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The flavonoids composition of Bulgarian foods – comparison with USDA database

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Flavonoids are polyphenolic antioxidants of plant origin, comprising more than 5000 individual compounds. Their protective role to human health is associated with reduced risk of development of series of degenerative diseases. Therefore the quantitative knowledge of the flavonoids composition is of crucial importance for elaboration of healthy nutrition diets. The aim of the study was to estimate differences between flavonoids content of Bulgarian foods and the USDA Flavonoids Database. For achieving the set aim, the Bulgarian Flavonoids Food Composition Database have been translated and transferred in Excel format, according the unified Access scheme of the USDA Database. The fast track and comparison of the data was made with automotive program written on Visual Basic for Application (VBA). The original data for representatives of 3 major classes of flavonoids in Bulgarian foods are presented. The data for the flavonols – myricetin, quercetin and kaempferol, flavan-3-ols – (+)-catechin and (-)-epicatechin and flavones – luteolin and apigenin were determined by validated HPLC methods. The food samples were collected according to a precise sampling plan and the origin of all foods was documented. In the current study data for 15 fruits, 30 vegetables and vegetable products and 3 leafy green condiments are reported. Our data are compared with the pool of results, summarized in USDA Database for the Flavonoids Content of Foods, where data for 506 foods are presented, covering 308 scientific literature papers. The current study revealed a number of topics whose further study would advance the development of nutritional databases.

Keywords: Flavonoids, Flavonols, Flavan-3-ols, Flavons, Data base

INTRODUCTION

Food is a source of essential nutritional compounds and provides energy and a broad spectrum of biologically active compounds for living organisms. It plays a vital role for the human body throughout its living cycle, ensuring growth and development and sustaining its physical and mental activity. Therefore, the knowledge on food composition is one of the most important tasks of food and nutrition sciences.

Flavonoids are representatives of the large class of phenolic compounds, which are known as powerful antioxidants and the initial interest in those compounds was mainly due to their pigment properties in plants [1]. The interest in flavonoids began to raise in 1993, triggered by the publishing of the first epidemiological survey (‘‘Zutphen Elderly Study’’) that revealed a reverse relationship between the high intake of flavonoids (flavonols and flavones) and the risk of cardiovascular disease (CVD) [2]. This survey was supported by numerous studies outlining the protective role of flavonoids concerning CVD [3-5], cancer [6-8] and neurodegenerative diseases like Parkinson’s and Alzheimer’s diseases [9].

Flavonoids are the biggest and most abundant class of phenolic compounds found in the plant kingdom. Structurally they diverse and vary from single molecules to highly polymerized structures [1]. Flavonoids are important plant pigments and the study of their chemical structure and properties started half a century ago. The classification of the flavonoids depends on two major factors – the place of the second phenyl radical bond to the heterocyclic skeleton and the oxidation state of the same heterocyclic ring. Different classes of flavonoids are differing by the number and the position of substituents in the aromatic rings. The main functional groups in flavonoids` structure are the hydroxyl groups (OH-) that could be methylated or glycosylated.

Flavonoids could act either as chain-breaking or preventive antioxidants and exert their antioxidant activity by different mechanisms: scavenging species that initiate peroxidation, chelating metal ions, thus making them unable to generate reactive species, decompose lipid peroxides, quenching O_2^-, breaking the auto-oxidation chain reactions, and/or reducing localized oxygen concentrations [10, 11].

The flavonoids – scavengers of free radicals

It has long been known that flavonoids act like scavengers of free radicals [12-14] and their antiradical activity in water and lipid media has been subjected to a thorough research [12, 15]. It is deemed that flavonoids’ activity as inhibitors of chain radical peroxidation is performed in two

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stages [11]. Initially one of the hydroxyl groups of the molecule (usually the 4'-OH group), is dissociated homolytically or heterolytically, thus leaving a flavonoid radical (aroxyl radical).

