Antioxidant activity and polyphenol content of Bulgarian fruits

Article *in* Bulgarian Journal of Agricultural Science · February 2013

Bulgarian Journal of Agricultural Science, 19 (No 1) 2013, 22-27
Agricultural Academy

ANTIOXIDANT ACTIVITY AND POLYPHENOL CONTENT OF BULGARIAN FRUITS

P. DENEV[1], A. LOJEK[2], M. CIZ[2] and M. KRATCHANOVA[1]
[1] *Bulgarian Academy of Sciences, Institute of Organic Chemistry with Centre of Phytochemistry, Laboratory of Biologically Active Substances, BG – 4000 Plovdiv, Bulgaria*
[2] *Institute of Biophysics, Academy of Sciences of the Czech Republic, Kralovopolska 135, Brno-612 65, Czech Republic*

Abstract

DENEV, P., A. LOJEK, M. CIZ and M. KRATCHANOVA, 2013. Antioxidant activity and polyphenol content of Bulgarian fruits. *Bulg. J. Agric. Sci*., 19: 22-27

The antioxidant activity of 26 Bulgarian fruits was measured by the Oxygen Radical Absorbance Capacity (ORAC) method. The total polyphenol content of the samples was investigated as well. Results varied considerably among the analyzed samples. Based on fresh weight, fruits of the elderberry *(Sambucus nigra)* showed the highest antioxidant capacity (205.4 ± 15.2 µmol TE.g^{-1}), followed by brier *(Rosa canina)* (201.1 ± 14.6 µmol TE.g^{-1}), chokeberry (*Aronia melanocarpa*) (160.8 ± 4.8 µmol TE.g^{-1}) and hawthorn (*Crataegus mollis*) (153.6 ± 9.1 µmol TE.g^{-1}). There was a good linear correlation between ORAC antioxidant capacity and polyphenol content, indicating that polyphenols are the major contributors to the antioxidant activity of the investigated fruits.

Key words: antioxidant activity, ORAC, polyphenols, fruits

Abbreviations: FL – Fluorescein; FW – Fresh Weight; ORAC – Oxygen Radical Absorbance Capacity; RNS – Reactive Nitrogen Species; ROS – Reactive Oxygen Species; TE – Trolox Equivalents

Introduction

A growing amount of evidence indicates the role of reactive oxygen species (ROS) such as peroxyl radicals (ROO$^{\cdot}$), hydroxyl radical (HO$^{\cdot}$), superoxide anion ($O_2^{\cdot-}$) and singlet oxygen ($^1O_2$) in the pathophysiology of aging and different degenerative diseases such as cancer, cardiovascular diseases, Alzheimer's disease and Parkinson's disease (Davies, 2000; Fenkel and Holbrook, 2000). Living cells possess a protective system of antioxidants, which prevents the formation and enables inactivation of ROS and reactive nitrogen species (RNS). To increase the antioxidant status of the body, external intake of antioxidants is necessary. The majority of antioxidants taken with the diet are of plant origin. The richest sources of antioxidants are fruits, vegetables, herbs, and grains. The compounds that are the most significant for the antioxidant properties of these raw materials are polyphenols, vitamins A, C, and E, carotenoids etc. The formulation of preventive and healthy nutrition requires information about the content of biologically active substances in raw materials and evaluation of their biological activity. In the last decade, there is an increased interest in the determination of antioxidant activity of foods. Plant materials contain various biologically active substances with different antioxidant capacities, and for the consumers it is difficult to estimate the intake of antioxidant units taken with the diet.

E-mail: petkodenev@yahoo.com; lbas@plov.omega.bg; milanciz@ibp.cz; alojek@ibp.cz

Therefore, it is necessary to characterize the antioxidant capacities of different plant materials.

