

## Research Article

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# Dietary intake of specific phenolic compounds and their effect on the antioxidant activity of daily food rations

**Abstract:** The determination of phenolic acids' and flavonoids' content in daily food rations (DFRs) of a selected group of male and female students and the development of chromatographic conditions is the primary goal of the study. The presence of 7 phenolic components were confirmed in the prepared extracts from all diets reconstructed within a period of 3 years. The highest concentrations were determined for hesperidin (124 and 55.6 mg for women and men, respectively), naringin (47.6 mg in female and 37 mg in male diets) and chlorogenic acid (19.7 and 19.8 mg for women and men). The antioxidant potential of the daily food rations, measured with a DPPH test, was higher for women (range 47.1–78.8%) than for men (range 34.5–78.0%) and was found to strongly correlate with the total phenolics content of the samples (Folin-Ciocalteu test) (correlation coefficient 0.90).

**Keywords:** Nutrition, Free Radical Scavengers, HPLC, Folin-Ciocalteu assay, DPPH

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## 1 Introduction

The vast majority of nutritional studies are concerned with the intake of building, energetic or regulatory components, such as macro-, microelements and vitamins. However, new light has been shed on the intake of polyphenols - substances present in plant originated

foods [1]. Phenols constitute one of the major groups of nonessential dietary components and have been classified as antinutritive compounds. The recent increased interest in these compounds has been because of their bioactivity. Their numerous pharmacological properties have been described as anti-allergic, antitumor and antioxidant properties. Therefore, it is believed that an increased intake of these compounds may result in the prevention of diseases, where free radicals play a direct or indirect role. Various studies have identified the positive role of that polyphenols play in the prevention and even treatment of cardiovascular and ophthalmological malfunctions, as well as in the therapy of AIDS [2-5]. One of the largest groups of exogenous antioxidants are polyphenols, among which flavonoids are perceived as the most structurally differential compounds [1,6]. Moreover, a number of meta-analyses have shown that an increased intake of flavonoids may reduce the mortality rate from cardiovascular diseases [7]. Bibliographic data show a clear connection of phenolic compounds on the antioxidant status of specific food groups. A positive correlation between these two values was demonstrated in studies on teas, honeys, juices or fruits [8-10]. Moreover, it is proposed that the antioxidant status of food rations can be determined based on the free radical scavenging activity of a specific food products. However, a bibliographic review reveals a lack of direct analytical models available to assess the phenolics content and antioxidant capacity of food products, as well as their correlation with daily food rations. Numerous scientific papers estimate a daily dietary intake of specific flavonoids among the European population based on the US database of flavonoid content in selected foods. Such calculations are greatly simplified and highly biased [11,12]. In light of these, the development and optimization of analytical procedures suitable for the recognition of phenolics in the daily food rations remains essential to estimate the daily human intake of food flavonoids.

The objective of this study is to develop and validate chromatographic conditions to separate and identify phenolic acids and flavonoids in reconstructed diets.

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The determination of the daily intake of specific phenolic compounds among young adult population in Poland was performed. Additionally, the authors' intention was to monitor the dietary habits of students coming from different backgrounds and studying at different universities: medical, agricultural and humanistic colleges, to observe if habitual differences in their interests influenced the selection of food products and scientific profiles could change antioxidant capacity of their daily diets.

The dietary habits of selected groups of students could be expanded so as their nutrition might be treated as representative of the East-European type studying population.

Based on a thorough quantitative and qualitative HPLC analysis of the phenolics in the obtained extracts, several observations based on the antioxidant potential and possible sources of phenolics in their diets. The results obtained were compared with the values observed in other European countries over different years. Finally, the assessment of antioxidant potential measured in a DPPH test, and of total polyphenols content (TP) obtained in a Folin-Ciocalteu assay were performed to quantitatively determine the influence of phenolics intake on the free radical scavenging activity of the human diet.