Homolytic dissociation of OH-group:

Flavo-OH → Flavo-O$^{\cdot}$+$^{\cdot}$H

Heterolytic dissociation of OH-group:

Flavo-OH → Flavo-O$^{\cdot}$ + e^-+H^+

The resulting flavonoid radical is stabilized through resonance conjugation of the unpaired electron with the π-electron system of the benzene ring. The stability of the flavonoid radicals is very important for their antioxidant activity, since the generated radical must have high stability and low reactivity in order to prevent the initiation of new free radicals in the system. The resulting stable flavonoid radical intercepts the chain reaction, giving various molecular products as a result. This is the so-called stage of interruption of the chain radical peroxidation [11, 12].

R$^{\cdot}$ + Flavo-O$^{\cdot}$→ stable non-radical products

The antioxIdant activity of flavonoids depends on two main factors: (1) the ability of the hydroxyl groups to bind to the aromatic ring and to yield hydrogen atom, which then binds the free radicals and (2) the fact that the system can sustain an unpaired electron through delocalization of the π-electron system.

The flavonoids – inhibitors of singlet oxygen

The oxygen molecule is in triplet state ($^3O_2$) and has two unpaired electrons (one for each of the constituting oxygen atoms), which are with parallel spins [16]. Therefore, the oxygen molecule is a biradical. Nevertheless, the oxygen molecule has low reactivity in biological systems, containing predominantly molecules with dominating covalent bonds [16]. The oxygen molecule can be activated in biological systems through monovalent reduction, which yields in succession $O_2^{\cdot-}$, H_2O_2, $HO^{\cdot}$and$^1O_2$ [17]. The singlet oxygen ($^1O_2$) is formed by the triplet oxygen in some photosensibilization processes [18] and unlike the triplet one has greater oxidation potential and readily engages in reactions with biomolecules. Flavonoids can inactivate singlet oxygen through its physical transformation to the more stable triplet form or through direct interception of its molecule (scavenging effect) [10, 19].

The flavonoids - chelating agents of metal ions

It is well known that ions of transition metals (Fe^{2+}/Fe^{3+}, Cu^+/Cu^{2+}) catalyse the formation of free radicals, such as hydroxyl or hydroperoxyl radicals [20, 21]. Therefore, binding these ions in stable chelate complexes decreases their role in the initiation of free radical-forming processes. Thus, chelating agents are acting as preventive antioxidants [20]. Stable chelate complexes of flavonoids and transition metals are mostly formed at the catechol group.

MATERIALS AND METHODS

In the present study original data for representatives of 3 major classes of flavonoids in Bulgarian foods are presented - the data for the flavonols – myricetin, quercetin and kaempferol, the flavan-3-ols – (+)-catechin and (-)-epicatechin and flavones – luteolin and apigenin .

The food samples are collected according precise sampling plan and the origin of all foods has been documented. In the current study data for 15 Bulgarian fruits, 30 Bulgarian vegetables and vegetable products and 3 leafy green condiments are reported.

The following apparatus and analytical methods are used in the analysis of Bulgarian foodstuffs:

Apparatus

Hewlett Packard Liquid Chromatograph with HP pump 1050; thermostat: HP 1100; UV detector: HP 1050; fluorescent detector; injector: Rheodyne 750; ChemStation Software for data handling. The chromatographic analysis was performed by using Alltima (100 × 4.6 mm, i.d. 3μm) C18 analytical column, connected to pre-column Alltima (4×4.6 mm, i.d. 3μm) C18, Alltech Association Inc. Isocratic elution with 53% MeOH in 2% acetic acid, with a flow rate of 0.8mL/min was applied.

HPLC analysis of flavonols and flavones: myricetin, quercetin, kaempferol, luteolin and apigenein were measured as free aglucones after acid hydrolysis and HPLC determination with UV detection.

HPLC analysis of catechins: (+)-catechin and (-)-epicatechin were determined in freeze-dried samples by RP-HPLC and fluorescence detection at λ_{EX}=280 nm and λ_{EM}=315 nm.

