

## Dietary Intake of Antioxidants in the Czech Republic

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### Abstract

JOUDALOVÁ K., RÉBLOVÁ Z. (2012): **Intake of antioxidants in the Czech Republic.** Czech J. Food Sci., **30**: 268–275.

The intake of extractable antioxidants in the Czech Republic was studied using the FRAP (ferric reducing antioxidant potential) method applied to water-methanol extracts. The daily intake of these antioxidants was 16.6 mmol generated Fe(II) for men and 15.0 mmol for women (i.e. 8300 and 7500  $\mu\text{mol}$  Trolox equivalents). The largest sources of antioxidants were coffee (43.1% of overall intake for men and 54.6% for women) and beer (15% for men vs. 1.8% for women). Other significant sources of antioxidants were tea, vegetables and vegetable products (including potatoes and potato products), fruit and fruit products, cereal products, wine, sugars and sweets, spices and meat and meat products. Small amounts of antioxidants (less than 1.0% of overall intake) were supplied by nuts and seeds, milk and milk products and fats, while pulses, eggs and egg products, convenience foods and cheese were insignificant sources of antioxidants. Within the fruit and fruit products category, apples were the most significant source of extractable antioxidants, and in the vegetable and vegetable products category, peppers were the largest source of antioxidants.

**Keywords:** food antioxidant capacity; extractable antioxidants; FRAP (ferric reducing antioxidant potential)

It is widely accepted that undesirable oxidation plays a role in the origin and development of many human diseases. This oxidation is initiated by reactive oxygen species (ROS), most notably free radicals. In humans, ROS are formed during every day metabolic processes and as a part of the immune response, but may also accumulate from external sources, such as air pollution, cigarette smoke, and ultraviolet radiation (WILLCOX *et al.* 2004).

Ideally, the production of these potentially dangerous substances is compensated by the antioxidant system, which includes compounds of both endogenous and exogenous (e.g. dietary) origin. However, if antioxidant protection is not effective, accumulation of excessive concentrations of free radicals may occur, causing damage to all types of biomolecules and leading to the development of

oxidative stress. This process is associated with many human diseases, including cancer, diabetes, cardiovascular diseases, and neurodegenerative diseases (Alzheimer's and Parkinson's diseases), and free radicals also reduce the function of the immune system and contribute to aging and degenerative diseases associated with aging (WILLCOX *et al.* 2004; SEIFRIED *et al.* 2007).

Therefore dietary antioxidants are commonly assumed to have a positive effect on human health. Although this hypothesis is supported by a significant body of evidence, not all epidemiological and clinical studies have confirmed this claim. In fact, high dietary antioxidant intake has even been reported to have a negative effect in some cases (WILLCOX *et al.* 2004). Therefore, the recommended daily intake of antioxidants has not been

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 6046137305, and by Specific University Research, MSMT No. 21/2011.

determined yet, and antioxidant consumption in the form of dietary supplements is discouraged. Nevertheless, the intake of antioxidants through ordinary diet (i.e. without food supplements) has been suggested as an indicator of a healthy diet (HALLIWELL 2006).

With respect to the above-mentioned, the study of antioxidant intake in different countries, and possibly in different population groups, and determination of the contribution of particular foods, and possibly of particular compounds, to antioxidant intake would be beneficial. In fact, similar research has already been conducted in some countries, for example in Spain (SAURA-CALIXTO & GOÑI 2006; TABERNETO *et al.* 2006), Italy (PELLEGRINI *et al.* 2007), Norway (SVILAAS *et al.* 2004), Japan (FUKUSHIMA *et al.* 2009), France (BRAT *et al.* 2006), and the USA (CHUN *et al.* 2005). However, for the Czech Republic, only estimates of antioxidant consumption, which were made by POKORNÝ (2007) several years ago, are available. Therefore, the aim of the starting project is to establish the level of antioxidant intake in the Czech Republic for the adult population and to study the contribution of various dietary sources to this intake.

