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# The flavone, flavonol and flavan-3-ol content of the Greek traditional diet

Vardis Dilis, Effie Vasilopoulou, Antonia Trichopoulou *

*Department of Hygiene and Epidemiology, School of Medicine, National and Kapodistrian University of Athens, Mikras Asias 75, 115 27 Athens, Greece*

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**Abstract**

Flavonoids are an important category of plant antioxidants and evidence is accumulating on their favorable effects against the development of heart disease and certain forms of cancer. We analytically determined the flavonol (quercetin, kaempferol, myricetin, isorhamnetin), flavone (luteolin, apigenin) and flavan-3-ol (catechin, epicatechin, epigallocatechin, epigallocatechin gallate, epicatechin gallate) content of a weekly menu representative of the Greek traditional diet. The overall daily average content was found 79.01 mg of which flavonols contribute 47% (37.17 mg/day), flavan-3-ols 40% (31.67 mg/day) and flavones 13% (10.17 mg/day). The levels of agreement between the analytical results and the respective theoretical flavonoid calculations conducted previously on the same weekly menu ranged widely, indicating that caution should be taken when calculated flavonoid values are to be used in epidemiological studies. Compared to northern European and American diets, the traditional Greek diet has a higher flavonoid content, at least with respect to flavones, flavonols and flavan-3-ols.


*Keywords:* Traditional greek diet; Flavonoid content; Flavones; Flavonols; Flavan-3-ols

## 1. Introduction

The 'traditional Mediterranean diet' can be described as the culinary culture developed over the centuries by populations bordering the Mediterranean sea until the 1960s, when yet the way of life in the Mediterranean region remained practically intact and populations still applied simple and time-honored approaches. In the 1960s, however, the food industrial revolution, which was gradually expanding in Europe after the Second World War, finally invaded the Southern European countries causing a serious disruption in the traditional dietary pattern of the Mediterranean populations. In the years to come, globalization further broadens this gap. However, emerging scientific evidence which attributes beneficial health effects to the traditional Mediterranean diet (Trichopoulou, Costacou, Bamia, & Trichopoulos, 2003; Trichopoulou et al., 1995) provoked a reversion of the Mediterranean populations to their traditional dietary habits (Naska, Oikonomou, Trichopoulou, Wagner, & Gedrich, submitted). Nowadays, the Mediterranean diet has been globally acknowledged as a healthy dietary model.

The traditional Mediterranean diet was largely shaped by climatic and socioeconomic conditions, and evolves around a key primary product, the olive oil. Bread and cereals, mostly unrefined, are also fundamental to this diet to produce or complement cooked and raw dishes. Vegetables and pulses are commonly present at the daily table, usually prepared or supplemented with olive oil, resulting in palatable dishes. Fresh fruits are consumed in vast amounts as a snack or dessert. Local livestock secures a moderate availability of homemade cheese and yogurt while red meat is rarely consumed, mainly in special occasions. Some poultry is consumed instead, while fish is eaten in moderate amounts depending on the proximity to the

* Corresponding author. Tel.: +30 210 746 2074; fax: +30 210 746 2079.
*E-mail address:* antonia@nut.uoa.gr (A. Trichopoulou).

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sea. Alcohol is consumed regularly in moderate amounts mainly in the form of red wine and within meals (Trichopoulou et al., 2003; Willet et al., 1995).

It has been suggested that the advantageous role of the Mediterranean diet may be partly attributed to the presence of large amounts of antioxidants, which exist in abundance in vegetables, fruits, beverages and extra virgin olive oil (Trichopoulou & Lagiou, 1997; Trichopoulou, Lagiou, & Papas, 1998; Trichopoulou, Vasilopoulou, & Lagiou, 1999; Willett, 1994). Moreover, an interest concerning polyphenols is growing, due to their antioxidant properties, and their potential impact on various diseases related to oxidative stress (Scalbert, Manach, Monard, Remesy, & Jimenez, 2005). Flavonoids are one of the most important categories of polyphenolic compounds ubiquitously present in foods of plant origin, and evidence is accumulating on their favorable effects against the development of heart disease and certain forms of cancer (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Lagiou, Samoli, Lagiou, Peterson, et al., 2004; Lagiou, Samoli, Lagiou, Tzonou et al., 2004; Peterson et al., 2003). They comprise of flavones, flavonols, flavan-3-ols (catechins), flavanones, anthocyanidins and isoflavones.

Recently, the nutrient and flavonoid content of a Greek traditional weekly menu was determined (Trichopoulou, Vasilopoulou, & Georga, 2005; Vasilopoulou, Georga, Joergensen, Naska, & Trichopoulou, 2005). The hypothesis actuating the determination of both the nutrient and non-nutrient content of the menu was that a high intake of antioxidant components in combination with the sufficient intake of macro and micro-nutrients might account synergistically for the health benefits apparent in Mediterranean populations. The determination of the flavonoid content of the menu was conducted theoretically using flavonoid databases (US Department of Agriculture, 2003; VENUS Phytoestrogen Database).

We reproduced the typical Greek menu (Trichopoulou et al., 2005; Vasilopoulou et al., 2005) and this work reports on its analytically determined flavone (luteolin, apigenin), flavonol (myricetin, quercetin, kaempferol, isorhamnetin) and flavan-3-ol (catechin, epicatechin, epigallocatechin, epigallocatechin gallate, epicatechin gallate) content. The analytical results were compared with the respective theoretical flavonoid values of the same weekly menu (Vasilopoulou et al., 2005).

