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## THE TOTAL PHENOLICS AND ANTIOXIDANTS FROM FRUIT AND VEGETABLES: AN EVALUATION OF DAILY INTAKE

### SUMMARY

Given the importance of antioxidant-rich food in the promotion of health and the prevention against damages caused by reactive species, this paper presents insights into the antioxidant activity of phenolics, total phenolics and antioxidants in fruits and vegetables. It also comprises the daily intake of phenolics and antioxidants from foods in the American and French diets, and the evaluation of Montenegro by using literature data.

In comparison to fruit, vegetables contribute less in the daily intake of phenolics and antioxidants. In the Montenegrin diet, the main dietary sources of phenolics and antioxidants are apples and potatoes, and relatively high daily intakes are achieved from bananas, plums, oranges, bell peppers and cabbage. The daily consumption of fruit and vegetables in Montenegro is significantly lower than in France, and especially the USA. Dietary modification through the balanced consumption of fruit and vegetables is necessary. Therefore, it is more important and more effective than taking nutritional supplements for the primary prevention of chronic diseases.

**Key words:** fruit, vegetable, phenolic, antioxidant, intake

### INTRODUCTION

The increased consumption of fruit and vegetables is associated with a lower risk of degenerative diseases (Vinson et al., 1998; Vinson et al., 2001). A significant inverse correlation of mortality with the increasing consumption of healthy food was found (Kant et al., 2000). There is also abundant evidence for fruit and vegetables decreasing the risk of heart disease (Rimm et al, 1996). Also, the consumption of fruit (especially of citrus fruit and juice) and vegetables is especially effective in the protection against the risk of strokes (Gillman et al., 1995; Joshipura et al., 1999). An additional benefit of fruit and vegetables is their association with lowering blood pressure (Ascherio et al., 1991). The risk of macular degeneration (Seddon et al., 1994) is diminished in people who consume large quantities of fruit and vegetables. Fruit and vegetable consumption prevents cancer (Steinmetz and Potter 1996). The intake of apples is inversely associated

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with the incidence of lung cancer (Knekt et al., 1997). Those who consume ten or more servings of tomato products a week are found to develop prostate cancer less often than when their intake was low (Giovanucci et al., 1995). The consumption of 200g of strawberries increases the plasma antioxidant capacity (Cao et al., 1998). The American National Cancer Institute and the National Research Council recommends at least five servings of fruits and vegetables daily (Vinson et al., 2001). It is generally assumed that the vitamin and provitamin antioxidants in these foods (ascorbic acid, tocopherols, and carotenoids) account for their beneficial effects. However, the consequences of the dietary intakes of these antioxidants are difficult to separate in epidemiological studies from the other important constituents, such as the phenolics e.g., flavonoids (Vinson *et al.*, 1998). These natural antioxidants are known to minimise the adverse effects of the reactive species generated by various metabolic processes and environmental stresses in the living system (Mishra et al., 2012). Free radicals and other 'reactive oxygen/nitrogen/chlorine species' are widely believed to contribute to the development of several age-related diseases.

The objective of the present paper is to point out the potential for applications of antioxidant-rich food in the promotion of health and in the prevention against the damages caused by radicals, and to provide an overview of the content of the total phenolic and antioxidant capacity in fruit and vegetables. In addition, the daily intake of phenolics and antioxidants in Montenegro has been evaluated by using of literature data (Chun et al., 2005). The data about the consumption of fruit and vegetables, according to COICOP, was provided by the Statistical Office of Montenegro (MONSTAT). On an annual level, the sample consists of 1560 households, with each month focussing on 26 enumeration areas, i.e., 130 households were selected. The 2011 survey examined 1287 households, which made the 82.50% response rate. The household budget survey has been harmonised with International standards and the recommendations from EUROSTAT and the UN, which enabled an international comparability of data (HBS MNE - 2011, page 1).