In the present study, the intake of extractable hydrophilic and slightly lipophilic antioxidants (extractable with water-methanol mixture) was studied. These antioxidants account for a predominant part of all extractable antioxidants (PULIDO *et al.* 2003). Due to their better mobility, they can be easily absorbed in the small intestine to express *in vivo* antioxidant activity in the prevention of some diseases (see above). However, the signification of non-extractable antioxidants (and antioxidants extractable after food polymer hydrolysis) cannot be marginalised. This part of antioxidants has an unsubstitutable role in the protection of the digestive system, above all the colon (SAURA-CALIXTO 2011). Therefore, the intake of these antioxidants will be studied in the future part of this project.

The results of the present project were compared with the data on antioxidant intake levels in Spain (SAURA-CALIXTO & GOÑI 2006; TABERNETO *et al.* 2006), Italy (PELLEGRINI *et al.* 2007), and Norway (SVILAAS *et al.* 2004) obtained by similar methodology. Most importantly, the same method was used to determine antioxidant capacity, since the antioxidant capacity of food is highly dependent on the method of determination as well as on the method of food extraction (SAURA-CALIXTO & GOÑI 2006; SAURA-CALIXTO *et al.* 2009).

## MATERIAL AND METHODS

**Samples.** Antioxidant capacity was determined for 128 commodities (Table 1), after their typical adjustment for consumption. Each commodity consisted of two samples at least, which were mixed before analysis or before culinary treatment, using a weight or volume ratio of 1:1; with the exception of spices, where the samples were mixed in proportion to their consumption in the Czech Republic (RUPRICH *et al.* 2006). All analysed samples were purchased in ordinary Czech markets and shops.

**Food intake.** Information on the consumption of particular foods (recorded separately for men and women, aged 18–59 years) was obtained from a study conducted in 2003–2004, based on the EU recommendations (RUPRICH *et al.* 2006). In addition, information on the consumption of poppy seeds (which was not available from the 2003–2004 study) was obtained from the Czech Statistical Office (2010), and information on the typical consumption of green and black teas was obtained from a marketing research (ANONYMOUS 2010).

**Determination of antioxidant capacity.** The antioxidant (reducing) capacity of the various food commodities was determined by the Ferric Reducing Antioxidant Potential (FRAP) method, which is based on the reduction of complex  $\text{Fe}^{3+}$ -2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) by antioxidants present in the sample. The antioxidant content was then expressed in millimoles of  $\text{Fe}^{2+}$  ions generated per 100 g (BENZIE & STRAIN 1999; HALVORSEN *et al.* 2002), which is a double of the antioxidant content expressed in Trolox equivalents (BENZIE & STRAIN 1999).

Samples were prepared as previously described (HALVORSEN *et al.* 2002). Samples were homogenised using a kitchen mixer (with a known amount of water), a kitchen coffee-mill or manual mixing (for fats and beverages). Subsequently, 1 g of homogenised sample was weighed into three test tubes and 9 ml of methanol was added. For dry food samples, 0.1 g was weighed and 0.9 ml of distilled water was added before methanol addition. Test tubes were then placed in an ultrasonic ice-water bath (or for fats, in a room-temperature bath) for 15 minutes. After extraction, the samples were centrifuged at  $3200\times g$  for 2 minutes.

FRAP analysis was conducted as previously described (BENZIE & STRAIN 1996, 1999; PULIDO *et al.* 2000). FRAP reagent (100 ml of 0.3M acetate buffer, pH 3.6, 10 ml of 10mM TPTZ in 40mM HCl,