## 2. Materials and methods

### 2.1. Development and preparation of the menu

A weekly menu, representative of the traditional Greek diet, was designed on the basis of the Greek dietary guidelines (Supreme Scientific Health Council, 1999), a pictorial presentation of which is presented in Fig. 1. The investigated menu (Table 1) refers to adults of both genders and incorporates abstinence of consumption of animal products every Wednesday and Friday as prescribed by the Greek Orthodox Church. Portion sizes were defined according to the Greek market regulations and refer to edible parts. All primary ingredients were purchased from super markets of Athens, and the preparation (cooking) of the foods took place in an average household using common cooking utensils. All meals, salads and beverages were prepared according to the recipes involved in the calculation of the flavonoid content (Vasilopoulou et al., 2005). These refer to most commonly used recipes, for which, detailed information is available elsewhere (Trichopoulou & Georga, 2004) and reflect primary foods and recipes typically consumed in a traditional Greek household.

### 2.2. Preparation of the samples

The primary and composite solid foods consumed daily (Table 1) were combined to form a composite sample. Seven composite samples were prepared representative of each day of the week. The solid foods included in each daily menu were cut into small pieces, mixed, deep frozen under liquid nitrogen, placed in food bags and stored in deep freeze (−40 °C) until lyophilized (LABCONCO FREEZONE 4.5) at 0.05mbar. Then each of the 7 freeze–dried daily samples was homogenized (IKA M20 Universal Mill) under liquid nitrogen, placed in airtight glass containers and stored in deep freeze (−40 °C) until shipment. The moisture content of the samples was estimated through the lyophilization procedure.

The liquid foods consumed each day (Table 1) are identical in terms of ingredients and quantities with the exception of Tuesday. Therefore, only two composite liquid samples were prepared, one for Tuesday and one for all the other days of the week. The liquids included in the daily menu were mixed and stored separately in deep freeze (−40 °C) until distribution. Water was not included in the menu since it does not contribute to the flavonoid content of the diet.

### 2.3. Determination of flavonoids

Flavonoid analyses were conducted by RIKILT – Institute of Food Safety, Wageningen, the Netherlands. Two flavones (luteolin, apigenin) and four flavonols (quercertin, kaempferol, myricetin, isorhamnetin) were quantitatively determined as follows (Hertog, Hollman, & Venema, 1992): 40 ml of 50% aqueous methanol were added to 0.5 g of freeze-dried sample material. To this extract 10 ml of HCL 6 M were added with careful mixing. The extraction solution thus obtained consisted of 1.2 M HCL in 50% aqueous methanol (v/v). After refluxing at 90 °C for 2 h with regular swirling, the extract was allowed to cool and was subsequently made up to 100 ml with methanol and sonicated for 5 min. Approximately 2 ml was filtered through a 0.45-μm filter for organic solvents prior to injection. The resulting aglycones were separated on a NOVA-PAK C18 column, protected by a Perisorb RP-18 guard column, using methanol/phosphate buffer (45/55,