The used foods composition tables and data are:

- Our data for flavonoid composition and quantity in Bulgarian fruit and vegetables [22, 23].
- USDA Database for the Flavonoid Content of Selected Foods – Nutrient Data Laboratory, USDA [24]. (S. Bhagwat, D. Haytowitz, J. Holden, 2014)
- Release 1 (March 2003) – Flavonoid content of 225 foods items.
- Release 3.1 (December 2013) The database contains values for 506 food items, covering 308 scientific literature papers and for 26

predominant dietary flavonoids that belong to the five subclasses reported below:

Flavonols: isorhamnetin, kaempferol, myricetin, quercetin;

Flavones: apigenin, luteolin;

Flavanones: eriodictyol, hesperetin, naringenin;

Flavan-3-ols: (+)-catechin, (+)-gallocatechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin 3-gallate, (-)-epigallocatechin 3-gallate, theaflavin, theaflavin 3-gallate, theaflavin 3'-gallate, theaflavin 3,3'-digallate, thearubigins;

Anthocyanidins: cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin

On figure 1 the database comparison algorithm is presented. The usage of MS Office product for American Database /MSACCESS/, and Bulgarian Database/ MSEXECEL/, allows us to use Visual Basic for Application as a programming language to compare information in both bases. The main moment in the process is the compliance test. We have three levels of comparison:

First Level – Identification of the foodstuffs by names. On this level the names of the individual products in the bases are characterized and compared. The VBA function for the text comparison is used. Below is provided a program code:

```
Dim LResultAs Integer
LResult = StrComp ("BG DB Food/Fl Name", "USDA DB Food/FL Name")
```

We checked the result from comparison: if the LResult was equal to 0 then the first level test was passed and we shifted to second level; if the LResult was not equal to 0 the first level test did not pass and we finished with this Food/Fl and selected the next Food/Fl from USDA database.

Second Level – Identification by biological family, spices variety. The comparison is manual by using additional information from different scientific sources.

Third Level – Parameter and value check – The third level is crucial for the comparison of the two databases. We established a difference between BG Data and USDA Data in mg/100 g.