## 2 Experimental

### 2.1 Investigated material

This study was performed from 2008 to 2010 and involved 648 randomly chosen students from three Lublin universities – Medical University, University of Life Sciences and Catholic University. The population was chosen to be a representative sample of the young adult population from the eastern part of European Union. The questioned population was different in each year of the survey. There were 487 questionnaires completed by women and 161 by men. All students were volunteers; and their lifestyles were characterized by moderate physical activity. Average age of the women was  $22 \pm 3$  and of the men  $24 \pm 4$ . Pregnant and lactating mothers were excluded from the study. The investigations were carried out using a 24-hour dietary recall technique. Dietary questionnaires were collected from January to March and the average diet duplicates were prepared in April each year. Respondents were asked to complete a form to record what they ate for the three main meals (breakfast, dinner and supper) and all other food consumed during a particular day. On the basis of this information, the qualitative and quantitative

composition of diets were determined by the students, the average diets for both, women and men, were reconstructed (3 universities  $\times$  2 genders  $\times$  3 years = 18 average daily food rations), using Dietetyk 2006 software. The composition and masses of average female and male diets are presented in Table 1.

Using Dietetyk 2006 software, the average quantities of all products consumed by particular groups were calculated to compose an average diet. Each average daily food ration was reconstructed five times, therefore 30 average diets per each year were obtained (6 diets  $\times$  5 reconstructions = 30 diets per year; in total 30 diets  $\times$  3 years = 90 diets). All products used to prepare food rations came from a retail market within the Lublin area. The daily rations included the plate portions for the main meals, beverages and all other foodstuff consumed daily, which were identical to the ones consumed by individuals. Diets' duplicates were prepared in accordance with the generally accepted culinary techniques. Each average diet duplicate was homogenized and subsequently extracted.

### 2.2 Extraction procedure

Three 75 g portions from each average daily diet were placed in a 300 mL conical flask and suspended in 75 mL of methanol. The diet portion was extracted three times

**Table 1:** Average daily intake of food by food group in the present study (3 years study period).

Food intake (g/day)		
Food group	Women	Men
1. Milk and dairy	174 $\pm$ 35.8	294 $\pm$ 88.6
2. Eggs	23.3 $\pm$ 10.4	59.0 $\pm$ 19.2
3. Meat and meat products	122 $\pm$ 17.6	213 $\pm$ 66.6
4. Fish	24.1 $\pm$ 14.5	43.2 $\pm$ 31.1
5. Cereals	247 $\pm$ 32.7	343 $\pm$ 72.5
6. Vegetables	209 $\pm$ 33.4	219 $\pm$ 40.1
7. Fruits	205 $\pm$ 73.3	164 $\pm$ 57.0
8. Potatoes	99.0 $\pm$ 27.3	163 $\pm$ 47.9
9. Fats and oils	17.9 $\pm$ 5.35	23.5 $\pm$ 3.61
10. Sugar and sweets	36.6 $\pm$ 7.45	38.8 $\pm$ 8.05
11. Water and beverages	697 $\pm$ 133	787 $\pm$ 228
12. Other foods	6.60 $\pm$ 4.04	9.90 $\pm$ 3.30
<b>Total</b>	<b>1862 <math>\pm</math> 152</b>	<b>2357 <math>\pm</math> 477</b>

over 48 h with an addition of fresh solvent, by shaking maceration. The extracts were filtered out, combined, and evaporated under reduced pressure at a temperature not exceeding 40°C using a rotary evaporator. The dry residue was redissolved in 100 mL of methanol-water (1:1 v/v).

