

The results showed that most often quercetin was the major flavonol representative and in many fruit samples it was the only representative of this flavonoids class. Generally the content of the other two flavonols representatives - myricetin and

Table 2. Quercetin content in fruits and ratios to total flavonols and total flavonoids

Fruit	Quercetin/		
	Quercetin mg/100g	Total Flavonols ¹ %	Quercetin/ Total Flavonoids ² %
Apple, red (peeled)	0.0	0.0	0.0
Apple, red (unpeeled)	1.59	100.0	3.0
Apple, green (peeled)	0.0	0.0	0.0
Apple, green (unpeeled)	1.39	100.0	1.0
Blackberry	2.70	84.4	5.0
Blueberry	9.92	72.9	5.0
Cherry	2.52	100.0	13.0
Fig	0.87	100.0	4.0
Grape, black	2.32	91.3	3.0
Grape, white	1.56	85.7	4.0
Peach	3.41	100.0	23.0
Pear (peeled)	0.0	0.0	0.0
Pear (unpeeled)	0.59	100.0	1.0
Plum	2.34	100.0	2.0
Raspberry	1.60	100.0	6.0
Sour cherry	1.08	100.0	1.0
Strawberry	1.02	44.0	1.0

¹Total flavonols as sum of myricetin, quercetin and kaempferol, determined by HPLC analysis;

²Total Flavonoids, determined by AlCl₃ colorimetric assay and expressed as mg catechin equivalents CE/100g fresh weight; determined by HPLC methods and expressed as mg/100 g fresh weight

kaempferol - was very small and was detected only in samples of grapes, blackberries and blueberries.

There was, though, a notable exception of the general rule – in strawberries quercetin was only 44.0% of the total flavonols that once again emphasized the need of a complex approach to analysis of data for flavonoids content.

The data also showed that the highest ratio quercetin/total flavonoids belonged to peaches – 23%, followed by cherries – 13.0% and raspberries – 6.0%.

The results for quercetin content in samples of Bulgarian vegetables, presented in mg/100 g fresh weight as well as the ratio quercetin/total flavonols and quercetin/total flavonoids (%) were listed in Table 3.

It is obvious that quercetin is the main flavonol in Bulgarian vegetables. Our previous studies have shown that myricetin was not detected in vegetable samples and kaempferol did not exceed amounts of 0.8 mg/100 g. In this sense quercetin ratio to total flavonoids in vegetables was not a surprise and the results were equal or close to 100 % in many of the tested samples. An interesting exception, though, were the results of the representatives of the *Cruciferae* family – broccoli and Brussels sprouts. Only those two vegetable species had a very high quercetin to total flavonoids ratio, reaching up to almost 50% in Brussels sprouts.

Table 3. Content of quercetin in Bulgarian vegetables and its ratio to total flavonols and total flavonoids

Vegetable	Quercetin mg/100g	Quercetin/ Total Flavonols ¹ %	Quercetin/ Total Flavonoids ² , %
Okra	20.03	100.0	41.0
Tomato	1.42	88.2	11.0
Red pepper	1.49	100.0	11.0
Green pepper	10.27	100.0	37.0
Lettuce	15.39	100.0	16.0
Brussels sprouts	2.63	49.8	8.0
Broccoli	2.94	36.6	16.0
Red onion	45.25	100.0	(241.9)
White onion	20.41	100.0	-
Spring onion	10.32	87.60	65.0
Leek	0.0	0.0	0.0
Beans green	2.13	100.0	52.0
Beans yellow	2.29	100.0	28.0

¹Total flavonols as a sum of myricetin, quercetin and kaempferol, determined by HPLC analysis;

²Total Flavonoids, determined by AlCl₃ colorimetric assay and expressed as mg catechin equivalents CE/100g fresh weight; determined by HPLC methods and expressed as mg/100 g fresh weight