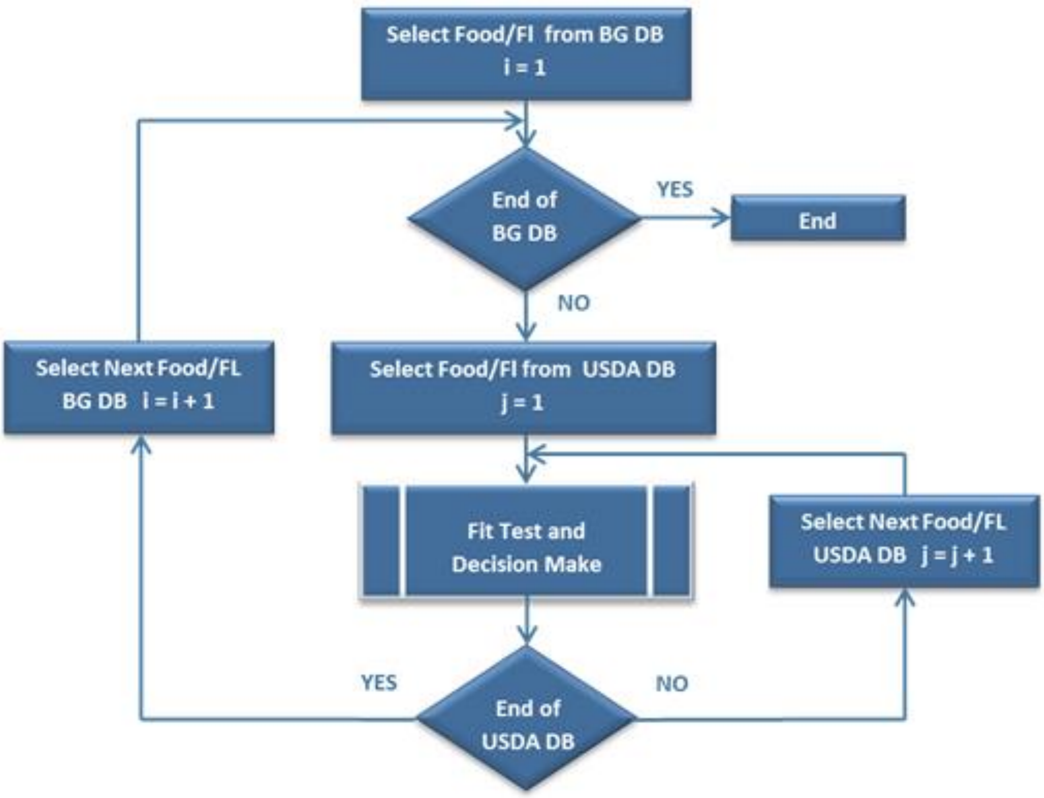

Figure 1. Database comparison scheme

RESULTS

The data of flavonoids composition in Bulgarian fruits are presented on Figure 2. The richest sources of flavonoids are blueberries. The myricetin, in significantly lower content was found only in blueberries. The comparison of our data and those, reported in USDA for the flavonoid content in fruits is presented on Figure 3.

Flavonoids in Bulgarian fruits

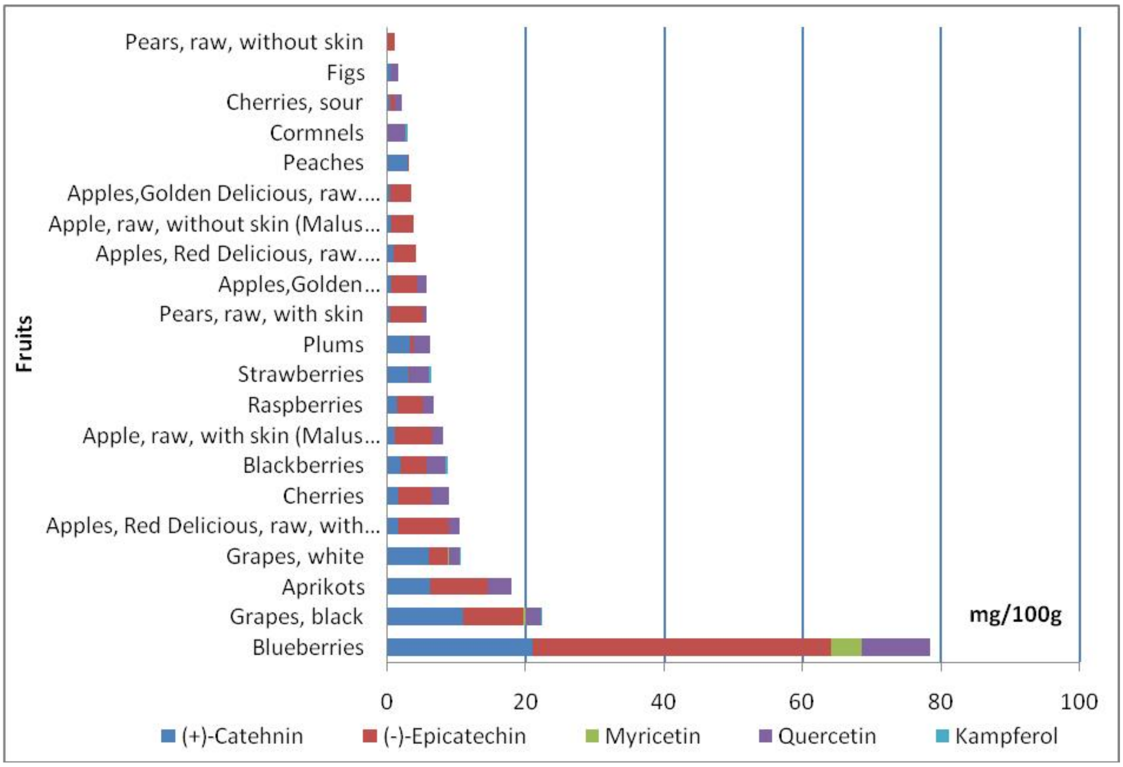

Figure 2. Flavonoids composition in Bulgarian fruits.