## 2.3 Determinations

**Total polyphenols:** The analysis was carried out employing the modified Folin-Ciocalteu methodology, as described previously [13]. For this purpose, 0.5 mL of the extracted solution was mixed with 30 mL of distilled water and 2.5 mL of Folin-Ciocalteu reagent. After 1 minute (but not longer than 8 minutes) 7.5 mL of 20% Na<sub>2</sub>CO<sub>3</sub> solution was added and the mixture was topped up with distilled water to a total volume of 50 mL. After two hours the absorbance was measured at a wavelength of  $\lambda = 760$  nm in 1-cm cuvettes on a UV-Vis spectrophotometer Thermo Fisher Scientific Evolution (Waltham, USA) and compared to a blank that had been prepared in a similar manner, but replacing the extract with distilled water. A calibration curve was created with an aqueous solution of gallic acid (50–500 mg mL<sup>-1</sup>). For this purpose, instead of the analyte, 0.5 mL of aqueous solutions of gallic acid was added at concentrations of 50, 100, 200, 300 and 500 mg mL<sup>-1</sup>. After plotting the calibration curve, total polyphenols content in the studied samples was read as mg gallic acid equivalents (GAE). Results were expressed as GAE per 1 g of total fresh daily diet.

**Free radical scavenging activity:** A previously described protocol [14] was used after a few modifications. 0.2 mL of methanolic extract from each diet was mixed with 3.8 mL 6 × 10<sup>-5</sup> M methanolic solution of 2,2-diphenylpicrylhydrazyl (DPPH) stabilized radical. The absorbance at 515 nm was read at  $t = 0$  (AC<sub>0</sub>) and in 5-minute periods until the reaction reached a *plateau* value (AC<sub>t</sub>). A control sample was prepared

by replacing the addition of extract with methanol. The obtained results were expressed as a percentage of inhibition using the following equation: inhibition [%] = [(AC<sub>0</sub> - AC<sub>t</sub>)/AC<sub>0</sub>] × 100.

**HPLC analysis of obtained extracts:** Phenolic profile assessments of the investigated daily food rations were performed by high pressure liquid chromatography on Agilent Technologies 1100 apparatus with an autosampler and DAD detector. The separation procedure was conducted on a Sigma – Aldrich, Supelco, 5µm, 25 cm x 4.6 mm column by Sigma Aldrich (St. Louis, MO, U.S.A). A gradient elution profile was used. The mobile phase applied two solvents: acetonitrile [B] and 2% aqueous solution of acetic acid [A]. The starting gradient at 0 min was set at 1% B in A, then at 40 min the concentration of B in A increased to 20%, at 60 min the content of B was 40% and later dropped to 1% in the 70 min. The flow rate was set at 1 mL min<sup>-1</sup>, temperature at 25°C, injection volume at 20 µL, and the postrun time at 4 min. The spectra were registered at three wavelengths:  $\lambda = 260$ nm,  $\lambda = 280$ nm,  $\lambda = 365$ nm.

A qualitative composition of the extracts were performed by comparing the retention times and UV spectra with standards of phenolics from the library of spectra performed previously where the quantitative analysis was performed by comparing peak areas with specific standards used in appropriate and known concentration. In this study 10 standards (quercetin, kaempferol, apigenin, kaempferol 3-glucoside, apigenin 7-glucoside, hesperidin, naringin, chlorogenic acid, ferulic acid and caffeic acid) were used at concentrations of 1 mg mL<sup>-1</sup>. All standards were produced by Sigma-Aldrich and were obtained in chromatographic purity (> 98%).

A quantitative analysis was conducted by comparing the peak areas of standard solutions with account samples' and diets' weights. The method was validated and the LOD, LOQ and linear range values were presented in Table 2 (only for compounds which were present in diets). All measurements were made in triplicate.

**Table 2:** LOD and LOQ parameters of the phenolics analysis in the daily food rations.

Phenolics	LOD [µg mL <sup>-1</sup> ]	LOQ [µg mL <sup>-1</sup> ]	R <sup>2</sup>	Linear range [µg mL <sup>-1</sup> ]
Gallic acid	0.11	0.33	0.9993	0.2–1.2
Chlorogenic acid	1.82	5.46	0.9991	4–12
Caffeic acid	0.34	1.02	0.9997	0.8–5.0
Ferulic acid	0.17	0.51	0.9990	0.4–4.0
Quercetin	0.08	0.24	0.9990	0.2–1.0
Naringin	0.08	0.24	0.9994	0.16–1.0
Hesperidin	0.21	0.63	0.9992	0.5–3.0

