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Flavonoids content in fresh and processed vegetable foods

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Flavonols and flavones are classes of flavonoids with antioxidant activity. Dietary flavonoids are assumed to have protective effect against different degenerative diseases; therefore, it is important to have reliable information about their content in various foods. Data for flavonoids composition in plant foods are compiled in several databases, however, data for specific processed traditional foods, which are substantial part of our diet, are missing. The aim of the study is to present new analytical data for flavonols and flavones content of 6 and 10 vegetable food varieties, widely consumed in Bulgaria. Flavonols – quercetin and kaempferol and flavones – luteolin and apigenin were determined by HPLC analysis with UV detection after acid hydrolysis with 1.2M hydrochloric acid. The results show that in lutenitsa (traditional vegetable spread) the level of quercetin is 1.42 mg/100g and of luteolin is 2.65 mg/100 g, very close to their level in the row ingredients. We can conclude that flavonoids are stable after food technological processing and in contrast to other antioxidants they do not degrade in canned foods.

Keywords: Flavonols, Flavones, HPLC, Processed foods, Food composition

INTRODUCTION

Flavonoids are polyphenolic antioxidants, ubiquous in plant foods. In the 1990s, flavonoids triumphantly returned to the scientific literature on nutrition due to their potent antioxidant activity [1, 2]. Dietary flavonoids have protective effect against the development of different degenerative diseases like cardiovascular, cancer, diabetes mellitus, and Alzheimer diseases [3-7]. Therefore, it is important to have reliable information about their content in various foods. Food composition information is the core of all nutritional programs. The development of nutrition science poses increasing requirements on the quality of food composition data to meet the needs in establishing the link food-nutrients-dietary intake-health. Knowledge of the flavonoid content of foods will allow a more precise and comprehensive evaluation of their quality, for elaboration of databases necessary for establishing relevant food policy and data exchange on national and international level.

Data for flavonoids content in plant foods are compiled in several international databases like USDA Database for the Flavonoid Content of Selected Food and Phenol-Explorer [8-10] but data for processed foods is scattered, moreover, for some specific traditional foods from different parts of the world such data are missing. The technological processing of food can affect the content of flavonoids, thereby reflecting on dietary intake assessment. According to the National Statistical Institute, Bulgarians consume yearly about 20 kg of tomatoes, 8 kg of peppers and 10 kg of vegetable canned food *per capita*, suggesting that processed vegetable foods also are a considerable dietary source of flavonoids [10]. Taking into consideration the rich biological activity of flavonoids, many epidemiological studies have shown their association with suppression of a large range of pathophysiological processes in the human body. The information in Bulgarian food composition database is constantly updated and a need for new original quantitative data for flavonoids is demanded.

The aim of the study is to present new analytical data for the content of flavonoids from the group of flavonols and flavones of fresh and processed vegetable food varieties, typical for Bulgarian diet and widely consumed in Bulgaria.

EXPERIMENTAL

Food sampling plan

The need for representative data places the development of a sampling plan as a key and starting point in the food analysis process, which has its specificity, according to the purpose of the study. In this work, following the current scientific approaches in the field [12, 13] a detailed sampling plan was developed.

Each individual sample of fresh vegetables is a composite sample of three single samples, which were purchased from three different items on the same day. The quantity of single samples was not less than 1 kg for fresh vegetables. For each single sample, a sampling protocol was filled up, reflecting the origin and identification of the samples.

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The samples were transported to the laboratory where they were mixed into combined samples – 3 kg. After removing the non-edible food parts, an average sample of 1 kg was prepared by random selection of vegetables, followed by freeze-drying. The freeze-dried sample was stored at -18^{0}C. Before analysis the average sample was finely ground and homogenized and analytical sample of 0.500 – 1.500 g was taken for determination of flavonoids. For processed foods three samples from three different producers were analyzed. Each composite sample consisted of three single samples of the respective product (packages, jars, cans) with different production dates, which were stored at 4^{0}C or -18^{0}C (for frozen foods). Before analysis an average sample was prepared and well homogenized using a blender. For flavonoids analysis an analytical sample of 10.00 g was taken.