Despite the vast interest in determination of antioxidant activity of natural products, in the literature there are only few papers reporting scarce data for the antioxidant capacities of Bulgarian plant materials. There is limited information about the antioxidant activity of Bulgarian herbs (Ivanova et al. 2005; Kiselova et al., 2006; Kratchanova et al., 2010; Nikolova et al., 2011) and vegetables (Ciz et al., 2010). To our knowledge there are not papers reporting the antioxidant activity of Bulgarian fruits. Fresh fruits are a good source of antioxidants and their use in human nutrition is of fundamental importance. To encourage fruit consumption among the population it is important to recognize which fruits have the highest antioxidant activity and to promote their regular consumption. Therefore, the main objective of the current work was to evaluate the ORAC antioxidant activity and total polyphenol content of 26 fruits of Bulgarian origin and to explore their relations. The current survey is a part of a long-term investigation, which aims to develop a database with antioxidant capacities of mushrooms, fruits, vegetables, grains and herbs cultivated in Bulgaria.

Materials and Methods

Chemicals and apparatus

2,2'-azobis[2-methyl-propionamidine] dihydrochloride, 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), fluorescein disodium salt and gallic acid were obtained from Sigma-Aldrich (Steinheim, Germany). Folin-Ciocalteu's phenol reagent was purchased from Merck (Darmstadt, Germany). All other solvents used were of analytical grade.

Fruit samples

Fruits were sampled at the time of peak production from retail outlets in the local supermarkets. After delivery they were frozen and kept at T = -18ºC in plastic bags. The following fruits, all of Bulgarian origin were investigated: apple *(Malus domestica)* – golden delicious var., apricot *(Armeniaca vulgaris)*, black currant *(Ribes nigrum)*, blackberry *(Rubus caesius)*, blackthorn *(Prunus spinosa)*, blueberry (*Vaccinium myrtillus)*, brier *(Rosa canina)*, cherry *(Cerasus avium)*, chokeberry (*Aronia melanocarpa*), cornel cherry *(Cornus mas)*, cranberry *(Vaccinium oxycoccos)*, elderberry *(Sambucus nigra)*, fig *(Ficus carica* L.), hawthorn *(Crataegus mollis)*, honeydew melon *(Cucumis melo).* peach *(Persica vulgaris)*, plum *(Prunus domestica)*, pomegranate (*Punica granatum)*, pumpkin *(Cucurbita pepo)*, raspberry *(Rubus idaeus)*, red grapes (*Vitis vinifera*), sour cherry *(Cerasus vulgaris)*, strawberry *(Fragaria ananassia)*,watermelon *(Citrullus lanatus)*, white grapes (*Vitis vinifera*).

Sample preparation

Approximately 2 g of the edible part of the fruits were weighed accurately and homogenized in a laboratory blender. Samples were transferred into extraction tubes and mixed with 20 ml of the extragent (0.2% formic acid in 80% acetone solution). Extraction was conducted on orbital shaker at room temperature for one hour. After that, the samples were centrifuged (6 000 x *g)* and supernatants were removed. The solid residues were subjected to the second extraction under the same conditions. Both supernatants were combined and further used for antioxidant activity and total polyphenol determination after a proper dilution.

ORAC assay

The ORAC method measures the antioxidant scavenging activity against peroxyl radical induced by 2,2'-azobis[2-methyl-propionamidine] dihydrochloride dihydrochloride at 37°C. Fluorescein (FL) is used as a fluorescent probe (Ou et al., 2001). The loss of fluorescence of FL is an indication of the extent of damage from its reaction with the peroxyl radical. The protective effect of an antioxidant is measured by assessing the area under the fluorescence decay curve as compared to that of blank in which no antioxidant is present. One ORAC unit was assigned to the net protection area, provided by solution of Trolox with concentration 1 μmol.l^{-1}. Results were expressed as μmol Trolox equivalents (TE) per gram fresh weight. Denev et al., (2010), describe the detailed experimental procedure used in the current study.

ORAC analyses were carried out on a FLUOstar Galaxy plate reader (BMG LABTECH, Offenburg, Germany), excitation wavelength of 485 nm and emission wavelength of 520 nm were used.

Total polyphenols analysis

Total polyphenols were determined according to the method of Singleton and Rossi, (1965) with Folin-Ciocalteu's reagent. Results were expressed as gallic acid equivalents (GAE) per 100 g of fresh weight.

Statistics

Statistical analyses and graphs were performed using Microsoft Excel (Microsoft Corporation). All experiments were repeated six times. The data were expressed as means ± standard deviation (SD).