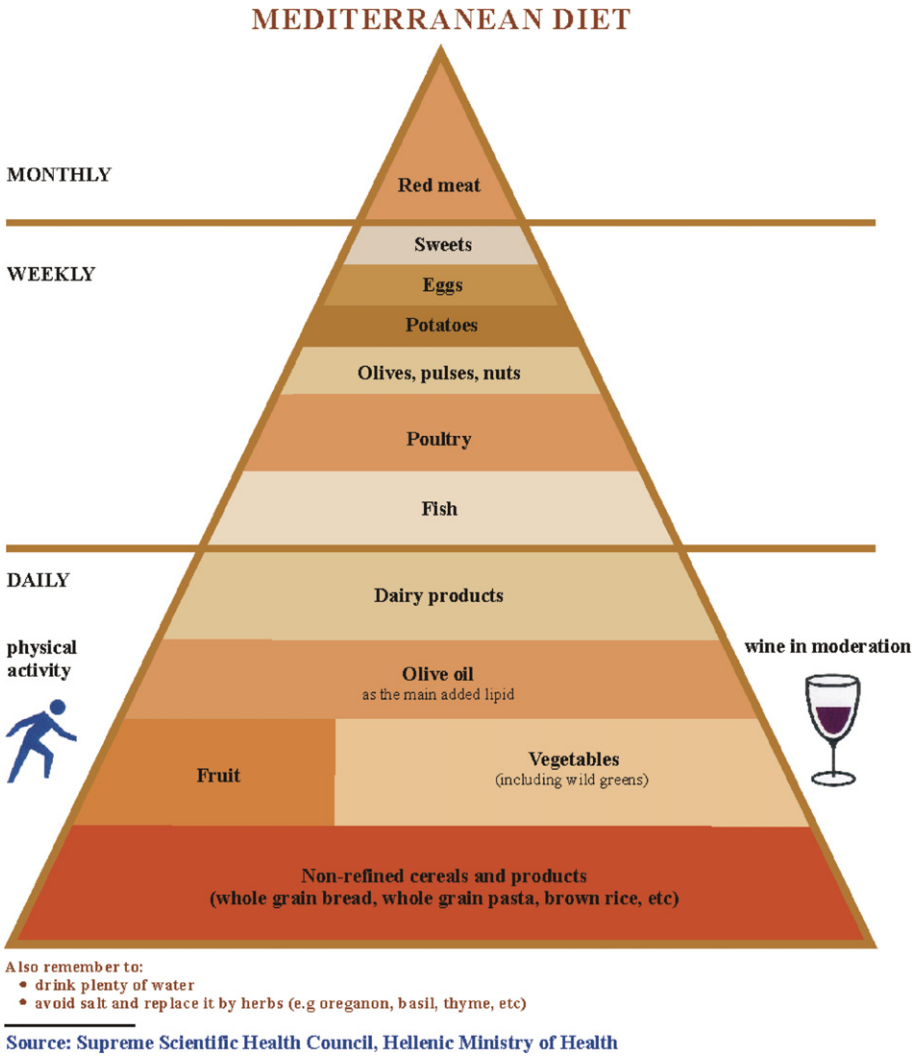


Fig. 1. The Mediterranean diet pyramid.

v/v, pH 2.4) as mobile phase, and UV detection (370 nm). Peak identity and purity was checked using a photodiode array detector to record UV-spectra of the flavonoids in samples on-line. Flavonoids were quantified using a calibration curve of pure aglycone standards. All flavonols and flavones analyses were conducted in duplicate. In each series of flavonol/flavone analyses, an onion quality control sample was analysed together with the samples. Results of this quality control sample were within two standard deviations of the average values (mg/kg): quercetin 2140 ± 240; myricetin 30 ± 7; kaempferol 33 ± 6; isorhamnetin 59 ± 6; luteolin 13 ± 3; apigenin 8 ± 2.

Five flavan-3-ols ((+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, (−)-epigallocatechin gallate) were quantitatively determined as follows (Arts & Hollman, 1998): 0.5 g freeze-dried sample was mixed with 25 ml of 90% v/v methanol/water. The extract was shaken in a mechanical shaker (250 rpm) for 60 min at room temperature. After extraction, the volume was made up to 50 ml with the same solvent, filtered over a 0.45 μm Acrodisc filter and injected without further processing. Beverages were injected directly after filtration over a 0.45 μm Acrodisc filter. The HPLC equipment consisted of a Gilson 234 auto injector which injects 10 μl of sample onto an Inertsil ODS-2 column, protected by a guard column both placed in a column oven set at 30 °C. The solvents used for separation were 5% acetonitrile (eluent A) and 25% acetonitrile (eluent B) in phosphate buffer (0.025 M, pH 2.4). The gradient was as follows: 0–5 min, 10% B; 5–20 min, linear gradient from 10% to 80% B; 20–22 min, linear gradient from 80% to 90% B; 22–25 min, isocratic at 10% B to re-equilibrate. The monitoring of the effluent was done by a fluorescence spectrophotometer (280 nm excitation, 310 emission wavelengths) and a UV detector (270 nm) which were connected in series. Catechin and epicatechin were detected with fluorescence detection, while the other flavan-3-ols were detected with UV. All flavan-3-ols analyses were conducted in duplicate. In each series of flavan-3-ol analyses, an apple quality control sample was analysed together with the samples. Results of this quality control

Table 1
The foods, recipes and servings of the investigated traditional Greek menu

| | Monday (g) | Tuesday (g) | Wednesday (g) | Thursday (g) | Friday (g) | Saturday (g) | Sunday (g) |
|---|---|---|---|---|---|---|---|
| Breakfast | Herbal tea[a] (165)<br>Sugar (5)<br>Feta (50)<br>Bread (60) | Yogurt (200)<br>Honey (40) | Herbal tea (165)<br>Sugar (5)<br>Black olives (30)<br>Bread (60) | Herbal tea (165)<br>Sugar (5)<br>Feta (50)<br>Bread (60) | Herbal tea (165)<br>Sugar (5)<br>Black olives (40)<br>Bread (60) | Herbal tea (165)<br>Sugar (5)<br>Feta (50)<br>Bread (60) | Herbal tea (165)<br>Sugar (5)<br>Feta (50)<br>Bread (60) |
| Morning snack | Grapes (150) | Apple (160) | Apple (160) | Apple (160) | Apple (160) | Apple (160) | Apple (160) |
| Lunch | Green beans (250)<br>Feta (50)<br>Bread (90)<br>Red wine (120)<br>Apple (160) | Fried wet salted cod (150)<br>Chicories (290)<br>Bread (90)<br>Red wine (120)<br>Pear (160) | Lentils with tomato (350)<br>Green olives (40)<br>Bread (90)<br>Red wine (120)<br>Orange (200)<br>Lettuce salad (140) | Chicken casserole (180)<br>Rice (pilaf) (150)<br>Bread (90)<br>Red wine (120)<br>Pear (160)<br>Mizithra (cheese) (10)<br>Cabbage salad (120) | Eggplants casserole (250)<br>Fish roe salad (50)<br>Bread (90)<br>Red wine (120)<br>Orange (200) | Baked vegetables (250)<br>Feta (50)<br>Bread (90)<br>Red wine (120)<br>Pear (160) | Roast lamb (110)<br>Baked potatoes (150)<br>Bread (90)<br>Red wine (120)<br>Orange (200)<br>Lettuce salad (140) |
| Afternoon snack | Raisins (60)<br>Greek coffee[b] (86)<br>Sugar (6) | Olive oil cookies (40)<br>Greek coffee (86)<br>Sugar (6) | Semolina cake (halvah) (65)<br>Greek coffee (86)<br>Sugar (6) | Pasteli, sesame bar (30)<br>Greek coffee (86)<br>Sugar (6) | Semolina cake (halvah) (65)<br>Greek coffee (86)<br>Sugar (6) | Raisins (60)<br>Greek coffee (86)<br>Sugar (6) | Olive oil cookies (40)<br>Greek coffee (86)<br>Sugar (6) |
| Dinner | Cheese pie (150)<br>Greek salad (200)<br>Bread (90) | Spinach rice (250)<br>Feta (60)<br>Bread (90) | Potatoes Casserole (250)<br>Lettuce salad (140)<br>Bread (90) | Feta (50)<br>Greek salad (200)<br>Bread (90) | Spinach pie (155)<br>Greek salad (200)<br>Bread (90) | Fried potatoes (115)<br>Greek salad (200)<br>Bread (90)<br>Fried egg (56) | Chilopites (pasta) (250)<br>Cabbage salad (120)<br>Bread (45)<br>Mizithra (cheese) (10) |

