Polyphenols and Antioxidant Capacity in Fruits and Vegetables Common in the Croatian Diet

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Summary

The objective of this study was to determine the polyphenol content (total phenols, flavonoids, nonflavonoids, anthocyanins) and antioxidant capacity in selected fruits (apple, 'Idared'; apricot, 'Madjarska najbolja'; blueberry, 'Bluecrop'; mandarin, 'Kuno'; orange, unknown; sour cherry, 'Marasca'; strawberry, 'Maya' and peach, 'Redhaven') and vegetables (broccoli, 'Belstar' -flower and steam; cauliflower, 'Favola'; kale, 'Melissa' and leek-leaf and root -unknown) commonly consumed in the Croatian diet. Total phenols, flavonoids and nonflavonoids were measured calorimetrically by using the Folin-Ciocalteu reagent with gallic acid as the standard; anthocyanins were determined using bisulphite bleaching method using cyanidin-3-diglucoside as standard, whereas antioxidant capacity was measured using DPPH method. The highest total polyphenols were observed in sour cherry as 2560 mg GAE/kg fresh weight (FW), followed by blueberry, orange, mandarin, strawberry, apple, apricot, and peach. Among vegetables the highest concentration of total polyphenols was determined in kale (1039 mg GAE/kg FW) followed by broccoli flower, broccoli steam, leek leaf, leek root and cauliflower. In all investigated fruits, as well as vegetables, total flavonoids were predominant polyphenols. Total nonflavonoids were present in low concentration especially in leek leaf, broccoli flower and leek root. The highest antioxidant capacity in fruit and vegetables was determined in samples with higher concentration of flavonoids. In selected fruits, there was a distinct correlation between total phenols or flavonoids and antioxidant capacity, while in vegetable the correlation was not observed.

Key words

polyphenols, anthocyanins, antioxidant capacity, fruits, vegetables

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Received: November 3, 2008 | Accepted: March 18, 2009

Introduction

Numerous epidemiological studies indicate that diet rich in phytochemicals and antioxidants execute a protective role in health and disease. Furthermore, fruit and vegetable based diet reduces the risk for development of chronic diseases, such as cancer, coronary heart disease, obesity, diabetes etc. (Arts et al., 2005; George et al., 2005). Phytochemicals are bioactive compounds that have been associated in the protection of human health against chronic degenerative diseases. Antioxidants are compounds that help delay and inhibit lipid oxidation and when added to foods tend to minimize rancidity, retard the formation of toxic oxidation products, help to maintain the nutritional quality and increase their shelf life (Fukumoto & Mazza, 2000). The major groups of biologically active compounds that may contribute to the total antioxidant capacity of plant foods include different group of polyphenols (phenolic acids, coumarins, flavonoids, stilbenes, hydrolysable and condensed tannins, lignans and lignins), carotenoids and vitamins. The total polyphenols and total anthocyanins are good indicators of antioxidant capacity and studies have reported a high correlation between antioxidant capacity and total polyphenols (Simonetti et al., 1997; Pellegrini et al., 2000). The phenolics in apple varieties are mostly hydroxycinnamic acid derivatives, flavan-3-ols (monomeric and oligomeric), flavonols and their conjugates, dihydrochalcones and procyanidins (Guyot et al., 2001). Chlorogenic acid was the major hydroxycinnamic acid identified in the apple fruit accounting for up to 87 % of the total amount (Guyot et al., 1998). Flavonoids identified in Citrus fruits cover over 60 types, according to the five classes mentioned flavones, flavanones, flavonols, flavans and anthocyanins (the last only in blood oranges) (Tripoli et al., 2007). Apricot and peach contain a lot of different classes of polyphenols which can be divided in phenolic acids and flavonoids (Bengoechea et al., 1997; Dragovic-Uzelac et al., 2005, 2007). Several flavonols, e.g. quercetin, kaempferol, myricetin, which are presented in much kind of fruits, are powerful antioxidants. Antioxidant capacity of red colored fruits (e.g., sour cherry, sweet cherry, strawberry etc.) is influenced by the total anthocyanin contents (Prior et al., 2000; Conner et al., 2002; Cordenunsi et al., 2002; Šimunić et al., 2005; Kim et al., 2005). Phenolic acids were the dominant polyphenols in some kind of vegetables, whereas vegetable such as, onion, broccoli, kale, lettuce contain also significant level of flavonoids, especially kaempferol and quercetin (Cantos et al., 2001; Romani et al., 2003; Cieslik, et al., 2006). Vinson et al. (1998, 2001) reported data on the total polyphenol content of various fruits and vegetables, determined calorimetrically by the Folin-Ciocalteu reaction after correction for ascorbic acid contribution. A food database on flavonoids, a class of polyphenols, was recently published by the USDA, based on the quality evaluation system reported by Holden et al. (2005).