Results and Discussion

Antioxidant activity rank order

There are different methods to evaluate the *in vitro* antioxidant activity of compounds, natural products, and biological fluids. These methods rely on generation of various radicals acting by different mechanisms. The current study employs ORAC method, which provides a unique and complete assessment of antioxidant activity, in which the inhibition time and inhibition degree are measured as the oxidation reaction goes to completion (Ou et al., 2001). The ORAC method has also been found to be the most relevant one for biological samples, since it assesses the radical scavenging activity of the sample against peroxyl radicals, which physiologically are the most important ones (Huang et al., 2005; Prior et al, 2005). It was found that ORAC method is more sensitive than other methods, thus indicating antioxidant properties in samples with very low quantities of polyphenols (Ciz et al., 2010). Therefore, we chose this assay to investigate the antioxidant activity in the current work, which to our knowledge is the most comprehensive study on the antioxidant properties of Bulgarian fruits. The total antioxidant activity varied considerably among the investigated fruits (Figure 1). For example, on the basis of fresh weight, elderberry showed the highest antioxidant capacity (205.38 ± 15.24 µmol TE.g^{-1}) followed by brier with (201.14 ± 14.59 µmol TE.g^{-1}). Pumpkin, watermelon and honeydew melon revealed the lowest ORAC antioxidant activity (4.92 ± 0.47, 3.80 ± 0.47 and 2.33 ± 0.12 µmol TE.g^{-1}, respectively). The beneficial properties of elderberry, brier and hawthorn are well known to the Bulgarian population as they have been widely used in ethnopharmacology and traditional medicine since ancient times. Another fruit with high antioxidant activity, chokeberry is of North American origin and was introduced to Bulgaria about 40 years ago. Nowadays it is cultivated successfully as an industrial crop and deserves attention, because it is recognized as an especially beneficial medicinal plant. There are many papers attempting to rank the antioxidant properties of different plant materials via different methods (Velioglu et al. 1998; Pellegrini et al., 2003), including ORAC (Wang et al., 1996; Ou et al, 2002; Wu et al., 2004). When comparing the results with other published data it is seen that Bulgarian fruits reveal different antioxidant properties. Our results 47.2 µmol TE.g^{-1} and 98.8 µmol TE.g^{-1} for strawberries and blueberries are 18.5% and 37% higher than the results reported by Wu et al. (2004) for the same plant species. On the other hand, results reported from the same authors for raspberry are 26% higher than the results in our study. The accumulation of biologically active substances in plants depends on several genetic and environmental factors including cultivar, climate, fertilization, irrigation, sun exposure, etc. Ou et al. (2002) reported variable results even for samples from one species depending on the variety, place of origin, and harvest time. Therefore, it is of a particular interest to evaluate the antioxidant activity of local natural products, since they are the most consumed by the Bulgarian population. The current survey is a part of a long-term investigation, aiming the development of a database with antioxidant capacities of Bulgarian raw materials. The development of such database will identify the major contributors to the antioxidant potential of our daily diet. In the literature similar ORAC databases are already reported for common foods in the USA (Wu et al., 2004) and fruits produced in south Andes region of South America (Speisky et al., 2012).

In one of the first attempts to quantify dietary antioxidant needs of the body Prior et al. (2007) demonstrated that consumption of certain berries and fruits such as blueberries, mixed grape and kiwifruit was associated with increased ORAC plasma antioxidant ca-