Table 1. Analysed food commodities

Categories	Commodities
Fats	margarine, vegetable oils, solid vegetable fats, butter, butter spreads, lard
Sugar and sweets	sugar, non-chocolate confectionery, cream cakes, wafers, cocoa powder and cocoa instant drink, chocolate, chocolate sweets and bars, chocolate nut spreads
Non-alcoholic beverages	fruit tea, black tea, green tea, herbal tea, instant coffee, coffee, coffee substitutes, coca-cola, 100% juice, lemonade, instant drinks, water: drinking, table and mineral, syrup
Alcoholic beverages	white wine, red wine, beer, spirits
Cereal products	bread, rolls and French loaf, short pastry, wholemeal bread, durable pastry, pasta, rice, flour and semolina, ready-to-eat cereals, cereal bars
Vegetables and vegetable products	cauliflower, cabbage, other cole crops, onions, garlic, cucumbers, green pepper, tomatoes, other fruiting and bulbous vegetables, celeriac, parsley, carrots, other root vegetables, leafy vegetables, young peas, butter-beans, potatoes, canned peas, tomato ketchup, tomato paste, pickled gherkins, sauerkraut, potato crisps, French fries
Pulses	lentils, others pulses
Fruit and fruit products	apples, pears, stone fruits, grapes, lemons, mandarins, oranges, others citrus fruit, bananas, strawberries, bilberries, others soft fruit, others subtropical fruit, fruit in syrup, raisins, others dried fruit, jams and marmalade
Meat and meat products	beef, pork, chicken meat, hen meat, goose and duck meat, turkey meat, other meat, mincemeat, offal, dry salami, cooked salami, sausages, cooked meat products, other meat products, canned meat, canned meals, sea fish, freshwater fish, fish products, canned fish
Milk and milk products	milk, yogurt, fermented milk products, ice cream, curd, others milk products
Cheese	hard cheese, soft cheese, mould cheese, processed cheese, other cheeses
Eggs	eggs, egg products
Convenience food and meals	dumplings, instant soups, fast food meals
Other food	honey, mustard, poppy seeds, nuts and seeds, fresh mushrooms, spices

and 10 ml of 20mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) was freshly prepared, and 3 ml of FRAP reagent was pipetted into spectrophotometric cuvettes, which were heated to 37°C. Then, 100 µl of extract or diluted extract was added to the reagent, and the cuvettes were incubated at 37°C for 30 minutes. Absorbance was measured at a wavelength of 595 nm against water. A blank sample (where the extract was replaced with distilled water) and calibration samples (where the extract was replaced with freshly prepared solutions of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in distilled water at a concentration range of 0–2 mol/l) were prepared and measured simultaneously every day.

## RESULTS AND DISCUSSION

### Antioxidant capacity of different food commodities

The antioxidant capacity of particular commodities was determined after their typical adjustment for consumption. However, the obtained results were

subsequently recalculated and expressed in mmol  $\text{Fe(II)}/100\text{ g}$  (100 ml) of purchased food, with respect to food consumption data taken from RUPRICH *et al.* (2006). These data (Table 2) are in agreement with the results determined in Spain (SAURA-CALIXTO & GOÑI 2006) and Norway (HALVORSEN *et al.* 2002). A very high antioxidant content (typically dwarfing 100-fold the content of antioxidants found in other commodities) was found in black, green tea and coffee, all known for their high antioxidant capacity (SVILAAS *et al.* 2004; SAURA-CALIXTO & GOÑI 2006; FUKUSHIMA *et al.* 2009). In addition, a relatively high antioxidant content was also found in cocoa products, consistent with the significant antioxidant activity reported for cocoa (TABERNETO *et al.* 2006), and in spices, which are well-known for their high antioxidant activity (SIKORA *et al.* 2008). Other commodities were found to have generally lower antioxidant capacity, with a few exceptions, such as nuts, seeds or red wine. Very low antioxidant capacity was detected for meat, cheese and milk and milk products (with the exception of some meat products).

Table 2. Antioxidant content in the particular food categories

Category	Antioxidant capacity (mmol/100 g)*		
	average	min	max
Fats	0.25	nd (lard)	0.55 (vegetable oils)
Sugar and sweets	2.79	0.34 (cream cakes)	11.55 (cocoa powder and instant cocoa drinks)
Non-alcoholic beverages	49.55	0.05 (water: drinking, table and mineral)	171.65 (coffee, instant)
Alcoholic beverages	0.83	0.03 (spirits)	2.46 (red wine)
Cereal products	0.40	0.12 (rolls, French loaf and pasta)	0.76 (wholemeal bread)
Vegetables and vegetable products	0.46	0.09 (celeriac)	2.77 (green peppers)
Pulses	0.87	0.84 (others pulses)	0.89 (lentils)
Fruit and fruit products	0.85	0.12 (bananas)	3.49 (others soft fruit)
Meat and meat products	0.16	0.02 (chicken and beef meat)	0.49 (other meat products)
Milk and milk products	0.20	0.06 (milk)	0.46 (ice cream)
Cheese	0.10	0.05 (hard cheese)	0.15 (soft cheese)
Eggs and egg products	0.06	0.03 (eggs)	0.09 (egg products)
Convenience food and meals	0.24	0.13 (dumplings)	0.43 (instant soup)
Other food	8.30	0.20 (fresh mushrooms)	45.5 (spices)