The aim of this study was to assess the amounts of total polyhenols, flavonoids, anthocyanins, non-flavonoids and total antioxidant capacity in edible part of fruit and vegetables purchased from Croatian (Zagreb) market. Furthermore, the second aim was to study correlation between polyphenols and antioxidant capacity of selected fruit and vegetables.

Material and methods

Samples. Samples of different commercially available fruits and vegetables were either obtained from grocery stores or market places in Zagreb. Sour cherry (cv. Marasca), blueberry (cv. Bluecrop), apple (cv. Idared), apricot (cv. Madjarska najbolja) and peach (cv. Redhaven) were pitted, whereas from mandarin (cv Kuno) and orange (unknown) the skin was removed. Flower and steam of broccoli (cv. Belstar) and leaf and root of leek (cv. unknown)- were separated. From cauliflower (cv. Favola) outer leaves were removed. Strawberry (cv. Maya) and kale (cv. Melissa) were also used in this investigation.

Determination of polyphenols. Before extraction all fruits or vegetables were chopped and homogenized in house blender (Mixy, Zepter, International) and analytical determination was carried out on a fruit or vegetable slurry. The slurries were used to evaluate the total phenols (TP), flavonoids (TF) and non-flavonoids (TNF), anthocyanins (TA) and antioxidant capacity (TAC). Extracted TP were determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965). TF were determined according to procedure described by Zhuang et al. (1992). TNF were calculated as difference between TP and TF. Concentration of TP, TF and TNF was expressed as mg gallic acid equivalent (GAE)/kg fresh weight (FW) of edible part of fruits. The total anthocyanin content in extract from selected fruits was determined using bisulphite bleaching method (Riberéau-Gayon & Stonestreet, 1965). Results were expressed as mg cyanidin-3-glucoside equivalent (Cy-gE)/kg FW of edible part of fruits.

Determination of antioxidant capacity. The antioxidant capacity was determined using 2,2-diphenyl-1-picrylhydrazil (DPPH) as a free radical (Brand-Williams et al., 1995). Results were expressed as mmol Trolox equivalent (TE)/kg of FW of edible part of fruits.

Results and discussion

The polyphenols content of some common fruits and vegetables obtained from the market in Zagreb, Croatia are shown in Tables 1 and 2. The data demonstrate that these compounds are widely distributed in the fruit and vegetables analyzed and their quantities vary. According to Bravo (1998), the presence of polyphenols in plant foods is greatly influenced by genetic factors, environmental conditions, degree of ripeness, variety, etc. The concentration of total phenols (TP) in investigated fruits ranged from 407 (peach) to 2560 (sour cherry) mg GAE/kg FW, whereas in vegetables, TP ranged from 654 (cauliflower) to 1039 (kale) mg GAE/kg FW. Considering this wide variation in the TP, the fruits and vegetables were divided into three groups namely high (>2000 mg GAE/kg), medium (1000-2000 mg GAE/kg) and low (<1000 mg GAE/kg) phenolic content. Among all the fresh fruits and vegetables analyzed, colored fruits such as sour cherry and blueberry had the significantly highest phenolic content (high phenolic content group), followed by orange, mandarin, strawberry,

Table 1. Polyphenols in selected fruits from Zagreb's market

Kind of fruits	Total phenols mg GAE/kg fw	Total flavonoids mg GAE/kg fw	Total nonflavonoids, mg GAE/kg fw	Total anthocyanins mg Cy-GE/kg fw
Orange	1278 ± 16	759 ± 5	519 ± 11	-
Mandarin	1161 ± 24	654 ± 43	507 ± 19	-
Apple	567 ± 18	388 ± 20	178 ± 20	-
Apricot	506 ± 32	260 ± 12	246 ± 26	-
Peach	407 ± 29	209 ± 11	197 ± 21	-
Blueberry	2196 ± 36	1717 ± 62	479 ± 36	1694 ± 102
Sour cherry	2560 ± 109	1969 ± 19	591 ± 38	1927 ± 20
Strawberry	1127 ± 21	968 ± 34	160 ± 17	1171 ± 11

Data presented are means of two replicates ± SE

Table 2. Polyphenols in selected vegetables from Zagreb's market

Kind of vegetables		Total phenols mg GAE/kg FW	Total flavonoids mg GAE/kg FW	Total non-flavonoids mg GAE/kg FW
Broccoli	flower	1031 ± 75	983 ± 55	47 ± 13
	steam	784 ± 75	372 ± 39	414 ± 36
Leek	root	709 ± 14	561 ± 8	148 ± 15
	leaf	753 ± 39	750 ± 4	3 ± 1
Kale		1039 ± 118	549 ± 47	490 ± 65
Cauliflower		654 ± 52	423 ± 39	231 ± 39