[a] Quantity of herb used for each tea cup: 1.33 g.
[b] Quantity of Greek coffee used for each coffee cup: 4 g.

sample were within two standard deviations of the average values (mg/kg): epicatechin 490 ± 20; catechin 40 ± 4; epigallocatechin 580 ± 55.

### 2.4. Comparison of analytical and calculated values

The analytical flavonoid values and the theoretical estimations conducted previously on the same weekly menu are presented in Table 2. The strength of the associations between the results of the two methods was determined by Spearman's rank correlation coefficient (denoted as $\rho$) using a 5% level of statistical significance. To assess the agreement between the two methods in determining the flavonoid content of the menu, difference plots presented in Fig. 2 were constructed as proposed by Bland and Altman (1986). The plots include the mean differences between the two methods which are presented in a continuous line. The discontinuous lines represent the 95% limits of agreement which indicate how far apart measurements by the two methods are likely to lie. All statistical analyses were carried out with STATA version 7.0 (Intercooled Stata 7.0 for WINDOWS 98/95/NT; STATA Corporation, College Station, TX).

## 3. Results

The results of the flavone, flavonol and flavan-3-ol analyses are presented in Table 2 compared to the respective results of the theoretical estimation (Vasilopoulou et al., 2005). The presented values include the contribution of both solid and liquid samples.

The daily average flavonol, flavone and flavan-3-ol content of the typical Greek traditional menu is 79.01 mg of which flavonols contribute 47% (37.17 mg/day), flavan-3-ols 40% (31.67 mg/day) and flavones 13% (10.17 mg/day). The liquid constituents of the traditional menu (red wine, Greek coffee and mountain tea) contribute 6.7% (5.33 mg/day) to the determined flavonoid content. The remaining 93.3% derive from the solid primary foods and recipes. However, the analytical data do not allow for a thorough identification of the specific food sources of flavonoids in the traditional Mediterranean weekly menu, because the analyses were conducted on total daily diet samples.

For quercetin, isorhamnetin, apigenin and total investigated flavonols, the correlation between analytical and calculated values was strong ($\rho > 0.8$) and statistically

Table 2
Analytically determined[a] and calculated[b] flavonol, flavone and flavan-3-ol content of a weekly traditional Greek menu