## 3 Results and Discussion

### 3.1 Total polyphenols (TP) intake and antioxidant potential of daily food rations

The average wet weight of the food consumed by a group of women was calculated as 1862 g and 2357 g for men. This marked variation in food weight may be attributed to different quantities of beverages (teas, coffees or beers) consumed by the respondents. The antioxidant capacity and phenolic content were determined for each reconstructed average daily food ration. The total polyphenols content was calculated for all obtained diets in a Folin-Ciocalteu assay. Significant variations were observed between the investigated diets. Male diets were of higher weight than those of women. That is why, the results of the TP were calculated for 100 g of fresh food portion.

The average daily intake of polyphenols in the group of women was calculated for 656 mg (range 452–822 mg), whereas in the group of men was 714 mg (range 472–1090 mg). These results are presented in Table 3.

### 3.2 Statistical analysis

The analysis indicated that the antioxidant potential of the daily food rations was highly correlated with the concentration of phenolics (in 1 g of fresh portion) as shown by the Spearman's rank correlation coefficient, which was high for these values. The data is presented in tables 1S, 2S and 3S (supplementary material).

### 3.3 Optimisation of HPLC based separation conditions

The HPLC chromatography is a powerful tool for fast food rations fingerprinting [15,16]. In the study, the flow rate, injection volume and gradient profile was adjusted to provide improved separation. The addition of acetic acid to the solvent system resulted in a higher resolution of the chromatograms. Under the optimal conditions, more than 30 peaks were detected and well separated from one another within 70 minutes. The proposed method was found very sensitive with LOD values below  $1 \mu\text{g mL}^{-1}$  (except chlorogenic acid, see Table 2). In the performed study, phenolic compounds identified in the extracts from obtained diets were classified into two groups: flavonoids and phenolic acids. The identification of 7 phenolic compounds (gallic

**Table 3:** Daily intake of food polyphenols and antiradical activity of diets among women and men from three universities in 2008, 2009 and 2010.

Parameter	Women	Men
<b>2008</b>		
Polyphenols content in 1g of fresh diet [mg GAE]	0.34 ± 0.03	0.31 ± 0.02
Polyphenols content in the total diet [mg GAE]	667 ± 41.1	838 ± 115
Antiradical activity obtained in DPPH assay [% of inhibition]	55.6 ± 8.98	44.5 ± 6.84
<b>2009</b>		
Polyphenols content in 1g of fresh diet [mg GAE]	0.41 ± 0.03	0.36 ± 0.04
Polyphenols content in the total diet [mg GAE]	787 ± 38.4	794 ± 127
Antiradical activity obtained in DPPH assay [% of inhibition]	73.7 ± 2.84	68.5 ± 10.9
<b>2010</b>		
Polyphenols content in 1g of fresh diet [mg GAE]	0.29 ± 0.02	0.24 ± 0.02
Polyphenols content in the total diet [mg GAE]	513 ± 70.4	511 ± 66.7
Antiradical activity obtained in DPPH assay [% of inhibition]	63.6 ± 14.5	41.6 ± 6.16
<b>Mean value (3 years period)</b>		
Average polyphenols content in 1g of fresh diet [mg GAE]	0.35 ± 0.06	0.30 ± 0.07
Average polyphenols content in the total diet [mg GAE]	656 ± 127 (range 452–822)	714 ± 105 (range 472–1090)
Average antiradical activity obtained in DPPH assay [% of inhibition]	64.3 ± 11.6	51.5 ± 8.12

acid, chlorogenic acid, caffeic acid, ferulic acid, quercetin, naringin and hesperidin) were possible based on the literature data, retention times and UV spectra was similar to the standards. The identification of the constituents of three sample diets is presented in the Fig. 1.

### 3.4 Daily intake of selected phenolic compounds

The average intake of all of the identified phenolic compounds were calculated in the population of women and men and the results are presented in Table 4.