The results for quercetin content and its ratio to total flavonols in samples of representatives of the family *Liliaceae* – leeks, spring, red and white onions showed that quercetin was not detected in leeks which complied with literature evidence [15]. The data for spring and mature onions were interesting, demonstrating the importance of botanical maturity for the quercetin/flavonols ratio in vegetables. For example, in fresh spring onions quercetin was 87.6 %, while in the mature white and red onions it was 100% of the studied amount of total flavonols. In this group of samples the results for the ratio quercetin/total flavonoids was also interesting. It was established that in spring onions quercetin accounted for 65% of all flavonoids – the highest determined value. Unfortunately we could not provide data for quercetin/total flavonoids ratio in white onions as total flavonoids were not determined for those samples. The results for red onions were startling – formally they were 241.9%, which was an unreal value. That was the only sample we tested where quercetin, determined by HPLC methods exceeded the total amount of flavonoids, determined by spectrophotometry. This result could be explained by the excessively high biological variation of flavonoids in food samples or by an analytical mistake in the determination of high amounts of total flavonoids by spectrophotometric methods. This once again supports the importance of a good sampling plan and assessment of the data quality

and emphasizes on critical interpretation of results obtained from analysis of single food samples.

The importance of the presented quercetin/total flavonoids ratios to the greatest extent was outlined by the results for green and yellow beans. The data showed that, while quercetin content in the two types of foods was very close, its percentage rate in green beans was about two times higher than that in yellow beans.

CONCLUSION

The rich plant biodiversity requires a broad spectrum of indicators for its identification. The analysis implemented in this survey showed that the inclusion of ratios between individual representatives of the flavonols group provided a more comprehensive and reliable assessment of the flavonols representation in the target plant species and could predict a value for their bioactivity that was closer to the real one. The completion of the data for composition and content of flavonoids with their ratios values will enable more correct identification of the biodiversity and compensation, though to a certain extent, for the effect of biological variation on the accuracy of the analysis and will enrich the information necessary for building up data bases for flavonoids in foods.

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СЪДЪРЖАНИЕ НА КВЕРЦЕТИН И СЪОТНОШЕНИЯТА МУ КЪМ ОБЩИТЕ ФЛАВОНОЛИ И ОБЩИТЕ ФЛАВОНОИДИ В БЪЛГАРСКИ ПЛОДОВЕ И ЗЕЛЕНЧУЦИ

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(Резюме)

Флавоноидите, като биоактивни съединения в растителните храни, са обект на голям брой изследвания. С мощната си антиоксидантна активност кверцетинът е във фокуса на изследванията върху идентифицирането на растителните видове и ролята му в здравословното хранене. В литературата има разнообразна информация относно съдържанието му в различните видове растения, но почти няма данни за съотношенията му с други представители на флавоноидите. Целта на настоящия преглед е да се събере информация относно анализа на кверцетин, съдържанието му като основен представител на флавонолите и съотношението му към общите флавоноли, изразени като сума от мирицетин, кверцетин и кемпферол, както и към общите флавоноиди в български плодове и зеленчуци. В прегледа са включени проби от 17 плодове и 13 зеленчуци, взети в съгласие със съвременните изисквания за пробоподготовка с оглед оценка на състава им. Анализът на кверцетин и други флавоноли е извършен с високоефективна течна хроматография; тоталното съдържание на флавоноиди е определено чрез колориметричен метод с алуминиев хлорид. Определено е съдържанието на кверцетин в плодове и зеленчуци и съотношението му към общите флавоноли и общите флавоноиди. Показано е, че кверцетинът е основният представител на флавонолите в повечето от изследваните проби. Има едно интересно изключение при представителите на сем. *Cruciferae* – броколи и брюкселско зеле, където съотношението на кверцетин към общите флавоноиди е много високо – почти 50%. В плодовете, например в ягодите, кверцетинът е само 44% от общите флавоноли. От получените резултати следва, че е нужен комплексен подход при анализа на данните за съдържанието на флавоноиди. Допълването на данните за състава и съдържанието на флавоноиди с данни за техните съотношения ще даде възможност за по-коректна идентификация на биоразнообразието, за компенсация, макар и частична, на влиянието на биологичната вариация върху точността на анализа и ще обогати информацията, необходима за създаване на база данни за флавоноиди в храни.