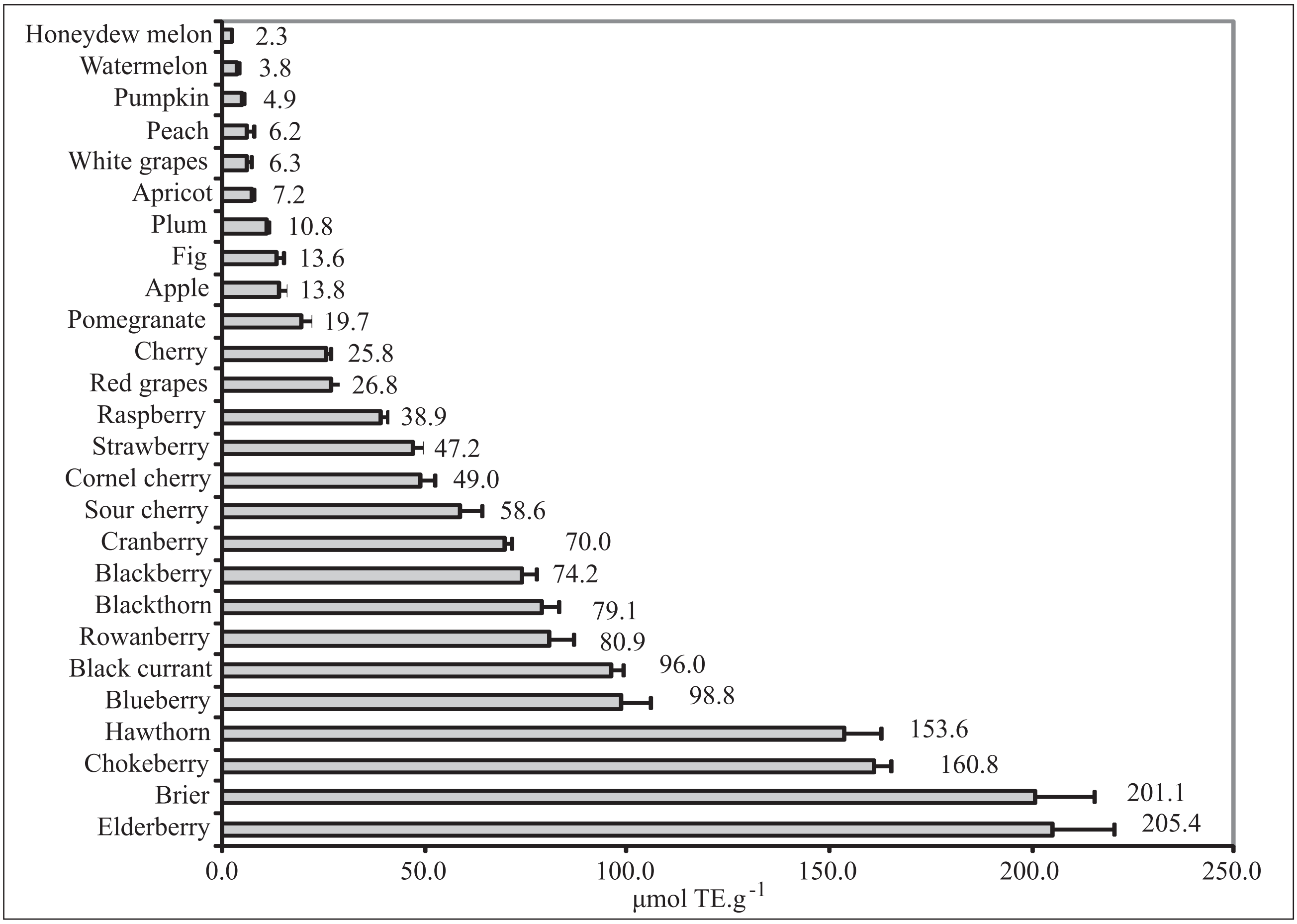

Fig. 1. Antioxidant activity (ORAC) of fruits. Data expressed as mean ± SD, n=6

pacity in the postprandial state and consumption of an energy source of macronutrients containing no antioxidants was associated with a decline in plasma antioxidant capacity. The authors estimated that according to the energy intake of the diet, 5000 – 15000 μmol TE are necessary to cover human daily antioxidant needs. Therefore, comparative studies such as the current are interesting not only from research point of view, but also for the consumers and nutritionists. The obtained results are a good tool for the medical professionals to promote the consumption of fruits with high antioxidant activity as a part of a healthy diet. For example, the consumption of only 25-75 g elderberries or briers, or 30-90 g chokeberries will cover the necessary antioxidant units per day. In contrast, approximately 2170 – 6500 g of honeydew melons will provide the same amount of ORAC units.