Fresh vegetable samples

In the present study 7 vegetable food items from 3 botanical plant families were included. The samples were selected according to their wide consumption in Bulgaria. From *Solanaceae* family tomatoes and peppers were included; from *Papilonaceae* family - green beans and from *Umbiliferae* family – carrots. Samples were collected randomly from open markets or supermarkets in Sofia during a 2-year period. In Bulgaria the vegetable production is season-determined and correspondingly they were collected during their ripening stage and presence in the market. Carrots are present throughout the year and were purchased in May, August and December. Tomatoes were purchased in August and September (field production) and in May (greenhouse production). The general requirement for plant species that differ in color to be present separately in the sample is reflected [12]. For example, pepper was purchased from two different types: "Kapia" variety within their green and red rape stage; two types of green beans: yellow and green variety.

Processed food samples

In the present study we have analyzed 6 processed vegetable products produced in Bulgaria, in which, according to preliminary data, we expected to find flavonols and flavones, and which are widely consumed in Bulgaria. We have selected the following processed vegetable products: tomato puree, canned tomatoes, ketchup, lutenitsa, green beans frozen and green beans canned. Lutenitsa is a typical for Bulgaria product, which is a vegetable spread produced from tomatoes, red peppers, carrots and condiments.

Analytical method for determination of flavonols and flavones

Extraction and hydrolysis. The analytical sample was weighed in a 200 ml Erlenmayer flask with stopper. 0.500 – 1.500 g of analytical freeze-dried sample (10 g for processed foods) was taken and then 25.0 ml of TBHQ, 19 ml of water and 6 ml of HCl were added. Internal standard morine was used so that the final concentration in the sample to be 2.5 μg/ml. In this study extraction and hydrolysis of flavonoids was performed at 1.2 M HCl in 50% MeOH in a water bath at 90^{0}C refluxing for 2 h. At the end of the hydrolysis, the sample was left to cool for about 5 min and then, to ensure stability of aglycones, one ml of ascorbic acid solution (1 mg/ml) was added. The sample was transferred to a 100 ml volumetric flask and the volume was brought to the mark with MeOH. The sample was placed in an ultrasonic bath for 3 min and then adjusted to the mark if necessary. The extract was homogenized and an aliquot of 2 ml was ultracentrifuged for 5 min at 14000 rpm. The supernatant was filtered through a membrane filter (HV-Millipore) with a pore size of 0.45μm and then 50 μl were injected into the liquid chromatograph.

HPLC analysis. The chromatographic separation of flavonols – quercetin, kaempferol and myricetin and flavones – luteolin and apigenin was performed on Alltima (100 × 4.6 mm i.d., 3 μm) C18 column attached to a pre-column Alltima (4 × 4.6 mm i.d., 3 μm) C18. Isocratic elution with 53% MeOH in 2% acetic acid with a flow rate of 0.8 ml/min was used, resulting in operational pressure of 18.0-18.5 MPa. Ultraviolet detection at a fixed wavelength = 365 nm was used to determine flavonols and flavones. For quantitative determination the method of internal standard was used. The results were expressed in mg/100 g f.w. (fresh weight). Applying the present analytical methodology, the following parameters of the method were obtained: limit of detection – 0.03 mg/100 g f.w; limit of determination – 0.09 mg/100 g f.w.

Statistical analysis

A Student's t-test was applied to compare two arithmetic mean values. All statistical values were calculated using SPSS 11.0 for Windows.

RESULTS AND DISCUSSION

The results for flavonols and flavones content of the analyzed fresh and processed vegetable foods are presented in Table 1.

