ICP 2012 - International Conference on Polyphenols Florence - Italy

Oxygen radical absorbance capacity (ORAC) of bilberry (*Vaccinium myrtillus*) powder and nettle (*Urtica dioica*) seed

Mari H. Jaakkola*, Marianne Mäki, Vesa T. Virtanen
University of Oulu, Kajaani University Consortium, CEMIS-Oulu, Analytical Chemistry and Bioanalytics, Salmelantie 43,FI-88600 Sotkamo, Finland *corresponding author: mari.jaakkola@oulu.fi

Abstract. The oxygen radical absorbance capacity (ORAC) of bilberry (*Vaccinium myrtillus*) powder and dried nettle (*Urtica dioica*) seed powder was studied. In addition, the affect of the following parameters in the ORAC analysis were investigated: 1) extraction solvent (acetone/water, ethanol and acidic methanol), 2) extract dilution, and 3) addition of ethylenediaminetetraacetic acid disodium salt hydrate (EDTA) as a metal chelating agent. Each extract was analysed with at least four different dilutions (from 50 to and 500 fold) and quercetin (3,3',4',5,7-pentahydroxyflavone) was studied as a reference standard. The use of EDTA in the buffer increased the ORAC values of quercetin and nettle seed 25% and 20%, respectively. This indicates that EDTA prevented antioxidants from autoxidation. Extraction solvent had a significant effect on the result. The highest ORAC values for both nettle and bilberry were obtained with acetone/water extraction, whereas lowest results were obtained with ethanol. Extract dilution affected the ORAC value with all studied samples but the affect was not remarkable. Average ORAC values obtained with acetone/water extract and addition of EDTA for the studied bilberry (n=12) and nettle seed (n=11) samples were at the same level: 614 µmolTE/g (SD=13%) and 543 µmolTE/g (SD=10%), respectively.

Introduction. Oxygen radical absorbance capacity (ORAC) is a method of measuring antioxidant capacities of samples *in-vitro*. Antioxidants are believed to have a significant role in the body defense system against reactive oxygen species that induces diseases such as inflammation, cancer, cardiovascular disease and aging-related disorders. Polyphenol rich plants such as berries are found to be efficient antioxidants in *in-vitro* studies. In addition, the berries have found to have positive effects on cardiovascular health in human clinical studies. Although ORAC analysis is one of the most commonly used and referred methods in the analysis of antioxidativity of food products, recent studies indicate that there is still need for further studies and optimization of certain parameters. For example, the presence of trace metal ions originating from the sample and/or reagents used in the analysis can induce rapid auto-oxidation of antioxidants and therefore result in underestimation of ORAC values of the sample [1]. In addition, the choice of extraction solvent and dilution of sample extract may affect significantly on the ORAC value [2,3]. In this study the antioxidant capacity of the extracts of bilberry powder and nettle seed were analysed by the ORAC method. The effects of three extraction solvents and different extract dilutions were evaluated as well as the effect of metal complexication by EDTA. A polyphenol standard, quercetin was studied as a reference standard compound in the study.

Materials and Methods. The tried plant materials, nettle seed and bilberry powder, were obtained from the local enterprises Osuuskunta Ärmätti Ky and Kiantama Oy, respectively. Samples were extracted using 1) acetone-water (1:1, v/v), 2) acidic methanol or 3) ethanol as an extraction solvent. At least four different dilutions (varying between 50 and 500 fold) were prepared from each extracts for the ORAC analysis, which was performed by slightly modified method of Huang et al. [4] using Varioskan Flash microplate reader (Thermo Scientific, Finland). EDTA was added to the buffer solution in the analysis of metal complexation. ORAC results were calculated as trolox equivalents (TE) using linear regression equation between trolox concentration and the net area under curve. In addition, the ORAC TE value for quercetin was calculated by dividing the slope of linear regression curve of quercetin by that of trolox.