This paper is important for the realisation of the scientific project titled the "Investigation of the effect of irrigation and fertilization on antioxidant activity and phenolic content in fruit crops".

### **Antioxidant activity**

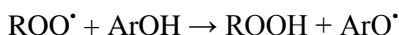
Reactive oxygen and nitrogen species (ROS/RNS) are essential for energy supply, detoxification, chemical signalling and immune function. They are continuously produced in the human body and they are controlled by endogenous enzymes (superoxide dismutase, glutathione peroxidase, and catalase). When there is an over-production of these species, an exposure to external oxidant substances or a failure in their defence mechanisms, damage to valuable biomolecules (DNA, lipids, proteins) may occur (Aruoma, 1998). This damage

has been associated with an increased risk in cardiovascular disease, diabetes, cancer and other chronic diseases (Dimitrios, 2006). If oxidative damage significantly contributes to disease pathology, then the actions that it decreases should be therapeutically beneficial. If the oxidative damage is involved in the origin of a disease, then a successful antioxidant treatment should delay or prevent the onset of that disease (Halliwell and Whiteman, 2004; Godjevac et al., 2009; Aljančić et al., 2010).

This explains the huge volume of research work, and the efforts from the many researchers who have linked diets that are rich in natural antioxidants with degenerative diseases. Many of naturally occurring antioxidants are now isolated, fully characterised and available for various applications (Dimitrios, 2006).

The efficiency of antioxidants is dependent on the ability of the free radical scavenger (FRS) to donate hydrogen to the oxidising free radical, to decrease the energy of the antioxidant radical and to prevent the autoxidation of the antioxidant radical into additional free radicals. Antioxidant radicals may undergo additional reactions, which remove radicals from the system, such as reactions with other antioxidant radicals or where the free radicals form nonradical-nonreactive species. This means that each FRS is capable of inactivating at least two of the free radicals.

Phenolics are effective FRS. These compounds primarily inhibit lipid oxidation through their ability to scavenge free radicals and convert the resulting phenolic radicals into a low-energy form that does not further promote oxidation (Decker, 2002).



As the oxygen-hydrogen bond energy of the FRS decreases, the transfer of the hydrogen to the free radical is more energetically favourable, and thus, more rapid.

If a compound has a reduction potential lower than the reduction potential of a free radical found in a food or biological tissue (e.g., a fatty acid based peroxy radical), it can donate hydrogen to that free radical, unless the reaction is kinetically unfeasible. The efficiency of an antioxidant FRS is also dependent on the energy of the resulting antioxidant radical. If a FRS produces a low energy radical, then the likelihood of the FRS radical to promote the oxidation of other molecules is lower, and the oxidation reaction rate decreases. Phenolics are effective FRS, because phenolic free radicals have low energy due to the delocalisation of the free radicals throughout the phenolic ring structure. Effective phenolic antioxidants also produce radicals that do not react rapidly with oxygen to form hydroperoxides, which could autoxidise, thus depleting the system of antioxidants (Decker, 2002).

Numerous studies have shown that grape skin, seed, and pomace extracts possess potent free radical scavenging activities. The beneficial effects of grape,

and relevant grape-derived food products, are believed to be related to phenolic antioxidants, typically including anthocyanins, flavan-3-ols, flavonols, resveratrol and phenolic acids (Stanković et al., 2008; Topalović and Mikulič-Petkovšek, 2009; Topalović and Mikulič-Petkovšek, 2010; Topalović et al., 2011; Topalović et al., 2012a; Topalović et al., 2012b).

Apples have shown strong antioxidant activities towards the oxidation of methyl linoleate (Kahkonen et al., 1999). Chlorogenic acid and phloretin glycosides are the major identifiable antioxidants in apple juice (Miller and Rice-Evans, 1997).

Neochlorogenic acid and chlorogenic acid, are the two predominant phenolic compounds found in plums, and are antioxidants towards the oxidation of human LDL (Yanishlieva-Maslarova and Heinonen, 2001).