\*(mmol/100 ml) for beverages; nd – non-detectable

For cereal products, the highest antioxidant capacity was detected for wholemeal products, probably because antioxidants are present in cereal grains in outer layers (MILLER *et al.* 2000), while the lowest antioxidant capacity was found in common light pastries. In agreement with previous studies, the highest antioxidant content in vegetables and vegetable products was detected in peppers; while within fruit and fruit products, berries, such as strawberries, blueberries, currants, blackberries, and raspberries, were found to have the highest antioxidant content (MILLER *et al.* 2000; HALVORSEN *et al.* 2002).

#### Contribution of particular food groups to the intake of antioxidants

The contribution of particular food groups to antioxidant intake (Table 3) depended on both the antioxidant capacity of the foods and their contribution to the consumption food basket. Thus, coffee was the most significant source of extractable antioxidants in the Czech Republic: 43.1% for men and 54.6% for women. In addition, for men only, the contribution from beer was

also significant (15% vs. 1.8% for women). Other important sources of the studied antioxidants were green and black tea (contributing in all to 7.5% of the antioxidant intake for men and 9.3% for women), vegetable and vegetable products (6.1% for men and 5.5% for women), fruit and fruit products (3.6% and 6.5%), cereal products (4.3% and 3.1%), wine (2.5% and 3.6%), sugar and sweets (2.7% and 2.5%), spices (3.2% and 1.8%) and meat and meat products (2.6% and 1.2%). Small amounts of antioxidants (between 0.5 and 1.0% of the intake) were supplied by nuts and seeds, milk and milk products, and fats. Pulses, eggs and eggs products, convenience food, and cheese were insignificant sources (< 0.5%) of antioxidant intake.

Within the fruit and fruit products category, apples were the most significant source of extractable antioxidants in the Czech Republic (37% of all fruit and fruit product derived antioxidants), while other important sources of fruit-derived antioxidants were oranges and stone fruits (17% and 14% of fruit and fruit product derived antioxidants). In the vegetable and vegetable products category (including potatoes and potato products) (RUPRICH *et al.* 2006), peppers were the largest

Table 3. Intake of antioxidants (AO) from the particular food groups

Category/commodity	Intake AO (mmol/day)		Share of intake AO (%)	
	men	women	men	women
Fats	0.102	0.082	0.6	0.5
Sugar and sweets	0.428	0.378	2.7	2.5
Coffee	7.168	8.184	43.1	54.6
Tea	1.241	1.400	7.5	9.3
Other non-alcoholic beverages	0.920	1.050	5.5	7.0
Beer	2.496	0.266	15.0	1.8
Wine	0.421	0.544	2.5	3.6
Spirits	0.002	0.001	0.0	0.0
Cereal products	0.717	0.459	4.3	3.1
Vegetables and vegetables products	1.014	0.827	6.1	5.5
Fruit and fruit products	0.604	0.970	3.6	6.5
Pulses	0.030	0.029	0.2	0.2
Meat and meat products	0.437	0.184	2.6	1.2
Cheese	0.022	0.018	0.1	0.1
Milk and milk products	0.090	0.127	0.5	0.8
Eggs and egg products	0.031	0.023	0.2	0.2
Spices	0.526	0.277	3.2	1.8
Nuts and seeds	0.157	0.102	0.9	0.7
Convenience food and meals	0.058	0.023	0.3	0.2
Other food	0.070	0.048	0.4	0.3

source of the studied antioxidant intake (providing 30% of all antioxidants coming from vegetable and vegetable products), while other important sources of antioxidants were potatoes (providing 16% of all antioxidants from this food group), sauerkraut and onions (each providing 10% of all antioxidants from vegetable and vegetable products).