Flavonoids in Bulgarian vegetables

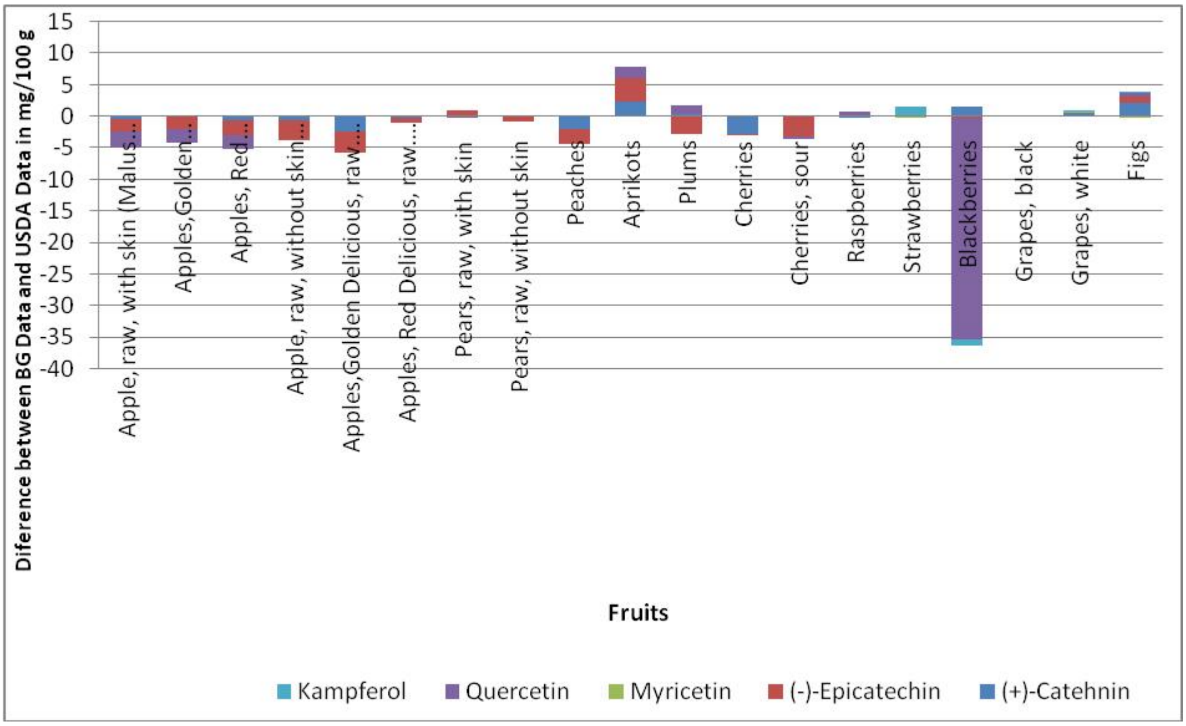

Figure 3. The comparison of Bulgarian and USDA data for the flavonoid content in fruits.

The results for flavonoids content in Bulgarian vegetables are shown in figure 4. We have to point out that the flavonol myricetin was not found in any of the vegetables analysed in our study, therefore data for myricetin were not included in figure 3. Very interesting results are the flavonoids data for parsley and dill. They show that parsley contains only one representative of flavonoids – apigenin, while dill is very rich in quercetin.