Miller and Rice-Evans (1997) demonstrated that the total antioxidant activity in orange juice could be accounted for by hesperidin and narirutin.

The antioxidant capacity in bananas may be attributed to their gallocatechin content (Someya et al., 2002).

The active phenolic compounds isolated from potatoes, especially in potato peelings, are the derivatives of caffeic acid, such as chlorogenic acid or caffeoylquinic acid derivatives with sugar moiety (Im et al., 2008).

Both yellow and red onions have strong antioxidant activity towards the oxidation of LDL. Onions are a good source of quercetin (Yanishlieva-Maslarova and Heinonen, 2001).

White cabbage extract was reported to show more than 80% inhibition of coupled oxidation of  $\beta$ -carotene and linoleic acid, and it was also an active hydroxyl radical scavenger (Yanishlieva-Maslarova and Heinonen, 2001).

### **Total phenolics and antioxidant capacity of fruit and vegetables**

In this review, the data for commonly consumed fruit and vegetables from an American and French study was used. Generally, there are significant differences between the results shown in Table 1, due to the differences in analysed cultivars, growing conditions and applied methodology. According to a study in France (Brat et al., 2006) for fruit, strawberries exhibited the highest content of total phenolics, followed by grapes and apples, while for vegetables the highest content was measured in broccoli, followed by celery and onions. The lowest content of total phenolics was found in kiwifruit and snap beans. In selected fruit and vegetables from the American diet (Chun *et al.*, 2005), plums showed the highest concentration, followed by strawberries and grapefruit and for vegetables it was bell peppers, garlic and cabbage. The lowest phenolic concentration was found in cherries and snap beans. Similarly, in the results of total phenolics, plums showed the highest antioxidant capacity. Among the selected vegetables, bell peppers showed the highest antioxidant activity, followed by cabbage and garlic. The average antioxidant capacity in the selected 12 fruits was 189.4 mg VCE/100 g, even about 6.7-fold higher than for 14

vegetables. Among them, Chun *et al.* (2005) determined the smallest antioxidant capacity in grapes and snap beans.

Table 1. The level of total phenolics, antioxidant capacity, antioxidant quality ( $IC_{50}$ ), total phenol antioxidant index (PAOXI) of fruit and vegetables based on a fresh edible portion (FEP), French and American daily food consumption