Correlation between polyphenol content and ORAC antioxidant activity

As reported by Prior et al. (1998), the major phytochemicals responsible for the antioxidant capacity of plant materials are most likely polyphenols, and particularly flavonoids. Flavonoids are known as secondary plant metabolites and their biosynthesis and content are influenced by a great number of factors, such as location, weather conditions, degree of maturity, variety, storage condition, processing etc. (Ayala-Zavala et al., 2004). Table 1 summarizes the polyphenols content of the investigated materials and their dry matter as well. The relationships between total polyphenol content and ORAC antioxidant capacity are shown on Figure 2. It is evident that, with few exceptions, there is a good linear correlation between these two entities with regression coefficient R^2 = 0.899. Such an exception is elderber-

Table 1
Polyphenol content of selected Bulgaria fruits

Sample	Dry matter, g/100g	Total polyphenols, GAE/100g
Apple	12.39	126.0 ± 5.6
Apricot	10.26	44.4 ± 0.4
Black currant	20.68	835.1 ± 19.1
Blackberry	21.53	688.2 ± 19.0
Blackthorn	29.59	858.3 ± 19.4
Blueberry	13.63	819.5 ± 9.7
Brier	34.48	1 934.3 ± 4.3
Cherry	13.97	118.4 ± 6.7
Chokeberry	28.97	1 817.8 ± 34.8
Cornel cherry	23.03	624.6 ± 1.3
Cranberry	16.78	705.5 ± 17.9
Elderberry	21.12	1 148.0 ± 11.9
Fig	18.32	98.7 ± 2.8
Hawthorn	29.42	1 184.4 ± 15.7
Honeydew melon	8.07	40.4 ± 1.1
Peach	20.93	41.1 ± 1.4
Plum	16.81	64.5 ± 1.7
Pomegranate	10.57	195.1 ± 8.0
Pumpkin	6.27	14.6 ± 0.9
Raspberry	15.02	369.1 ± 1.7
Red grapes	21.12	195.5 ± 8.9
Rowanberry	34,15	733,6 ± 7,4
Sour cherry	15.24	529.9 ± 10.0
Strawberry	10.44	386.5 ± 15.2
Watermelon	9.31	39.8 ± 0.8
White grapes	14.51	112.1 ± 0.3

Data expressed as mean ± SD (n=6)

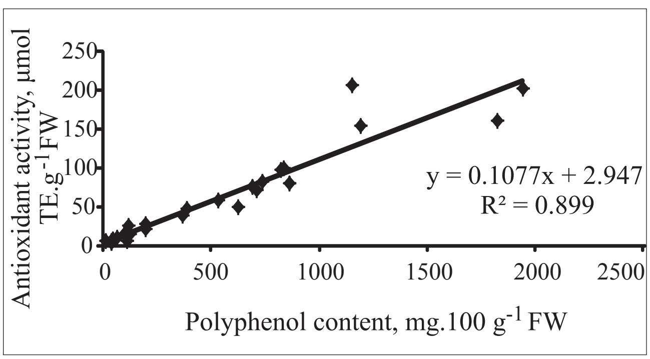

Fig. 2. Correlation between total polyphenols and ORAC antioxidant activity of fruits

ry, whose polyphenol content is comparable to that of hawthorn, but its antioxidant capacity is higher. This discrepancy could be explained with the different polyphenol composition of the tested fruits, since individual flavonoids differ significantly in their antioxidant capacities (Ou et al., 2002).

Conclusions

In summary, the ORAC antioxidant activity and the total polyphenol content of 26 fruits was measured in the most comprehensive study on the antioxidative properties of Bulgarian fruits. There is a good linear correlation between the total polyphenols content and the ORAC antioxidant activity of the investigated fruits. The results for the antioxidant activity of selected Bulgarian fruits are a good tool for the medical professionals to promote the consumption of fruits with high antioxidant activity as a part of a healthy diet. Such kind of information will be useful to not only for doctors and nutritionists, but to food scientist and technologists for the development of fruit functional foods, rich in natural antioxidants. The results from the current study will enrich the national database for antioxidant activity in foods and will help the identification of the major contributors to the antioxidant potential of our daily diet.

Acknowledgments

This study was supported by grant № DMU 03/74 of the Bulgarian Ministry of Education and Science and Youth.

References

Ayala-Zavala, J., S. Wang, C. Wang and G. González-Aguilar, 2004. Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. *Lebensmittel-Wissenschaft und Technologie*, **37** (7): 687-695.