In general, these results are in agreement with results obtained for the same type of antioxidants in other countries, such as Norway, Italy and Spain.

In Norway, coffee also contributed most to the antioxidant intake (66% of the antioxidant intake), while other important sources of antioxidants were fruits and berries (9% of the antioxidant intake), tea (8.5%), cereals (4.5%), wine (3.5%) and vegetables (including potatoes) (2.6%). Edible fats and cakes provided a small share of the antioxidant intake (between 1% and 2%), while milk, meat, sweets and beer were insignificant sources of antioxidant intake (< 0.6%) (SVILAAS *et al.* 2004).

In Italy, coffee and tea (38.1% of the antioxidant intake for women and 26.8% for men) together with alcoholic beverages (providing 13.9% of the antioxidant intake for women and 37.2% for men;

75% of which consisted of red wine) were the most significant sources of antioxidant intake. Other sources of antioxidants were fruits (11.7% of the antioxidant intake), vegetables (including potatoes) (6.9%), bread and cereals (4.4%), chocolate (0.9%), and oils and fats (0.7%) (PELLEGRINI *et al.* 2007).

In Spain, coffee was also the largest source of antioxidant intake (44.5%), similar to results obtained in other countries. Other important contributors to the antioxidant intake included wine (approximately 14% of all antioxidants), fruits (13%), vegetables (including potatoes) (7%), nuts (4.4%), and pulses (3.5%). Cereals provided only 3% of the antioxidant capacity of the Spanish diet, while tea provided only 2.7% and vegetable oils less than 1% (SAURA-CALIXTO & GOÑI 2006). Unfortunately, this study did not evaluate the contribution of cocoa products to the intake of antioxidants. However, in a subsequent study, cocoa products were found to contribute between 7.3% and 9.5% of the antioxidant intake in Spain (depending on the method used for antioxidant capacity determination; TABERNETO *et al.* 2006).

### Overall intake of extractable antioxidants and its interpretation

The average intake of extractable antioxidants in the Czech Republic was 16.64 and 14.99 mmol/day for men and for women, respectively; lower than the intake of these antioxidants in Italy (18.75 mmol/day) (PELLEGRINI *et al.* 2007) and Norway (17.45 mmol/day) (SVILAAS *et al.* 2004). However, the dietary antioxidant capacity in the Czech Republic was higher than in Spain (12.03 mmol/day) (SAURA-CALIXTO & GOÑI 2006). This result was relatively surprising, because the Spanish diet (as well as the Italian diet) is considered to be a typical Mediterranean diet, which has been associated with the prevention of the development of cardiovascular diseases, also due to its high antioxidant content (VISIOLI & GALLI 2001).

This contradiction may be due to the fact that in the Spanish study, only the plant-derived antioxidant intake was evaluated (SAURA-CALIXTO & GOÑI 2006), in contrast with the other countries – Czech Republic, Norway (SVILAAS *et al.* 2004) and Italy (PELLEGRINI *et al.* 2007). Therefore, the absolute intake (i.e. in mmol/day) of extractable antioxidants provided by different types of food was also compared between the particular countries (Figure 1).

For this comparison, the unequal *in vivo* effectiveness of antioxidants from various types of food was also taken into account. For example, after drinking an antioxidant-containing beverage, the antioxidant

capacity in plasma rises to a maximum relatively early following its consumption (after 30–60 min) and then decreases back to its original value quite quickly. In contrast, the consumption of solid food containing antioxidants causes the antioxidant capacity in plasma to increase to a maximum value after 2–3 h, returning to baseline values approximately 4–5 h later. Thus, the antioxidant activity of the plasma is enhanced for a longer time following the consumption of solid food (SERAFINI *et al.* 2006).

Furthermore, the consumption of coffee (which represents the largest share of the antioxidant intake in many countries, see above) is also connected with a higher occurrence of risk factors for cardiovascular diseases (including increased blood pressure and increased concentration of plasma homocysteine), though not all studies confirmed it (HIGDON & FREI 2006). The intake of antioxidants through alcoholic beverages may also be questionable. Though alcohol increases the absorption of antioxidants in the digestive tract (PORRINI & RISO 2008), it is also associated with cancer proliferation (BROWN 2005).