	France (Brat <i>et al.</i> , 2006)		USA (Chun <i>et al.</i> , 2005)			USA (Vinson <i>et al.</i> , 2001; Vinson <i>et al.</i> , 1998)	
	Food consumption (per capita) g day <sup>-1</sup>	Total phenolics <sup>a</sup> mg GAE 100 g <sup>-1</sup>	Food consumption (per capita) g day <sup>-1</sup>	Total phenolics <sup>b</sup> mg GAE100 g <sup>-1</sup>	Antioxidant capacity <sup>b</sup> mg VCE 100 g <sup>-1</sup>	$IC_{50}$ (μM)	Total PAOXI* 10 <sup>-3</sup>
<b>Fruits</b>							
Apples	52.8	179.1	53.9	118.30 ± 1.40	205.40 ± 5.60	0.31	20.6
Bananas	16.9	51.5	33.1	112.79 ± 6.70	173.57 ± 5.21	0.39	28.7
Cherries	2.0	94.3	2.1	55.77 ± 2.94	139.82 ± 5.52	0.10	109
Grapefruit	5.0	43.5	15.9	161.72 ± 7.66	123.88 ± 25.00	0.19	4.7
Grapes	9.0	195.5	22.7	83.59 ± 4.35	72.33 ± 23.61	0.20-white 0.27-red	33.5-white 50.3-red
Kiwifruit	4.4	28.1	0.7	61.21 ± 1.82	110.98 ± 5.27		
Lemons	2.9	45.0	8.1	108.78 ± 6.72	228.50 ± 22.81	0.29	8.28
Oranges	21.2	31.0	104.3	112.29 ± 4.50	140.58 ± 41.86	0.34	4.1
Peaches	-	-	11.7	98.56 ± 3.66	142.89 ± 7.75	0.46	5.22
Pears	12.3	69.2	7.6	70.19 ± 1.33	105.80 ± 5.06	0.51	12.9
Plums	-	-	3.6	368.66 ± 12.66	481.43 ± 15.03	0.50	15.6
Strawberries	6.9	263.8	7.2	225.00 ± 2.60	347.20 ± 9.10	0.12	38.3
<b>Vegetables</b>							
Bell peppers	2.7	22.5	8.3	52.49 ± 1.07	64.91 ± 1.39	0.61	2.69
Broccoli	1.2	98.9	9.4	25.02 ± 0.85	30.53 ± 1.24	0.88	4.1
Cabbage			12.8	45.28 ± 1.17	58.51 ± 2.52	1.16	1.6
Carrots	23.1	10.1	17.4	8.40 ± 0.12	11.25 ± 0.44	0.69	2.33
Cauliflower	4.0	12.5	2.6	10.40 ± 0.06	16.39 ± 0.43	0.79	2.22
Celery	0.9	84.7	8.2	17.09 ± 1.36	13.41 ± 0.41	0.73	1.67
Garlic	1.3	59.4	3.0	47.66 ± 2.16	49.98 ± 0.53	0.41	31.5
Lettuce (head)			29.2	9.82 ± 0.27	12.65 ± 0.44	0.60	1.27
Mushrooms			4.8	11.25 ± 0.31	14.87 ± 0.27	0.75	2.03
Onions	8.8	76.1	23.5	24.27 ± 0.26	21.76 ± 0.09	0.38-red 0.20-yellow	10.5 11.9
Potatoes	203.4	23.1	171.4	35.28 ± 0.56	35.45 ± 2.05	0.25	4.64
Snap beans	2.9	10.0	9.7	4.51 ± 0.16	1.92 ± 0.04	0.27	5.7
Spinach			2.5	32.54 ± 0.35	35.16 ± 2.38	0.86	1.98
Tomatoes	36.1	13.7	103.0	23.69 ± 0.21	29.44 ± 1.60	0.39	3.25

<sup>a</sup> Values are means, n = 3; RSD < 5% for each lot analyzed.

<sup>b</sup> Each value is the mean ± SD (n = 6)

The quality of the antioxidants was determined by the  $IC_{50}$  (the concentration to inhibit 50% oxidation of lower density lipoproteins – LDL + VLDL), with the lower numbers indicating the higher quality of antioxidants in analysed food (Vinson *et al.*, 1998; Vinson *et al.*, 2001). For fruit, the  $IC_{50}$  range was from 0.1 for cherries to 0.51 for pears (Vinson *et al.*, 2001), and for vegetables from 0.2 for yellow onions to 1.16 for cabbage (Vinson *et al.*, 1998). Cherries, with the  $IC_{50}$  of 0.1 μM, equalled the best for pure polyphenol, epigallocatechin gallate, the main antioxidant in green tea (20). Taking into account the range from 1.45 μM for ascorbate to 4.30 μM for beta-carotene, the antioxidant quality of fruit and vegetable extracts were superior to vitamin antioxidants. The same situation is also applies in comparison to most pure

phenols (Vinson et al., 1995). This suggests synergism among the antioxidants in the mixture, such as those found in wine (Ghiselli et al., 1998).

The phenol antioxidant index (PAOXI) is one of the most comprehensive parameters for comparing food antioxidants (Vinson et al., 1998; Vinson et al., 2001). This combined measure of quantity and quality of phenol antioxidants is determined by dividing the total phenol concentration ( $\mu\text{mol/kg}$ ) by the  $\text{IC}_{50}$  value ( $\mu\text{M}$ ). According to PAOXI (Table 1), cherry is at number one, distantly followed by the red grape and the strawberry. Those with low values of PAOXI are peaches, grapefruit and oranges. For vegetables, garlic and red and yellow onions are in high PAOXI values, and celery, cabbage and lettuce (head) are low. One of the reasons that fruit has better protective effects than vegetables, for reducing the risk of chronic diseases, such as cancer and heart disease, may be due to the improved quantity and quality of the antioxidants in fruit when compared to those in vegetables.