Table 1. Flavonoids in selected fresh and processed vegetable foods

Food Products	n	Flavonoids	Mean value	SD	Min	Max
			mg/100 g f.w.			
Tomatoes	7	Quercetin	1.42	0.55	0.78	2.59
	7	Kaempferol	0.19	0.44	0.07	0.44
Tomatoes, greenhouse	3	Quercetin	0.51	0.12	0.43	0.65
Tomato puree	3	Quercetin	4.10	2.16	1.67	5.64
	3	Kaempferol	0.08	0.13	0.06	0.15
Tomato, canned	3	Quercetin	0.32	0.26	0.24	0.63
Tomato, ketchup	3	Quercetin	0.45	0.09	1.67	5.64
	3	Luteolin	2.48	1.15	1.53	3.76
Pepper, red	5	Quercetin	1.49	0.91	0.71	3.03
	5	Luteolin	0.79	0.36	0.23	1.38
Pepper, green	5	Quercetin	10.27	5.92	4.92	19.49
	5	Luteolin	2.79	1.66	1.19	5.42
Carrots	5	Luteolin	0.88	0.47	0.31	1.43
Lutenitsa	3	Quercetin	1.42	0.43	0.93	1.70
	3	Luteolin	2.65	1.42	1.75	4.29
Green beans, green	5	Quercetin	2.13	0.83	1.11	3.05
Green beans, yellow	5	Quercetin	2.29	0.60	1.96	3.05
Green beans, frozen	3	Quercetin	1.95	0.38	1.56	2.32
Green beans, canned	3	Quercetin	0.84	0.16	0.66	0.96

n – number of samples; f.w. – fresh weight

The results are expressed in mg/100 g fresh weight or mg/100 g processed food. Data below limit of detection are not included in the table. Tomatoes and peppers are the most widely consumed vegetables in our country and are the basic ingredient of various canned vegetable product that are present at the table during the winter. The results show that the mean value of quercetin in tomatoes is 1.42 ± 0.55 mg/100 g (n=7). This value is statistically significantly higher than the results of Hertog *et al.*, 1992 (0.80 ± 0.31 mg/100 g, n=4), of Crozier *et al.*, 1997 (0.59 ± 0.31 mg/100 g, n=8) and Stewart *et al.*, 2000, (0.67 ± 0.44 mg/100 g, n=13); that in USDA Database for the flavonoids content of selected foods 3.2 (2015) is a combined value for row red ripe tomatoes: 0.58 ± 0.98 mg/100 g, n=96 and is comparable to the data for quercetin in tomatoes presented by Justesen *et al.*, 1998 (1.40 ± 0.8 mg/100 g, n=5) [13-15, 8, 16]. Since flavonoids biosynthesis is a light-dependent process, it is suggested that tomatoes produced in direct sunlight have higher flavonol content than tomatoes grown in greenhouses. The influence of cultivation conditions was examined by Stewart *et al.*, 2000, according to which the flavonols content of tomatoes produced in Spain and South Africa is 4-5 times higher than those grown in the UK, where their production is almost entirely concentrated in greenhouses [15]. Our results confirm the cited relation. The content of quercetin in the early greenhouse tomatoes we examined (n=3) purchased in May is 0.51 mg/100 g, and kaempferol was not detected. It should be noted that Bulgarian tomatoes have very high values for quercetin and kaempferol, in some cases close to data for Cherry tomato presented in USDA database [8]. It seems that the cultivated tomato Bulgarian varieties in combination of microclimatic conditions in our country are favorable for accumulation of high amounts of flavonoids.

In Table 1 the results for the content of quercetin in pepper of two different types - “Kapia” green and red are presented. Kapia peppers in their technological maturity are dark green in color, and in their botanical maturity are red. In our country the most widely consumed peppers are Kapia, both in their red and green color. The results for Kapia sweet pepper show that in red pepper the mean value for quercetin is 1.49 mg/100 g, while the green peppers Kapia are extremely rich in quercetin – 10.27 mg/100 g. This fact can be explained by the general tendency for flavonoids to be synthesized in large quantities in the early stage of development and their quantity decreases during ripening with different rate for different plant species. The results for the flavone luteolin show higher amount in green Kapia variety. We could compare our data for red and green pepper with USDA data, where quercetin in red sweet pepper has a mean value of 0.23 mg/100 g (n=7) as in both cases the USDA data [8] were statistically significantly lower than our results. As the consumption of green pepper Kapia is significant in Bulgaria, we can assume that it is one of the most important sources of flavonoids in the Bulgarian diet.