The analysis showed that the analytical results of total polyphenols intake were correlated with those obtained using the US database or with previous investigations

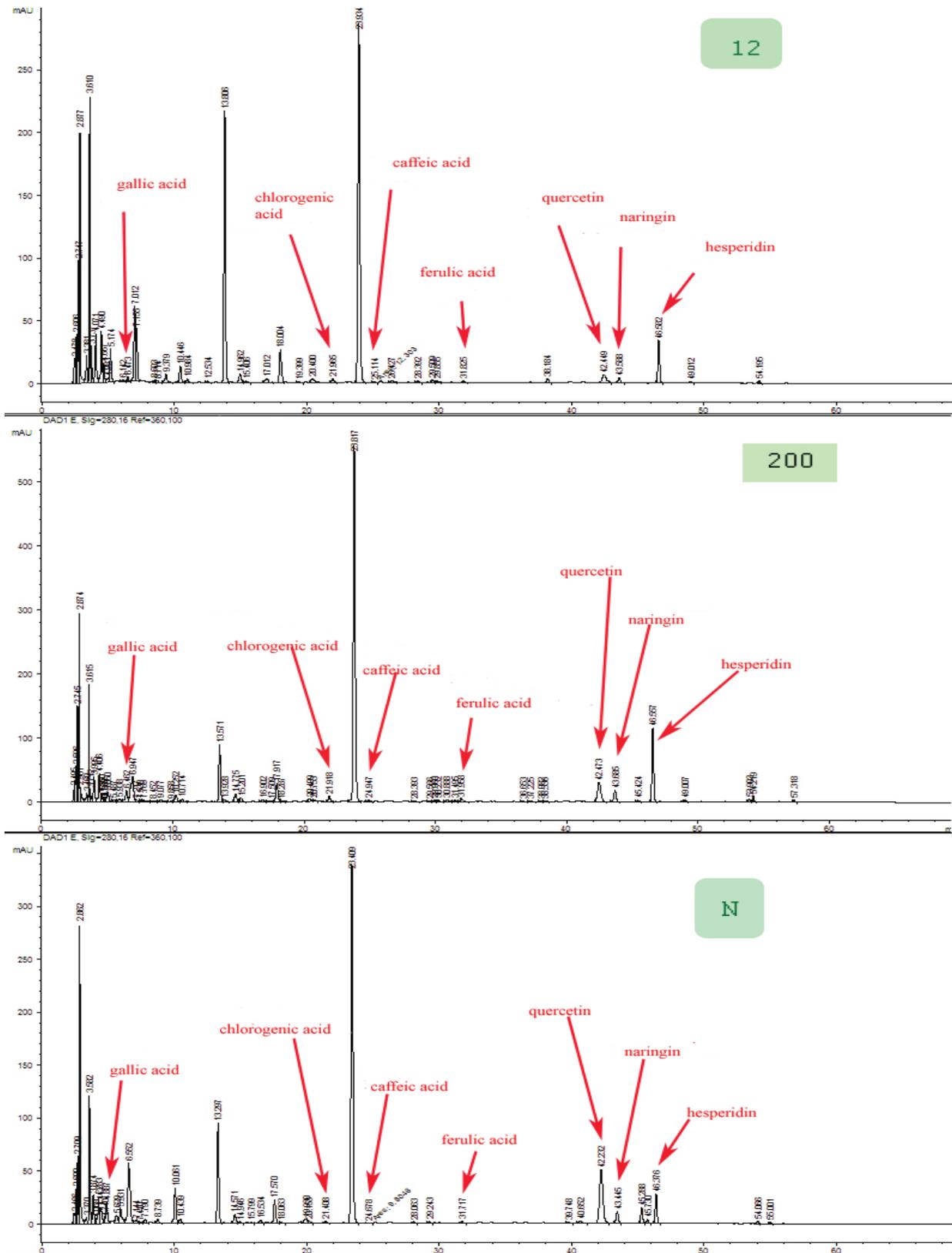


Figure 1: Representative chromatograms of extracts obtained from selected daily food rations (“12” – women from Medical University in 2008; “200” – women from Catholic University in 2009 and “N” – women from Catholic University in 2010).