Data presented are means of two replicates ± SE

kale, broccoli flower, which were included in the medium phenolic content group. Broccoli steam, leek root and leaf, apple, apricot and peach represented the low phenolic content group. The range of polyphenols concentration, in fruits and vegetables investigated in this work are in accordance with findings of other authors (Cieslik et al., 2006; Brat et al., 2006; Pedisić et al., 2007; Tripoli et al., 2007; Dragović-Uzelac et al., 2007a, 2007b; Giovanelli et al., 2009). Generally, in some kind of fruits (sour cherry, blueberry, orange, mandarin, and strawberry) the total phenols content was very high as compared to the vegetables. Otherwise, in apple, apricot and peach total phenols were lower compared to other investigated kind or part of vegetables. Furthermore, the polyphenols contents found in investigated plant materials were different compared to the values reported by other authors, especially in apple, apricot and peach where all polyphenols were lower. These differences may be due to multiple reasons including genetic factors, different environmental conditions stage of maturity, cultivar or varietals differences, growth stage, soil fertilization and the part of the plant used, amongst other factors that affect quantitative variation in these phytochemicals (Herrman, 1976; Hertog et al., 1992; Crozier et al., 1997).

The total flavonoids (TF) contents of the fruit investigated in this study varied from 209 (peach) to 1969 (sour cherry) mg GAE/kg FW. Among investigated vegetables, broccoli flower contain the highest content of TF (983 mg GAE/kg) followed by leek-leaf and root, kale, cauliflower and broccoli steam. In fruits and vegetables, the TF content was predominant compared to non-flavonoids (TNF), especially in colored fruits, broccoli flower and leek leaf. In broccoli the amount of polyphenols was determined separately in flower and steam, and in leek in root and leaf. Broccoli flower contain remarkable content of total phenols, and predominant are flavonoids. Otherwise, steam contains significantly lower content of total phenols compared to broccoli flower, while flavonoids and non-flavonoids were presented in similar amounts. The amount of TP in broccoli flower and leek leaf is probably caused by the action of sunlight. According to Herrman (1976) the concentration of flavonols (class of flavonoids) in the leaves is many times higher than that in other tissues of the same plants, probably due to the action of sunlight. Investigations into several varieties of lettuce and endives have showed that the flavonol concentration drops markedly from the outer to the inner leaves. Furthermore, seasonal variation in flavonol content was reported and the flavonol contents of leafy vegetables were three to five times higher in summer than in other seasons (Hertog et al., 1992). Non-flavonoids were presented almost in lower concentration in fruits except in apricot, which contained similar amount of both, TF and TNF. Among the fruits and vegetables used, only broccoli steam and kale contained similar amounts TF and TNF; and only blueberry, sour cherry and strawberry, contained anthocyanins (1693; 1927 and 1171 mg Cy-GE/kg, respectively). Presented values are in accordance with findings of other authors for same kind of fruits (Giovanelli & Buratti, 2009; Dragović-Uzelac et al., 2007b; Pedisić et al., 2007). In general, in fruits and vegetables investigated, flavonoids were predominant group of polyphenols.

Because it is well known that different kind of fruits and vegetables have high antioxidant capacity, it was investigated, too. Total antioxidant capacity (TAC) of fresh fruits and vegetables (Fig. 1) as determined by the DPPH radical scavenging method decreased in fruits in the following order: sour cherry > blueberry > strawberry > orange > mandarin > apple> apricot > peach; and by vegetables: broccoli flower > kale > broccoli steam > cauliflower > leek root > leek leaf.

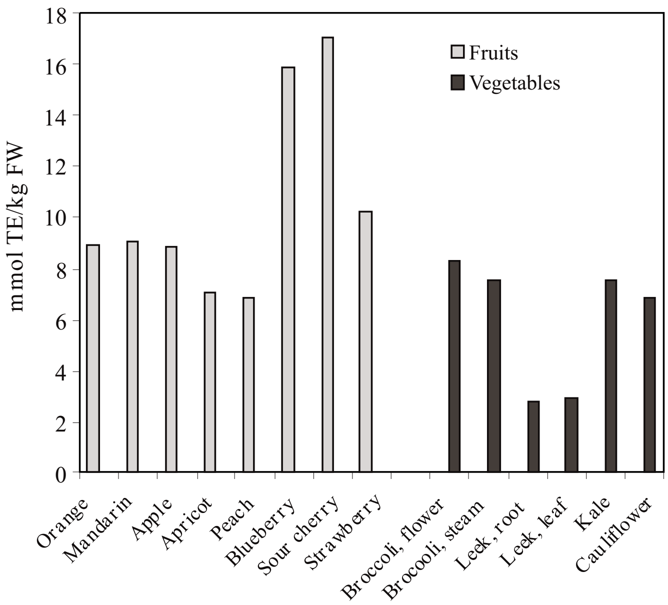

Figure 1. Antioxidant capacity in selected fruit and vegetables from Zagreb's market, determined by DPPH method