| Flavonoids | Daily menu | | | | | | | Weekly total (mg) | Correlation between methods | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Monday (mg) | Tuesday (mg) | Wednesday (mg) | Thursday (mg) | Friday (mg) | Saturday (mg) | Sunday (mg) | | $\rho$[c] | $p$ |
| *Flavonols* | | | | | | | | | | |
| Myricetin | **2.66** | **4.50** | **2.49** | **2.19** | **2.95** | **2.65** | **2.16** | **19.60** | 0.536 | 0.215 |
| | *1.88* | *0.95* | *1.16* | *0.88* | *1.39* | *1.03* | *0.92* | *8.21* | | |
| Quercetin | **21.34** | **37.21** | **31.32** | **12.04** | **30.74** | **20.73** | **9.27** | **162.65** | 0.857 | 0.014 |
| | *26.36* | *27.08* | *27.83* | *14.54* | *31.63* | *15.56* | *13.34* | *156.34* | | |
| Kaempferol | **5.64** | **7.80** | **19.16** | **0.37** | **5.66** | **4.18** | **1.79** | **44.60** | 0.721 | 0.068 |
| | *1.44* | *1.44* | *1.11* | *0.36* | *2.25* | *0.69* | *0.65* | *7.94* | | |
| Isorhamnetin | **0.96** | **11.86** | **4.81** | **nd**[d] | **11.91** | **2.86** | **0.95** | **33.35** | 0.929 | 0.003 |
| | *1.06* | *3.31* | *2.00* | *1.03* | *3.18* | *1.34* | *0.48* | *12.40* | | |
| Total investigated flavonols | **30.60** | **61.37** | **57.78** | **14.60** | **51.26** | **30.42** | **14.17** | **260.20** | 0.893 | 0.007 |
| | *30.74* | *32.78* | *32.10* | *16.81* | *38.45* | *18.62* | *15.39* | *184.89* | | |
| *Flavones* | | | | | | | | | | |
| Luteolin | **nd** | **30.57** | **2.62** | **1.45** | **16.32** | **1.90** | **nd** | **52.86** | 0.703 | 0.078 |
| | *1.31* | *1.75* | *2.72* | *1.29* | *5.63* | *1.27* | *0.05* | *14.02* | | |
| Apigenin | **4.79** | **nd** | **1.31** | **nd** | **8.38** | **3.81** | **nd** | **18.29** | 0.927 | 0.003 |
| | *13.81* | *0.00* | *8.50* | *0.31* | *17.96* | *6.08* | *0.32* | *46.98* | | |
| Total investigated flavones | **4.79** | **30.57** | **3.93** | **1.45** | **24.70** | **5.71** | **nd** | **71.15** | 0.536 | 0.215 |
| | *15.12* | *1.75* | *11.22* | *1.60* | *23.59* | *7.35* | *0.37* | *61.00* | | |
| *Flavan-3-ols* | | | | | | | | | | |
| Catechin | **7.67** | **2.37** | **5.68** | **1.45** | **nd** | **2.86** | **nd** | **20.03** | 0.623 | 0.135 |
| | *16.13* | *11.07* | *10.65* | *11.07* | *10.65* | *12.85* | *10.65* | *83.07* | | |
| Epicatechin | **18.61** | **21.24** | **28.78** | **23.58** | **27.25** | **28.99** | **20.83** | **169.28** | 0.090 | 0.848 |
| | *20.13* | *23.24* | *18.39* | *23.24* | *18.19* | *23.67* | *18.28* | *145.14* | | |
| Epigallocatechin | **nd** | **nd** | **9.18** | **23.23** | **nd** | **nd** | **nd** | **32.41** | na[e] | – |
| | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | | |
| Epigallocatechin gallate | **nd** | **nd** | **nd** | **nd** | **nd** | **nd** | **nd** | **nd** | na | – |
| | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | | |
| Epicatechin gallate | **nd** | **nd** | **nd** | **nd** | **nd** | **nd** | **nd** | **nd** | na | – |
| | *0.65* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | *0.65* | | |
| Total investigated flavan-3-ols | **26.28** | **23.61** | **43.64** | **48.26** | **27.25** | **31.85** | **20.83** | **221.72** | 0.126 | 0.788 |
| | *36.91* | *34.31* | *29.04* | *34.31* | *28.84* | *36.52* | *28.93* | *228.86* | | |
| Total daily content | **61.67** | **115.55** | **105.35** | **64.31** | **103.21** | **67.98** | **35.00** | **553.07** | 0.429 | 0.337 |
| | *82.77* | *68.84* | *72.36* | *52.72* | *90.88* | *62.49* | *44.69* | *474.75* | | |

[a] Analytical values presented in bold.
[b] Calculated values presented in italics (Source: Vasilopoulou et al., 2005).
[c] Spearman's rank correlation coefficient.
[d] Not detected.
[e] Not applicable.