[11,12,17]. However, it should be emphasized that the results presented in this paper were based on an analytical investigation of diet's replicates identically with those consumed by all participants, and not on calculations based on computer software. Therefore the results show the real dietary intake of particular phenolics compounds as well as total phenolic content in daily diet. The performed investigations revealed that female diets were characterised by higher antioxidant potential compared to daily food rations of men. Moreover, the concentration of phenolic compounds (in 1 g of fresh diet) was also higher in the group of women compared to men. However, because of higher male diet masses related to dishes and food products consumed in higher amounts, daily food rations of men contained more polyphenols in total. The authors assumed that the diets of students from Medical University will be characterized by higher antioxidant activity (DPPH test) related to higher intake of fruits, vegetables and teas based on the knowledge obtained in their education process. However, no differences between

particular universities were observed which could suggest that nutrition among young people is not correlated with their education.

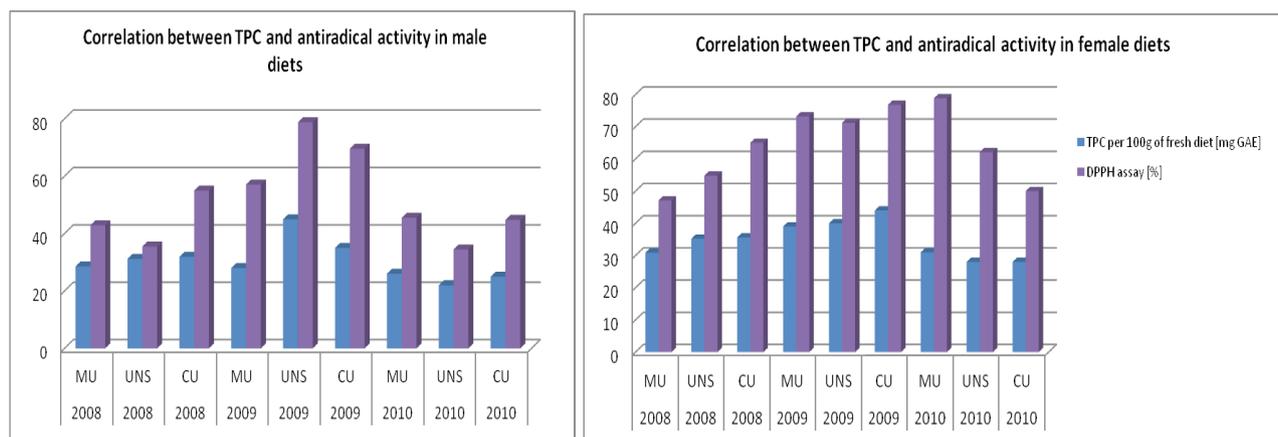
The analyses also show that the antioxidant status of daily food rations was highly correlated with the concentration (per 1 g of fresh portion) and not with the total amount of daily consumed phenolics. Statistical analyses confirmed this hypothesis as calculated Spearman's rank correlation coefficient between antioxidant potential of daily food rations and phenolic compounds concentration was significant and indicated high positive correlation between these two values (Fig. 2 and supplementary material).

According to Table 5 the intake of various phenolic compounds varied within a wide range and the differences may be as high as several hundred percent. This could be due to the marked instability of these substances and the influence of various factors on their content in foods. The method of cultivation, climatic conditions, time of harvest, storage conditions, culinary techniques or even the preparation of tea infusions, might have had a decisive influence on phenolic content and caused significant differences in the total intake of these group of compounds with food [18-20]. The present study sheds new light on the intake of selected phenolic compounds in comparison to other investigations made over the last 20 years in different countries (Table 5). In a study performed by De Vries et al. the daily intake of quercetin and kaempferol among various populations in many countries was assessed with a very wide range 1–81 mg person<sup>-1</sup> day<sup>-1</sup> was observed [21].