Ciz, M., H. Cizova, P. Denev, M. Kratchanova, A. Slavov and A. Lojek, 2010. Different methods for control and comparison of the antioxidant properties of vegetables. *Food Control*, **21**: 518–23.

Davies, K. J., 2000. Oxidative stress, antioxidant defenses and damage removal, repair and replacement systems. *IUBMB life*, **50**: 279-289.

Denev, P., M. Ciz, G. Ambrozova, A. Lojek, I. Yanakieva and M. Kratchanova, 2010. Solid-phase extraction of berries' anthocyanins and evaluation of their antioxidative properties. *Food Chemistry*, **123**: 1055–1061.

Fenkel, T. and N. J. Holbrook, 2000. Oxidants, oxidative stress and the biology of aging. *Nature,* **408**: 240-247.

Huang, D., B. Ou and R. L. Prior, 2005. The Chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry,* **53:** 1841-1856.

Ivanova, D, D. Gerova, T. Chervenkov and T. Yankova, 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology,* **96:** 145-150.

Kiselova, Y., D. Ivanova, T. Chervenkov, D. Gerova, B. Galunska and T. Yankova, 2006. Correlation between the In Vitro antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. *Phytotherapy Research*, **20** (11): 961–965.

Kratchanova, M., P. Denev, M. Ciz, A. Lojek and A. Mihailov, 2010. Evaluation of the antioxidant activity of medicinal plants containing polyphenol compounds. Comparison of two extraction systems. *Acta Biochimica Polonica*, **57**: 229-234.

Nikolova, M., L. Evstatieva and T. D. Nguyen, 2011. Screening of plant extracts for antioxidant properties. *Botanica Serbica*, **35** (1): 43-48.

Ou, B., M. Hampsh-Woodill and L. R. Prior, 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry,* **49**: 4619-4626.

Ou, B., D. Huang, M. Hampsch-Woodill, J. A. Flanagan and E. K. Deemer, 2002. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *Journal of Agricultural and Food Chemistry,* **50**: 3122–3128.

Pellegrini, N., M. Serafini, B. Colombi, D. Del Rio, S. Salvatore, M. Bianchi and F. Brighenti, 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy by tree different in vitro assays. *Journal of Nutrition,* **133:** 2812–2819.

Prior, R. L., G. Cao, A. Martin, E. Sofic, J. McEwen, C. O'Brien, N. Lischner, M. Ehlenfeldt, W. Kalt, G. Krewer and C Mainland, 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of *Vaccinium species. Journal of Agricultural and Food Chemistry,* **46:** 2686-2693.

Prior, R. L., X. Wu and K. Schaich, 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry,* **53:** 4290–4302.

Prior, R. L., H. Hoang, L. Gu, X. Wu, R. Jacob, G. Sotoudeh, A. Kader and R. Cook, 2007. Plasma antioxidant capacity changes following a meal as a measure of the ability of a food to alter *in vivo* antioxidant status. *Journal of the American College of Nutrition*, **26** (2): 170–181.

Singleton, V. and J. Rossi, 1965. Colorimetry of total phenolic with phosphomolibdiphosphotungstic acid reagents. *American Journal of Enology and Viticulture,* **16:** 144-158.

Speisky, H., C. López-Alarcón, M.Gómez, J. Fuentes and C. Sandoval-Acuña, 2012. First web-based database on total phenolics and oxygen radical absorbance capacity (ORAC) of fruits produced and consumed within the South Andes region of South America. *Journal of Agricultural and Food Chemistry*, Article in press, DOI: 10.1021/jf205167k.

Velioglu, Y., G. Mazza, L. Gao and B. Oomah, 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products *Journal of Agricultural and Food Chemistry,* **46** (10)**:** 4113-4117.

Wang, H., G. Cao and R. L. Prior, 1996. Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry,* **44:** 701-705.

Wu, X., L. Gu, J. Holden, D. Haytowitz, S. Gebhardt, G. Beecher and R. L. Prior, 2004. Development of a database for total antioxidant capacity in foods: a preliminary study. *Journal of Food Composition and Analysis,* **17**: 407–422.

Received April, 2, 2012; accepted for printing December, 2, 2012.