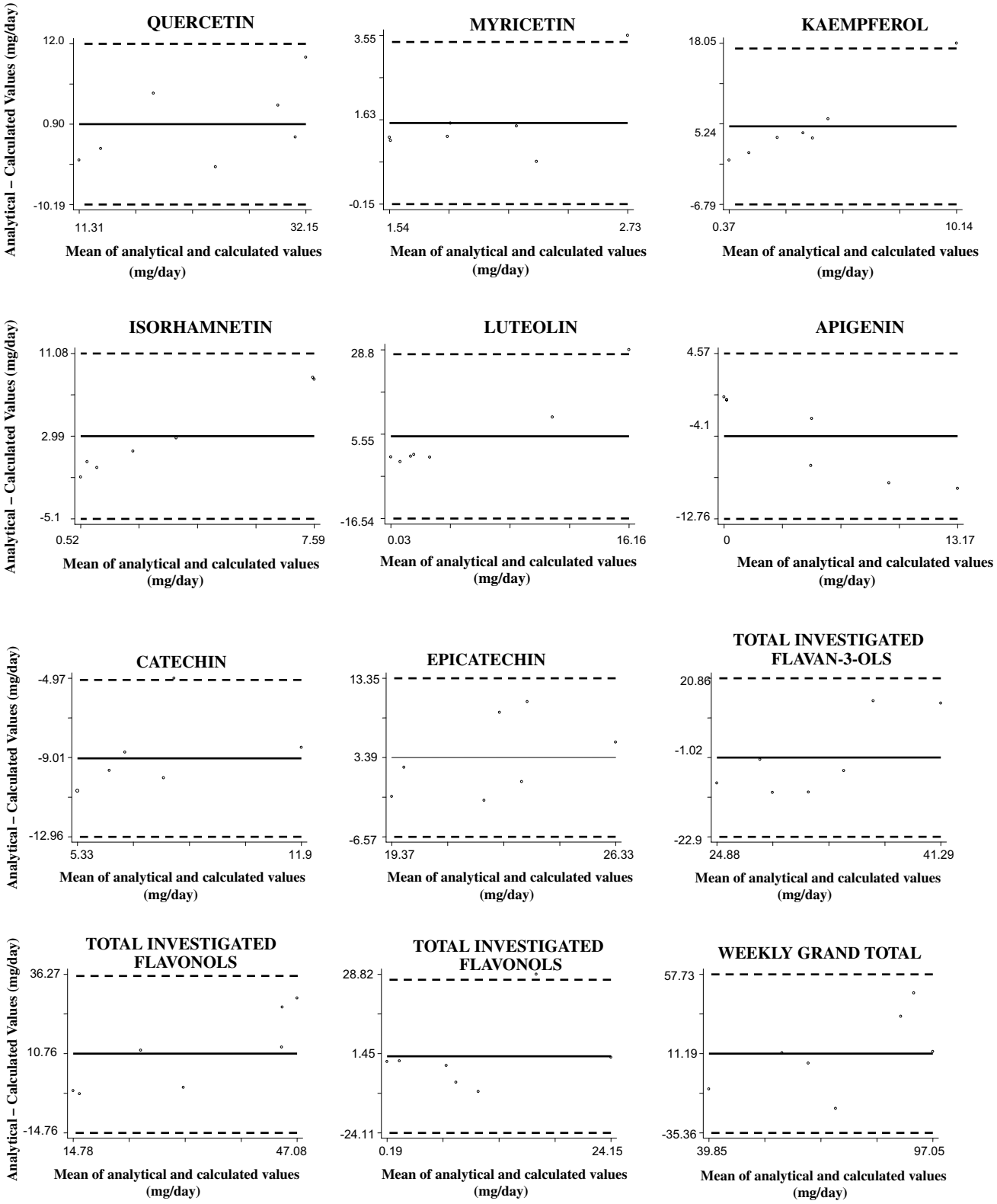


Fig. 2. Bland and Altman agreement plots of the investigated flavone, flavonol and flavan-3-ol content of the weekly traditional Greek menu, as determined analytically or when calculated using databases. The continuous lines represent the mean differences while the discontinuous lines the 95% limits of agreement between the two methods. All values are expressed in mg/day. ( Plots for epigallocatechin, epigallocatechin gallate and epicatechin gallate not shown.)

significant ($p < 0.05$), while for epicatechin on the contrary, the correlation was very weak ($\rho = 0.09$, $p = 0.848$) (Table 2). Correlations for kaempferol and luteolin were marginally not significant at the 5% statistical level ($p = 0.068$ and 0.078 respectively, Table 2). Large differences were observed between the two methods from the Bland and

Altman analysis (Fig. 2), with the 95% of the differences of the values varying widely for the investigated flavonoids as well as for their derived classes. Also, the systematic differences (mean differences) between the methods were generally high with some exceptions such as for quercetin (0.9 mg), total flavones (1.45 mg) and total flavan-3-ols (−1.02 mg) (Fig. 2).

## 4. Discussion

Over the last years, several epidemiological and clinical studies have correlated the Mediterranean diet with prolonged survival and reduced incidence of undesired health conditions such as heart disease and cancer (De Lorgeril et al., 1994; Estruch et al., 2006; Osler & Schroll, 1997; Trichopoulou et al., 2003; Trichopoulou et al., 1995). The evidence however on the protective properties of the individual traditional foods and recipes that this diet incorporates, as well as the synergies and complementation that they provide when combined in the diet, is limited.

A traditional Greek weekly menu has been compared to the nutritional recommendations of the Scientific Committee for Food of the European Commission for macronutrients and certain micronutrients (Commission of the European Communities, 1993). The menu under investigation was found compatible with the European recommendations (Trichopoulou et al., 2005). In addition to the nutrients analyses, the flavonoid content of the traditional weekly menu was calculated, using data derived from flavonoid databases (US Department of Agriculture, 2003; VENUS Phytoestrogen Database). The theoretical indications showed that the traditional Greek diet has high flavonoid content in comparison to diets in northern European countries (Vasilopoulou et al., 2005).

Our results provide supporting analytical evidence on the high flavonoid content of the Greek version of the traditional Mediterranean diet. The traditional Mediterranean menu contains substantially higher amounts of flavones and flavonols as compared to a US population. The estimated intake in the US population was found 20–22 mg/day (Sampson, Rimm, Hollman, de Vries, & Katan, 2002) while the estimated intake from the menu under investigation is twice as high (average content of 47.34 mg/day). In the US estimations, isorhamnetin was not included in the flavonol content. Its contribution, however, to the Greek traditional investigated menu is relatively low (4.76 mg/day) and therefore does not alter the correlation. Furthermore, quercetin contributed approximately 75% to the total flavones and flavonols in the US population while the respective percentage is 49% (or 54% if we exclude isorhamnetin from the calculations) in the Mediterranean menu, implicating a broader range of flavonoid compounds contributing to the overall intake of the Mediterranean populations. Similar findings were reported in a Dutch population with average intake of flavones and flavonols equal to 23 mg/day and mean quercetin intake 16 mg/day (or 70%) (Hertog, Hollman, Katan, & Kromhout, 1993). The respective daily quercetin intake in the investigated Mediterranean menu is 23 mg/day, meaning that in Mediterranean populations quercetin intake alone equals the total amount of flavones and flavonols intake of other populations.

More recently, in another study in the Netherlands, the average flavone, flavonol and flavan-3-ol intake was found 58 mg/day (Tabak, Arts, Smit, Heederic, & Kromhout, 2001), while the respective intake of the investigated Mediterranean menu is 79 mg/day. The total flavonol (quercetin, kaempferol and myricetin) intake of a Finn population was found about 4 mg/day (Knekt et al., 2002), while the respective intake of the investigated Mediterranean menu is 32 mg/day. Furthermore, the average daily flavonoid intake of the Brazilian population aged 17–88 was 79 mg/day for women and 86 mg/day for men (Arabbi, Genovese, & Lajolo, 2004). However, the Brazilian values derived from a wider range of flavonoids (flavones, flavonols, flavan-3-ols, flavanones and chalcones), with the principal flavonoid contributor in the diet being the oranges, which are significant sources of flavanones. The investigated Mediterranean menu has a flavonoid content of 79 mg/day without taking into account the flavanones and chalcones classes. The calculated flavanone intake of the investigated menu was found 38.45 mg/day (Vasilopoulou et al., 2005), meaning that this flavanone contribution would increase the flavonoid content of the Greek menu to 117.45 mg/day. These findings suggest that compared to northern European and American diets, the traditional Mediterranean diet has a higher flavonoid content, at least with respect to flavones, flavonols and flavan-3-ols.