In contrast, fruits and vegetables contain other compounds which act to prevent the development of some diseases traditionally associated with oxidative stress, such as fibre (HALLIWELL 2006). Although fibre is also contained (together with a relatively high concentration of antioxidants) in other foods such as wholemeal bread or nuts, these commodities have a higher content of unsaturated fatty acids, requiring a higher intake of antioxidants (KAMAL-ELDIN & PICKOVA 2008). However, these commodities are significant sources of non-extractable antioxidants (SAURA-CALIXTO *et al.* 2007) effective in the colon protection (as it was described in the introduction).

The bioavailability of antioxidants is affected by many other factors (SERAFINI *et al.* 2006; PORRINI & RISO 2008). However, considering the above, the intake of extractable antioxidants through fruits and vegetables is probably a better indicator of the quality of a diet (in terms of the intake of these antioxidants and the *in vivo* prevention of diseases associated with oxidative stress) than the overall intake of these antioxidants. Accordingly, a number of epidemiological studies have found an indirect relationship between the intake of fruits and vegetables and the risk for cardiovascular diseases, neurodegenerative diseases, cancer, diabetes and some other problems associated with aging (HALLIWELL 2006). In addition, other authors have

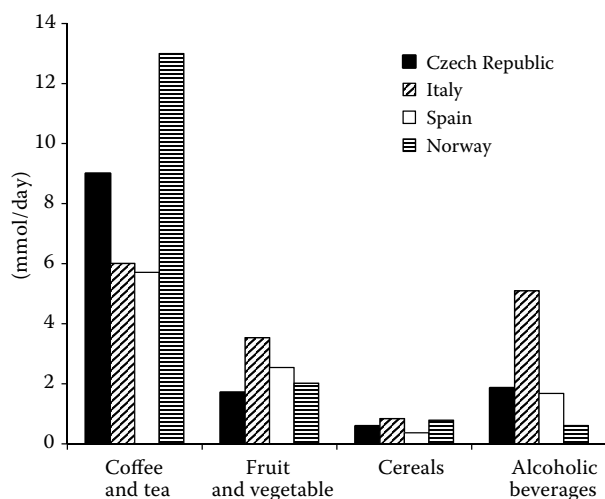


Figure 1. Intake of antioxidants from the particular food groups in the Czech Republic, Italy (PELLEGRINI *et al.* 2007), Spain (SAURA-CALIXTO *et al.* 2006) and Norway (SVILAAS *et al.* 2004)

recommended an increase in antioxidant intake by the increased intake of fruits and vegetables, even though a high content of antioxidants is present in other foods, such as coffee, tea and red wine (CORNELLI 2009).

As can be seen in Figure 1, the intake of extractable antioxidants through fruit and vegetable consumption is lower in the Czech Republic than in Spain, Italy and Norway. Thus, the quality of diet in the Czech Republic (in terms of *in vivo* oxidative stress prevention) is probably worse than in other European countries. Therefore, nutrition education programmes in the Czech Republic should focus on the increased intake of fruits and vegetables. In this context, the intake of all fruits and vegetables should be increased, not only of those species with significantly higher antioxidant activity (i.e. berries, stone fruits or peppers); fruits and vegetables are sources of other beneficial compounds, such as fibre and many other phytochemicals, which may prevent the development of some diseases associated traditionally with oxidative stress (HALLIWELL 2006).

## CONCLUSIONS

In the Czech Republic, the daily intake of extractable antioxidants was found to be 16.64 mmol/day for men and 14.99 mmol/day for women; less than in Italy and in Norway, but slightly more than in Spain. However, the intake of these antioxidants from fruits and vegetables (probably better sources of these substances for the prevention of *in vivo* development of diseases associated with oxidative stress than other foods and drinks, above all coffee and alcoholic beverages) was lower than in Spain, Italy and Norway. Therefore, nutrition education programmes in the Czech Republic should focus on increasing the intake of fruits and vegetables.

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Received for publication April 18, 2011

Accepted after corrections October 5, 2011

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