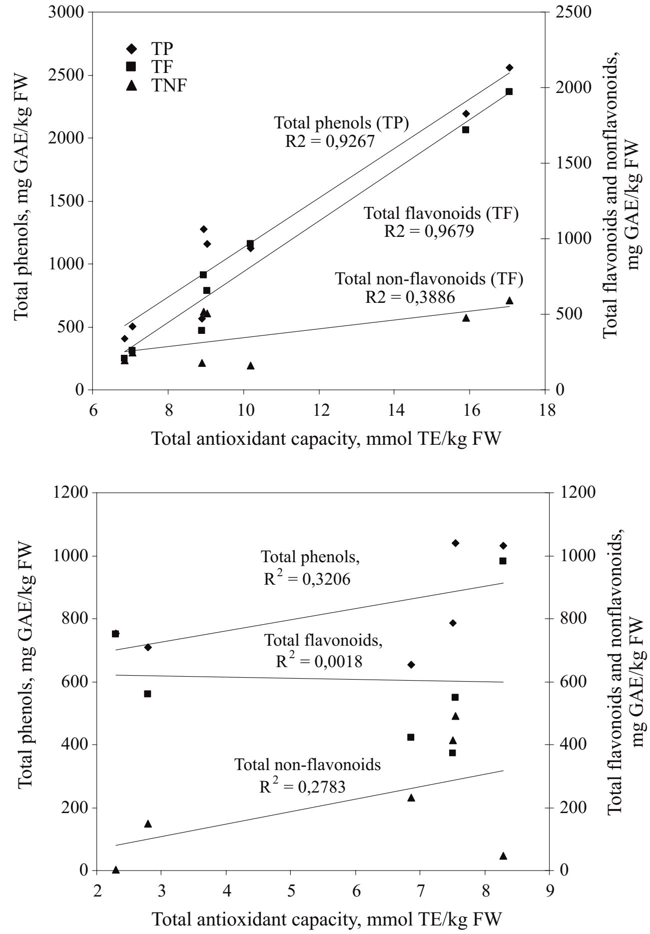

Figure 2. Correlation between total phenols, flavonoids and non-flavonoids and antioxidant capacity in selected fruit and vegetables from Zagreb's market

Our findings are in accordance with findings of other authors (Dragović-Uzelac et al., 2007b; Turkmen et al., 2005). Among all investigated vegetables broccoli showed the highest and leek the lowest antioxidant capacity, whereas antioxidant capacity was higher in colored fruits. It could be due to high amount of TP and TF and especially anthocyanins which contributed to the AOC.

One of the objective of this investigation was to consider the correlation between polyphenols and antioxidant capacity of analyzed fruits and vegetables extracts, as phenolic compounds contribute directly to antioxidant capacity (Duh, 1999). The TAC as a function of TP, TF and TNF content is shown in Fig. 2, respectively. In this study, there was a distinct correlation between TP, TF and TAC in selected fruits, while between TNF and TAC the correlation was not observed. In the case of antioxidant capacity of fruits, measured by DPPH method the correlation coefficients, calculated from linear regression analysis were high for total phenolics ($r = 0.96$) and especially for total flavonoids ($r = 0.98$). By vegetables, the correlation was not observed between TP, TF, TNF and TAC. Considering all vegetables with the exception of leek-root and leaf, a correlation was found between both antioxidant capacity and total phenols content ($r = 0.81$) and antioxidant capacity and flavonoids content ($r = 0.85$). The results obtained for leek-root and leaf indicate that the antiradical activity of this vegetable is particularly low, even they contained relatively high amount of polyphenols.

Conclusion

This study indicated that selected fruits and vegetables on the market in Zagreb, Croatia are rich sources of different group of polyphenols and possess significant antioxidant capacity. Predominat polyphenols in almost all investigated plant materials were flavonoids and high correaltion coefficients indicated that flavonoids significantly cotribute to the antioxidant capacity. The correlation coefficients exhibited a positive relationship between antioxidant capacity of all investigated fruits and contents of total phenols and flavanoids. Presented results showed thad only some kind of vegetables (broccoli, kale and cauliflower) contributed to the antioxidant capacity. Obtained results indicated that the fruits and vegetables evaluated may provide a potential source of dietary antioxidant and therefore their consumption should be stimulated.

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