The estimated amounts of total phenolics from 10 fruits and 10 vegetables in daily consumption examined in the French study were 160.8 and 65.1 mg GAE, respectively. In the French diet, the high daily intakes of total phenolics, as well as antioxidants, were achieved from apples and potatoes and also from the relatively high consumption of strawberries and grapes and from onions. The corresponding results in the American study, with 294.8 mg GAE in total for 10 pieces of fruit and 102.3 mg GAE for 10 vegetables, showed a significantly higher daily intake of phenolics than in France. Brat et al. (2006) emphasised the overestimation of the total content of phenolics due to effect of ascorbic acid as a reducing compound in colorimetric assays. In the French study, solid phase extraction was carried out on the raw extract to eliminate the water-soluble reducing interferences, including vitamin C. Apart from effect of cultivar, geographical origin, agronomic practice, it was one of possible reasons of difference among results.

The estimated amounts of total phenolics from 12 pieces of fruit and 14 vegetables in daily consumption as examined in American study were 319.7 and 112.3 mg GAE, respectively. The average quantity of antioxidants provided from fruit and vegetable consumption was 440.2 mg VCE per day and 122.4 mg VCE, respectively. In the American diet, the high daily intake of total phenolics, as well as antioxidants, were achieved from oranges, apples, bananas, potatoes and tomatoes, and a considerable amount from cabbage.

The consumption of a fresh edible portion (FEP) of fruit and vegetables in Montenegro (Fig. 1) was obtained by using data from the 2011 survey, which examined 1287 households (HBS MNE - 2011, page 1) and data about the waste percentage (Vukićević, 1991). The comparison of the daily intake of phenolics and antioxidants for individual fruit and vegetables are given in Fig. 2 and 3.

By using data about total phenolics and antioxidant activity for selected fruits and vegetables from the American study, the daily consumption of total phenolics from 12 pieces of fruit and 11 vegetables

for Montenegro are 81.7 and 60.2 mg GAE, respectively. The sum of the antioxidants provided by fruit and vegetable consumption are 125.7 mg VCE per day and 66.4 mg VCE, respectively. Therefore, the mentioned daily consumption of phenolics and antioxidants from fruit is 3.9 and 3.5-fold lower, and from vegetables about 1.8-fold lower in comparison to American diet.