Carrots contain no flavonols. In our study, we detected luteolin in all carrot samples, ranging from 0.31 to 1.43 mg/100 g with a mean value of 0.88 mg/100 g (n=5). In the USDA study, luteolin in carrots has a mean value of 0.11 mg/100 g (n=7).

The study includes some processed vegetable foods that take part in different national dishes or are widely consumed as ready-to-eat foods in various combinations, such as tomato puree, ketchup and lutenitsa. Tomato puree is a rich source of quercetin with a mean value of 4.10 mg/100 g (n=3). Our results are similar to data from USDA database, where high levels of quercetin in tomato puree were found (4.12 mg/100 g, n=9) [8]. This can be explained by the effect of concentrating the starting material (fresh tomatoes) during the production of tomato paste and the stability of flavonols during heat treatment.

Three ketchup samples prepared from tomato paste, modified starch and various spices were analyzed. In consistence with the lower amount of tomatoes in this product, the results for quarcetin content in different types of ketchup are not high (0.45 mg/100 g) and vary within a narrow range (SD = 0.09 mg/100 g). These results are higher than the data presented in Phenol-Explorer [9, 17], obtained from compilation of 26 samples from 2 literature sources (4.23×10^{-3} mg/100 g quercetin).

The amount of flavonols in three different samples of lutenitsa was determined, which, according to product label is made from a mixture of pepper, tomato puree, carrots, modified starch and various condiments. For the first time original analytical data for lutenitsa (national typical vegetable spread) are presented, showing that quercetin content is 1.42 mg/100 g and luteolin content is 2.65 mg/100 g. Taking into consideration the traditionally high consumption of lutenitsa in the Bulgarian diet, it is an important source of qurecetin, especially during the winter season. Luteolin is relatively high in lutenitsa and ketchup – 2.65 mg/100 g and 2.48 mg/100 g respectively, which might be due to the presence of carrots or different spices.

Leguminous crop and especially green beans, have been extensively studied for the content of myricetin, quercetin and kaempferol by many research groups in the field of food science, as they are staple food in many regions of the world. This study presents data on the amount of quercetin in two types of green beans – flat green peppers pods and flat yellow peppers pods. The results show that myricetin, kaempferol and flavones are not detected in the samples. The level of quercetin in green beans is not affected by the color of peppers (2.13 mg in green beans and 2.29 in yellow beans / 100 g). the mean values for quercetin in green beans published in the USDA database are close to our results – 2.73 mg and 3.03 mg/100 g for green and yellow beans, respectively [8].

The three samples of frozen green beans consisted of green cylindrical pods. Two of the canned green beans were flat pods and one of them was cylindrical pod. Frozen green beans are characterized by a slight decrease in quercetin level (1.95 mg/100 g), whereas in classical preservation in cans the amount of quercetin is less (0.84 mg/100 g) due to the passage of flavonols into the aqueous environment of the finished food product. It is important to note that in beans, flavonols were found only in green beans but not in mature white beans (USDA) [8], which is confirmed by our study.

CONCLUSION

Flavonoids are present in the studied fresh vegetables and in their processed products, which is a proof of their stability at different production temperatures, assuming that the tested products retain the antioxidant activity of the fresh ones. A specific characteristic of the flavonoid composition in both target groups is the high content of quercetin. The present data can be used to fill the gaps in the Bulgarian food composition database and to be integrated into international networks for data exchange and scientific information. This study proves the need to preserve traditional foods in Bulgarian diet, due to the high amount of bioactive antioxidant compounds.

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