Other studies confirmed the results that one of the major flavonoids in the human diet is quercetin [17,20,29]. However, the present study revealed that naringin and hesperidin were also consumed in large quantities. This

**Table 4:** Average intake of phenolic compounds among women and men (3 years study period).

Substance	Women [mg/diet]	Men [mg/diet]
Gallic acid	9.48 ± 5.02	12.0 ± 10.3
Chlorogenic acid	19.7 ± 6.75	19.8 ± 10.6
Caffeic acid	6.53 ± 3.15	8.74 ± 4.82
Ferulic acid	5.89 ± 2.69	3.75 ± 1.33
Quercetin	32.6 ± 19.4	35.8 ± 29.8
Naringin	47.6 ± 33.5	37.0 ± 68.3
Hesperidin	124 ± 71.5	55.6 ± 38.9



**Figure 2:** Correlation of TPC (total polyphenols content) and DPPH free radical scavenging assay (% of inhibition) (MU – Medical University, UNS – University of Life Sciences; CU – Catholic University).

**Table 5:** Daily intake and major sources of food flavonoids in different countries.

Country	Daily intake [mg]	Diet's major sources	Bibliography
Denmark	26	tea, onion, apples	[22]
Finland	3–10	fruits and vegetables	[23]
	0–41	apples and onion	[24]
Greece	15	fruits and vegetables	[23]
Italy	23–34	red wine	[23]
	35	fruits and vegetable soups	[25]
Japan	60–68	green tea	[23]
	17	green tea	[26]
Netherlands	33	tea, onion, apples	[23]
	23	tea, onion, apples	[27]
United States	20	black tea and apples	[28]

may be connected with high consumption of citrus fruits which significantly increased in the European countries within last two decades. Furthermore, the high intake of phenolic acids – chlorogenic and gallic should be also noted. These substances together with ferulic and caffeic acids may have a significant role in the antioxidant potential of daily food rations.

In general, the daily food rations of women were characterized by higher amounts of hesperidin, naringin and ferulic acid while the intake of the other identified phenolics was higher in of men. The intake of almost all of the investigated phenolic compounds was comparable among both genders except from hesperidin, which was present in significantly higher quantities among women. Detailed studies performed by Hertog et al. in Netherlands estimated the intake of quercetin as 16 mg person<sup>-1</sup> day<sup>-1</sup> and of the other investigated flavonoids (kaempferol, miricetin, luteolin and apigenin) on a much lower level [15]. The present study did not reveal the presence of these flavonoids, however, the intake of quercetin found to be higher.

## 5 Conclusions

In conclusion, a significant correlation between polyphenol concentration and the antioxidant status of daily food rations of students has been proved. A separation of methanolic extracts obtained from averaged daily food

rations was investigated using a high performance liquid chromatography and revealed the presence of 7 phenolic compounds. The major phenolic compound in the average daily food rations, of both women and men, was hesperidin. Marked amounts of naringin and quercetin were found in the obtained extracts. Chlorogenic acid was found to be the major phenolic acid present in the extracts from students' diets. Even though the authors identified 7 phenolic compounds in the investigated diets, the proposed chromatographic conditions create a selective analytical platform capable to perform the analyses of complex matrices rich in polyphenols for future applications on different food samples.

Some more critical findings of this investigation may be summarized as follows:

1. The antioxidant potential of the daily food rations of women was significantly higher as compared to men's diets.
2. Total intake of polyphenolic compounds determined with Folin-Ciocalteu reagent ranged 452–1090 mg person<sup>-1</sup> day<sup>-1</sup> and confirmed the assumption presented in other investigations that consumption of the sum of phenolics is close to 1 g.

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## Abbreviations

DFR – Daily Food Rations, TP – Total Polyphenols, GAE – Gallic Acid Equivalents, Rt – Retention Time

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**Supplemental Material:** The online version of this article (DOI: 10.1515/chem-2015-0100) offers supplementary material.