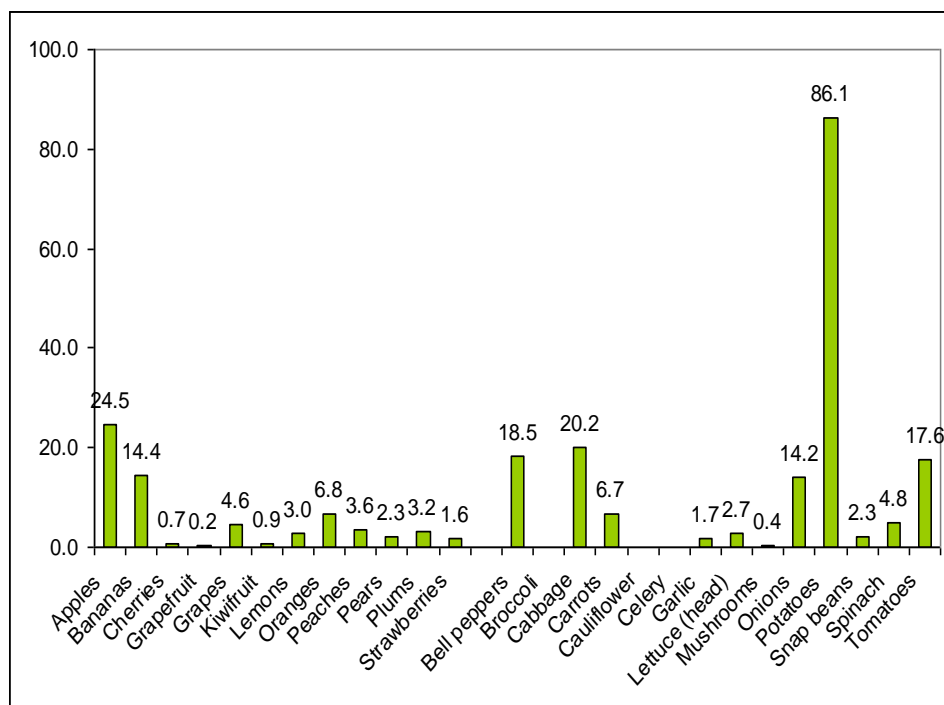
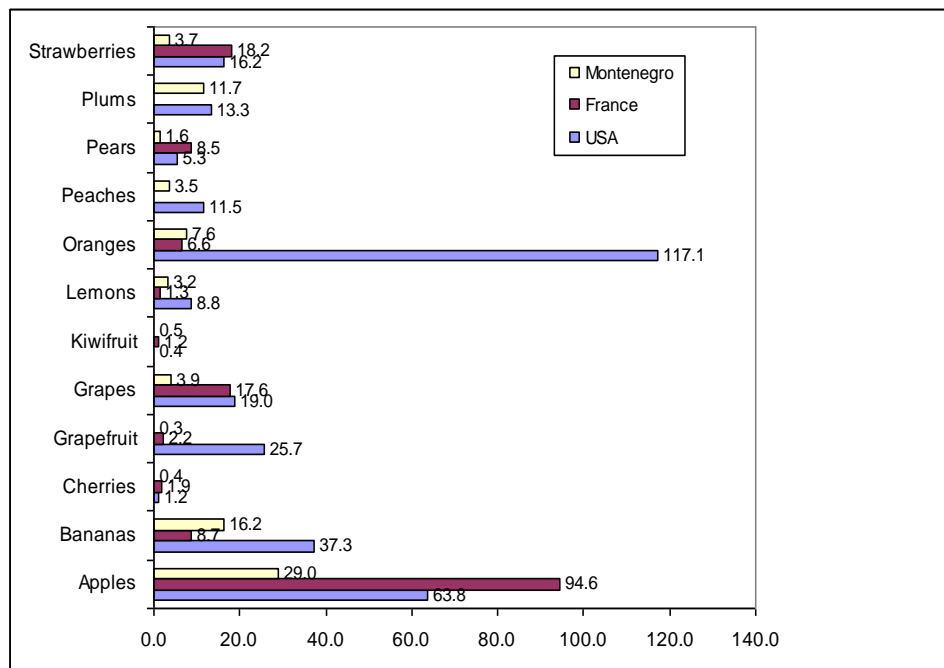