With respect to the contributors to the flavonoid content, as already mentioned, the analytical data do not allow for a thorough identification of the specific food sources of flavonoids in the traditional Mediterranean weekly menu, because the analyses were conducted on total daily diet samples. Tea was found the major source of flavonoids in the Netherlands contributing up to 48% of the total flavone and flavonol content (Hertog, Hollman et al., 1993), and 72% of the total catechin content (Tabak et al., 2001). Tea was also a major source of flavonols and flavones in a US population (Sampson et al., 2002). In the theoretical flavonoid estimation of the Greek traditional menu herbal tea *(Siderites)* was not considered a major contributor, since it is a poor source of flavonoids. The calculations indicated that in the case of the Greek diet the flavones and flavonols derived from a wide range of foods including apples (20%), herbs such as parsley (19%) and dill (6%), onions (16%), olives (7%), spring onions (7%), red wine (6%) and spinach (5%), while apples (44%) and red wine (44%) were equal contributors to the total catechin intake (Vasilopoulou et al., 2005).

The average daily flavone, flavonol and flavan-3-ol content of the Mediterranean menu derived from liquid foods (red wine, Greek coffee and mountain tea) was

5.33 mg. According to Atoui, Mansouri, Boskou, and Kefalas (2005), flavones, flavonols and catechins are absent from Greek mountain tea (except from an indication of apigenin presence). Coffee is not a source of flavones and flavan-3-ols, and contains trivial amounts of quercetin (0.05 mg/100 g of prepared coffee) and myricetin (0.05 mg/100 g of prepared coffee) (US Department of Agriculture, 2003). Values, however, on the flavonoid content of Greek coffee, to our knowledge, do not exist. It is more likely that red wine is the major flavonoid contributor between the liquid foods in the investigated weekly menu. Mean values for the investigated flavonoids found in red dry wine have been reported as 4.29 mg/100 g for epicatechin, 7.61 mg/100 g for catechin, 0.73 mg/100 g for myricetin, 0.84 mg/100 g for quercetin, 0.02 mg/100 g for isorhamnetin and 0.05 mg/100 g for kaempferol meaning in total 13.54 mg/100 g (US Department of Agriculture, 2003). Nevertheless, while the analyses of the liquid sample of the investigated Greek diet showed a similar content level of epicatechin, myricetin, quercetin and kaempferol as the reported values, it also showed a total absence of catechin which obviously accounts for the apparent difference.

The theoretical estimation of the flavonol, flavone and flavan-3-ol content of the Greek menu (Vasilopoulou et al., 2005) was based on the USDA flavonoid database (2003), on unpublished Greek data (97-DIATRO-30 Report-Hellenic General Secretariat of Research and Technology) for olives, dill and oregano, and on Atoui et al. (2005) for Greek mountain tea. The analytical values are in general accordance with the calculated values with respect to the total flavonoid (flavone, flavonol and flavan-3-ol) content of the weekly menu, although the analytical values are somewhat higher (553.07 mg/week analytical value and 474.75 mg/week calculated value). Similar consistency appears when comparing the total flavonoid classes (total flavonols, total flavones and total flavan-3-ols). The largest difference appears in total flavonols (260.20 mg/week analytical value and 184.89 mg/week calculated value). With respect to individual flavonoids, this consistency applies for epicatechin and quercetin which are however the most abundant flavonoids in the menu under investigation. For the other investigated flavonoids, the apparent differences between the weekly analytical and calculated values may be greater.

More specifically, with respect to flavones, the theoretical estimation indicated that the majority of flavone content derived from apigenin (77%), with the main contributing food source being parsley and 23% from luteolin (Vasilopoulou et al., 2005). The analytical data shows a reverse correlation with the majority of flavone content deriving from luteolin (74.3%) and 25.7% from apigenin. We are not in a position to identify the specific food sources, however we should point out that the highest luteolin content is found in the menu of Tuesday and Friday, where spinach dishes are included. Spinach is considered a good source of luteolin.

With respect to flavonols, the theoretical estimation indicated that the majority of flavonol content derived from quercetin (85%) (Vasilopoulou et al., 2005). The analytical data also shows that the majority of flavonol intake derives from quercetin but with a lower percentage 62.5% since the contribution of the other flavonols is higher. On the other hand the theoretical estimation of the flavan-3-ol content indicated that the majority derived from (−)-epicatechin (63%) (Vasilopoulou et al., 2005). The analytical data also shows that the majority of flavan-3-ol content derives from (−)-epicatechin but with a higher percentage 76%. The major catechin source according to the theoretical calculations is red wine contributing 63.92 mg/week. However, as already mentioned catechin was not detected in the liquid samples and this could explain the difference between the theoretical (83.07 mg/week) and analytical (20.03 mg/week) catechin values. Furthermore, while in the theoretical estimation epigallocatechin did not contribute to the total intake, the analytical data shows that epigallocatechin has a significant contribution (15%), possibly indicating an inconsistency in the calculation due to the lack of data for a contributing food source.