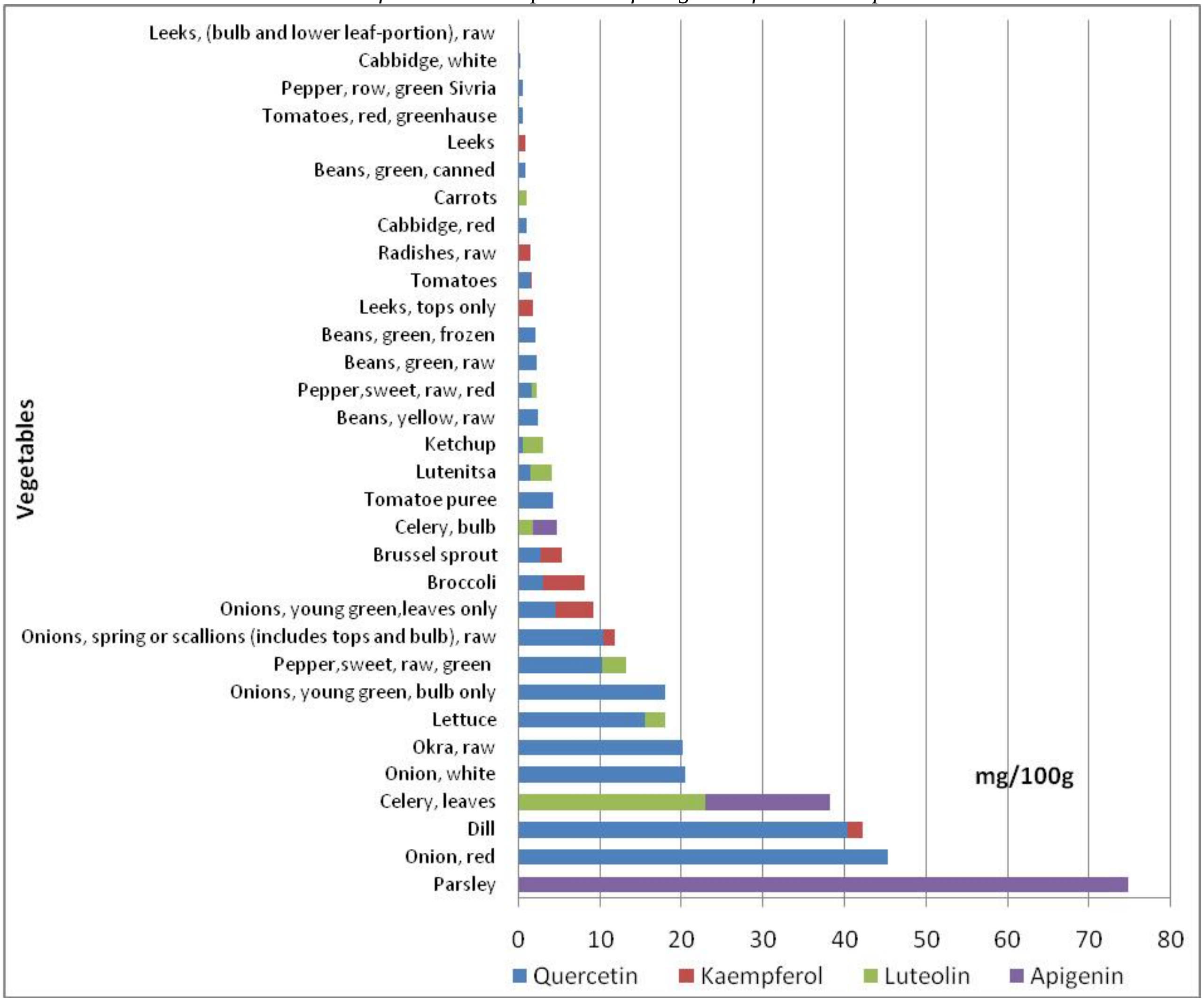

Figure 4. Flavonoids composition in Bulgarian vegetables..

The establishment of differences between our data for flavonoids in vegetables, and those in the USDA is presented in figure 5.

The determined differences between our data and those from USDA for flavonoid composition in fruits and vegetables can be due to various factors. In this aspect the biological variability of the plants products have the crucial role.

CONCLUSION

In the present study data of flavonoids content in Bulgarian fruits and vegetables, along with their comparison with the USDA Flavonoids Database are evaluated. Automation approach – VBA script for individual flavonoids' quantitative differences with three levels of data fit checks. The major differences between our data and the USDA ones were found for blackberries (- 35 mg/100 g) and parsley (-140 mg/100 g).

The current study revealed a number of topics whose further study would advance the development of nutritional databases. The validity of nutritional epidemiological studies depends on accurate food composition data. Nutritional database is an essential basic tool for virtually all quantitative nutritional research, dietary assessment, and development of food and nutritional policies. The nutritional data base programing should be an integral part of any national nutritional politic.