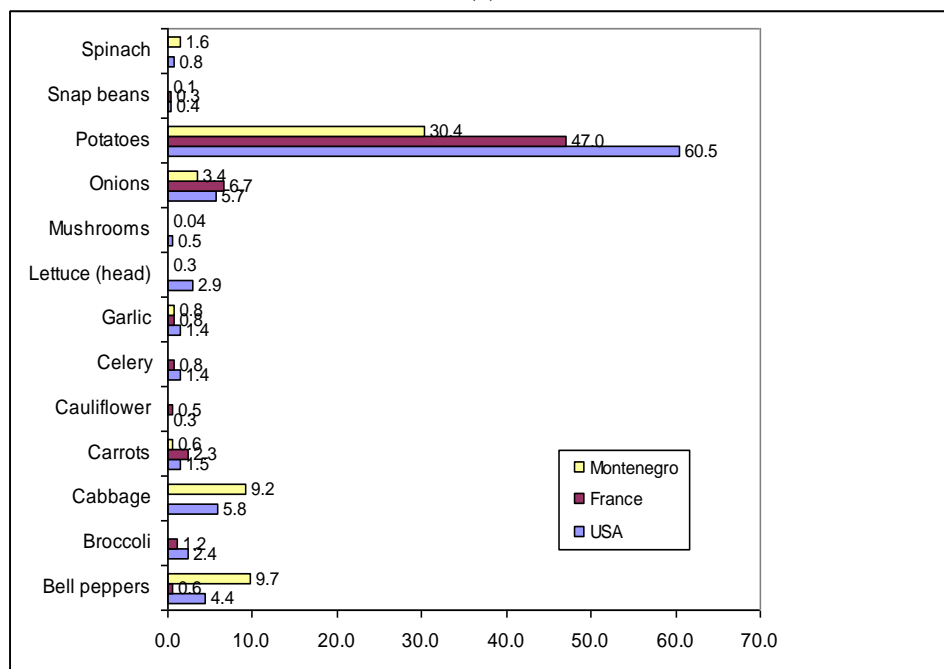
Fig. 1. The fruit and vegetable consumption (g FEP/day per capita) in Montenegro

The ratio between the daily consumption of phenolics from selected fruit, without peaches and plums in France and Montenegro is 2.4. This ratio is 1.3 for vegetables such as bell peppers, carrots, garlic, onions, potatoes, snap beans and tomatoes.

In the Montenegrin diet, the highest daily intake of the total phenolics, as well as antioxidants, are achieved from apples and potatoes and with relatively high intakes from bananas, plums, oranges, bell peppers and cabbage. Dietary modification through the balanced consumption of fruit and vegetables is necessary. Therefore, it is more important and effective than nutritional supplements for the primary prevention of chronic diseases.



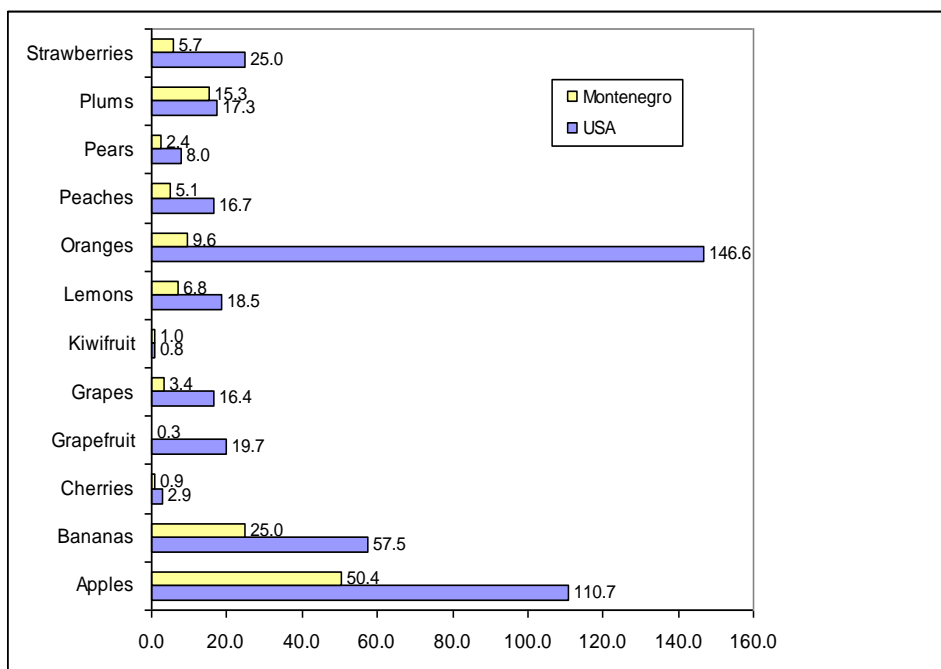
(a)