The assessment of the concordance between the two methods of determining the flavonoid content of the menu was performed using two statistical approaches. It has been argued that correlation measures the strength of the relation between the different methods of measurement, and that this is not analogous to whether the methods agree or not (Bland & Altman, 1986). The same authors suggested that correlation depends on the range of the values measured and that it does not take into account changes in the scale of measurements. They concluded considering this approach inappropriate for measuring agreement between methods of clinical measurement. However, correlation coefficients have been widely used in such comparisons (Altman & Bland, 2002). In our study, all observed associations between the two methods with regard to the specific flavonoids investigated (except epicatechin) had a correlation coefficient $> 0.5$ (Table 2). A few of these associations however were statistically significant (for quercetin, isorhamnetin and apigenin). For the summarized values representing the investigated flavonoids classes, only flavonol values were strongly correlated between the two methods ($\rho = 0.893$, $p = 0.007$). However, one should always consider sample size, which in our study was small ($n = 7$) and this could compromise the statistical interpretation.

The utilization of the Bland–Altman plot (Bland & Altman, 1986) for assessing agreement between methods of measurement is increasing (Dewitte, Fierens, Stock, & Thienpont, 2002). This method takes into account the absolute differences of the measurements derived from the methods compared ($y$ axis of the plot), and the means of the results of the two methods ($x$ axis of the plot). Whether a new method can be established, replace or be used interchangeably with an old one, will be judged upon clinical rather than statistical criteria. The estimation of the limits of agreement between the compared methods (mean

difference ± 2SD) is of critical importance. If differences within the limits of agreement are not important for the use intended, the new method studied may be regarded acceptable. Other researchers have proposed the use of analytical quality specifications as a criterion of acceptability of a new method (Petersen et al., 1997). Flavonoids databases represent an economical and convenient way to assess the flavonoid content of foods as well as population consumption. In the context of the present study however, we would not expect calculated data to fully agree with the analytical values because the calculated values represent means derived from studies undertaken through diverse agricultural and experimental conditions. Thus, our primary objective was to assess the magnitude of the differences, not to conclude whether these two methods can be used interchangeably. The wide 95% limits of agreement derived by the Bland and Altman analysis indicate that the estimation of the flavonoid content of foods or diets using databases-derived values should be done with caution, especially in circumstances requiring the estimation of flavonoids less prevalent in the diet, or when absolute flavonoid values and not their relative differences in consumption between or within groups are to be used. Further comparative studies are needed in order to gain a more thorough insight on the concordance of analytically determined and calculated flavonoid values.

These findings may reflect the fact that the composition of foods in minor constituents such as flavonoids may vary considerably due to agricultural factors. Hertog, Hollman, and Katan (1992) reported large variations in the flavonoid content of leafy vegetables (lettuce: 1.9–30 mg/kg quercetin, endive: 15–95 mg/kg kaempferol, leek: 11–56 mg/kg kaempferol), due to seasonal influences. Likewise, the total flavonoid content due to the time of harvesting varied for Brazilian vegetables such as chicory (18 and 38 mg/100 g of Fresh Weight, FW), smooth lettuce (2 and 4 mg/100 g FW) and red onion (40 and 100 mg/100 g FW) (Arabbi et al., 2004). In another study (DuPont, Mondin, Williamson, & Price, 2000), the total flavonoid content in eight varieties of lettuce ranged between 0.3 and 229 μg/g FW while in three varieties of endive between 44 and 248 μg/g FW. Furthermore, Schaffer, Schmitt-Schillig, Muller, and Eckert (2005) found substantial differences in the polyphenol content of the same edible wild plants originating from different geographical locations. Such multi-factorial variations in the flavonoid content of individual foods can have an impact on the individual flavonoid content. Nevertheless, in the weekly traditional Mediterranean menu investigated these differences within foods are most probably equalized in the context of a composite diet where an individual flavonoid derives from a variety of foods.

The favourable impact of the traditional Mediterranean diet on health has been well established. The role of antioxidants, however, on the observed benefits has not yet been completely elucidated. Future investigations have to take into consideration various factors that may affect the flavonoid content in foods as well as their bioactive role (Aherne & O'Brien, 2002). Also, other simple antioxidant phenols such as cinnamic and benzoic derivatives were found to be more widespread in plant foods than flavonoids (Sakakibara, Honda, Nakagawa, Ashida, & Kanazawa, 2003). The inclusion of such compounds in epidemiological and analytical studies may strengthen the antioxidant theory, providing us with further insight on the role of traditional foods in the Mediterranean diet. At the same time, attention should be focused towards the potency of flavonoids and other antioxidant constituents to exert prooxidant activity when subjected to specific circumstances (Chan, Galati, & O'Brien, 1999; Galati, Sabzevari, Wilson, & O'Brien, 2002). The elucidation of the latter attribute may supply important knowledge to the health implications associated with antioxidant compounds. In any case, the need for a reliable phytochemical database is beyond doubt. Steps towards this direction have been achieved by the European Food Information Resource Network (EuroFIR – FOOD-CT-2005-513944) funded under the EU 6th Framework Food Quality and Safety Programme (EuroFIR).

The composition of the Mediterranean diet and particularly the traditional Greek diet favors plant foods with antioxidant potential. The high phytochemical intake in conjunction with the sufficient intake of macronutrients and inorganic constituents may account for the advantages that the traditional Mediterranean diet may have over and beyond those conveyed by other types of diets.

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