Results and Discussion. The use of EDTA as a metal complexing agent in the buffer clearly increased the ORAC values of quercetin and acetone-water extract of nettle seed: quercetin and nettle seed ORAC values were increased from 12 to 15µmolTE/g and from 254µmolTE/g to 305µmolTE/g, respectively. This result supports the earlier finding [1] that trace amounts of metals even in the high purity standards autoxidize the antioxidants and thus decreases the ORAC values. Nettle generally contains quite high amount of metals, which can have affect on the ORAC result, in addition to the metals present in the reagents used in the method. Extraction solvent had a significant effect on the ORAC values of both matrices: the highest ORAC values were obtained with acetone/water extraction, whereas lowest results were obtained with ethanol (Figure 1). Due to the low ORAC activity of ethanolic and methanolic extracts of nettle, the maximum dilution of those extracts was 100 fold and 300 fold, respectively. The methanol extract of bilberry showed nearly as high ORAC activity as acetone/water extracts, whereas for nettle sample the ORAC activity of methanol extract was almost half of that obtained with acetone/water. This implies the difference in the chemical composition of antioxidants in these two matrices. Phenolic compounds are the main antioxidants in bilberry and they are generally extracted by acidic methanol. However, the antioxidant activity measured by ORAC method was in the same level in the studied nettle seed and bilberry: The average ORAC values obtained using acetone/water extract of bilberry powder (n=12) and nettle seed (n=11) were 614 µmolTE/g (SD=13%) and 543 µmolTE/g (SD=10%), respectively. However, the concentration effect was not very remarkable for these matrices. As a conclusion, this study shows that ORAC values of nettle seed and bilberry extracts depends on extraction conditions and strongly emphasize the importance of reporting the extraction protocol and sample dilutions together with ORAC values.

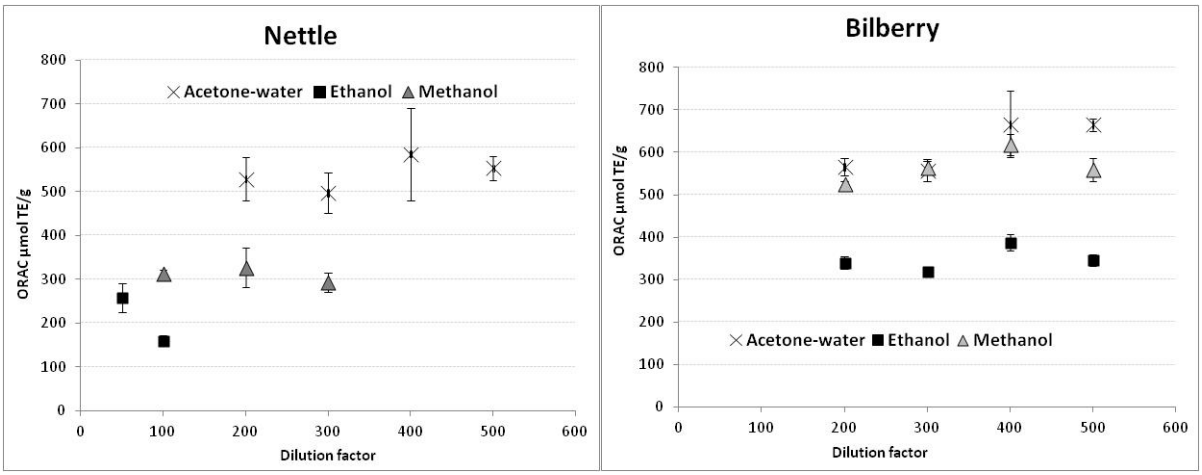

Figure 1. Effect of extraction solvent and concentration in the ORAC value of nettle seed and bilberry powder.

References

[1] Nkhili *et al.* (2011) *Anal.Bioanal.Chem.* 400: 1451-1458.
[2] Hengst *et al.* (2009) *Eur. Food. Res. Technol.* 230: 249-254.
[3] Bolling *et al.* (2012) *J. Food Sci.*77: H69-H75.
[4] Huang *et al.* (2002) *J. Agric. Food Chem*. 50:4437-4444.