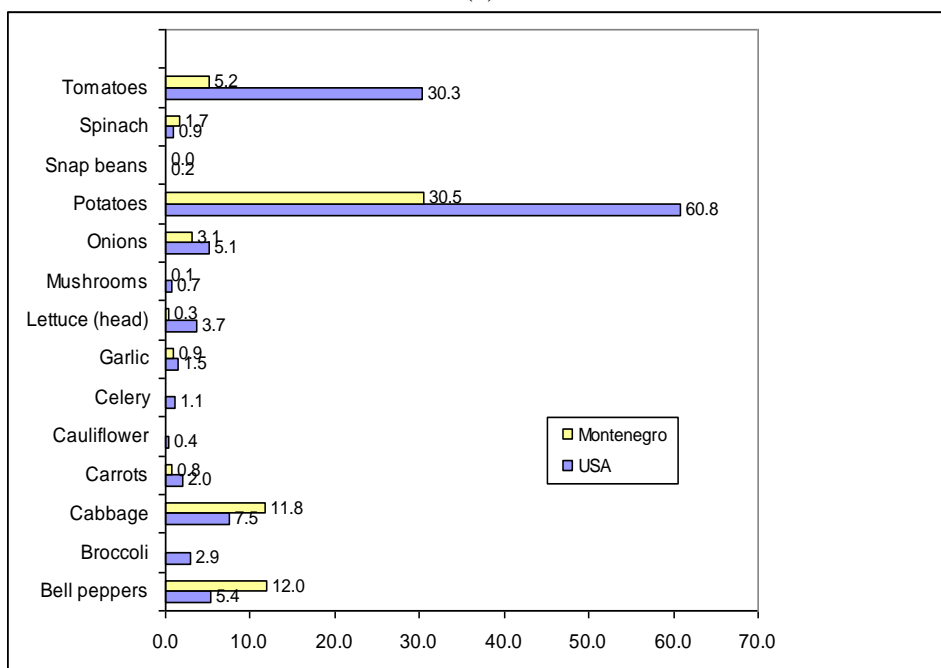
(b)

Fig. 2. Comparison of total phenolics from daily consumption (mg GAE/day per capita) of fruit (a) and vegetables (b)





(a)



(b)

Fig. 3. Comparison of antioxidant intakes from daily consumption (mg VCE/day per capita) of fruit (a) and vegetables (b)

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## **UKUPNI FENOLI I ANTIOKSIDANSI U VOĆU I POVRĆU: PROCJENA DNEVNOG UNOŠENJA**

### **SAŽETAK**

S obzirom na značaj hrane bogate antioksidansima u promociji zdravlja i sprečavanju oštećenja izazvanih reaktivnim vrstama, ovaj rad predstavlja uvid u antioksidativnu aktivnost fenola, ukupnih fenola i antioksidanasa u voću i povrću. Pored toga, obuhvata i dnevno unošenje fenolnih jedinjenja i antioksidanasa iz ove hrane u američkoj i francuskoj ishrani, a korišćenjem literaturnih podataka i procjenu ovog parametra ishrane za Crnu Goru.

U poređenju sa voćem, preko povrća se u manjem stepenu dnevno unose u organizam fenolna jedinjenja i antioksidansi. U crnogorskoj ishrani, glavni izvor fenolnih jedinjenja i antioksidanasa predstavljaju jabuke i krompiri, a relativno visoko dnevno unošenje se ostvaruje preko banana, šljiva, pomorandži, paprika i kupusa. Dnevna potrošnja voća i povrća u Crnoj Gori je značajno niža nego u Francuskoj, a naročito u SAD-u. Neophodne su modifikacije u ishrani kroz uravnoteženo unošenje voća i povrća, što je važnije i efikasnije od dijetetskih suplemenata u primarnom sprečavanju hroničnih oboljenja.

**Ključne riječi:** voće, povrće, fenoli, antioksidans, unos