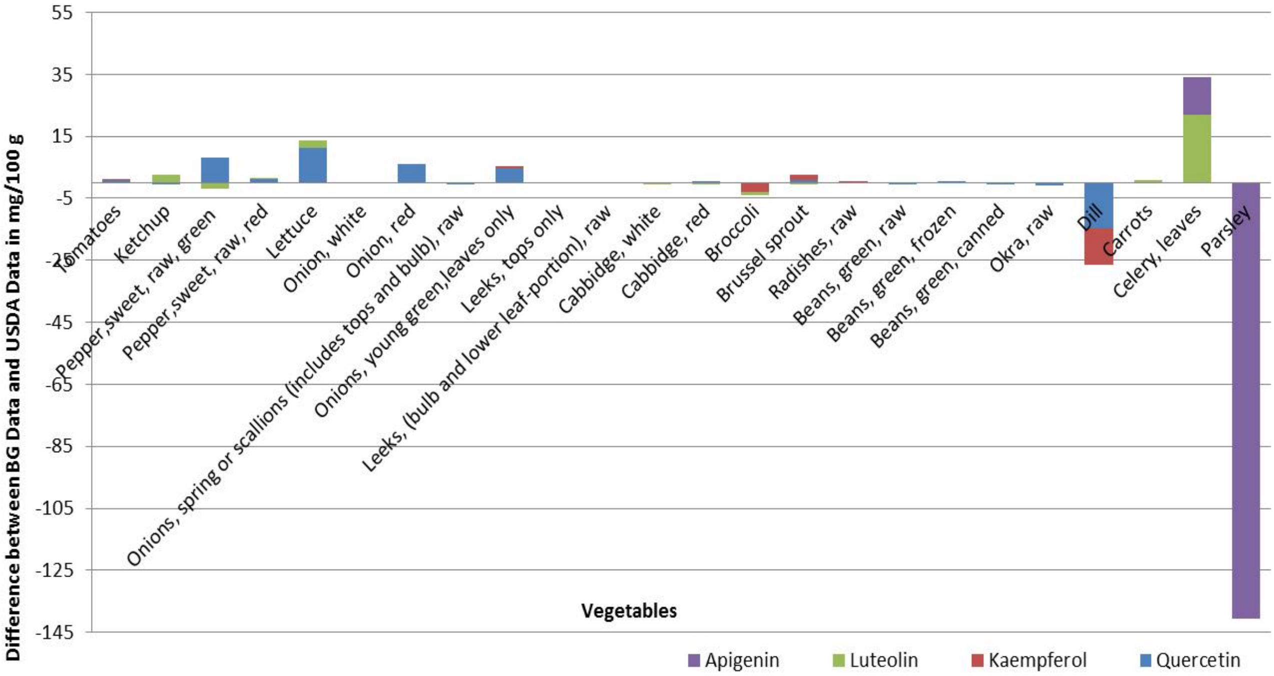

Figure 5. The comparison of Bulgarian and USDA data for the flavonoid content in vegetables

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ФЛАВОНОИДЕН СЪСТАВ НА БЪЛГАРСКИ ХРАНИ - СРАВНЕНИЕ С БАЗАТА ДАННИ НА САЩ

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(Резюме)

Флавоноидите са полифенолни съединения с антиоксидантна активност от растителен произход, включващи повече от 5000 индивидуални съединения. Защитната им роля за човешкото здраве се изразява в намаляване на риска от развитие на редица дегенеративни заболявания. Количественото познаване на състава на флавоноидите в различните растителни храни е от решаващо значение за разработването на диети за здравословно хранене. Целта на изследването е да се оценят различията между съдържанието на флавоноиди в български храни и съдържанието на флавоноиди, предоставено в американската база данни. За постигането на поставената цел, българската база данни за флавоноиди в храни е преведена и прехвърлена в Excel формат, съгласно унифицираната схема за достъп до USDA. Сравнението на данните е автоматизирано, като се използва програма написана на Visual Basic for Application (VBA). Предоставени са оригиналните данни за представители на 3 основни класа флавоноиди в български храни. Данните за флавонолите - мирицетин, кверцетин и камферол, флаван-3-ол (+)-катехин и (-)-епикатехин и флавони - лутеолин и апигенин са определени чрез валидирани HPLC методи. Пробите са набирани съгласно прецизен план за вземане на проби и техният произход е документиран. В настоящото проучване са включени данни за 15 плода, 30 зеленчуци и зеленчукови продукти и 3 зелени подправки. Данните ни са сравнени с тези, предоставени в базата данни на USDA за съдържанието на флавоноиди в 506 храни, обхващащи 308 научни статии. Настоящото проучване разкри редица въпроси, чието по-нататъшно изучаване би подпомогнало изграждането на хранителните бази данни.