Antioxidant Activity in Variously Prepared Elderberry Foods and Supplements

K. CEJPEK, I. MALOUŠKOVÁ, M. KONEČNÝ and J. VELÍŠEK*

*Department of Food Chemistry and Analysis, Institute of Chemical Technology in Prague, 166 28 Prague, Czech Republic, *E-mail: karel.cejpek@vscht.cz*

Abstract: Antioxidant capacity of foods and food supplements based on berries and flowers of medicinal plant elderberry (*Sambucus nigra* L.) was assessed. Reducing properties of the samples and extracts were evaluated using amperometric detection at working electrode potential –0.8 V after HPLC separation. Moreover, antiradical activity of selected samples was determined by the means of spectrophotometric DPPH radical scavenging method. Electrochemical activity (EA) of fresh juice pressed from elder fruits amounted to 0.71 g AAE/l with anthocyanins as minor contributors (10.2%). Catechins and phenolic acids were the major active groups. During production of elder berry spread, even more than 90% of the EA compounds found in raw elder berry material can be destroyed. Comparable activity may be found also in the products from elder flowers. Although elder blossom syrups possessed similar EA regardless of the technology used (0.033–0.054 g AAE/kg), their chromatographic patterns were often very different. For example, no flavonols were present in the syrups, if traditional preparation comprising 24-h maceration with citric acid was applied. Analyzing the chromatographic patterns, one can distinguish different base materials and technology, which can be used for the authenticity confirmation. Herbal infusions from elder flowers, which contain more flavonols than are in syrups, were 16–27 times richer in EA than drinks prepared from the syrups after recommended dilution. Only the syrup designed for preventing and treating upper-respiratory viral infections showed the EA (0.09 g AAE/kg) comparable to that of herbal infusion (0.13 g AAE/l).

Keywords: elderberry; elder flowers; antioxidants; reducing power; processing

INTRODUCTION

One of the most important sources of antioxidants among dietary plants is small red fruits. Moreover, other parts of these plants, such as flowers, leaves and bark, used commonly in traditional medicine and healing are often extraordinary rich in antioxidants as well. Black elder (*Sambucus nigra* L.) is an excellent example of such a beneficial plant. Its processing is well established especially in Europe and Mediterranean where numerous food, beverages, and medicinal products are available. Elderberry has been used for generations in traditional herbal medicine as a remedy for colds, sinusitis, and herpes. The berries also have a laxative effect in small doses. Infusions of flowers are known to have diaphoretic, anti-catarrhal, expectorant, diuretic, and anti-inflammatory actions (Wightman 2004; Zakay-Rones *et al*. 2004).

Elderberry medicinal potential comes from its antioxidant potential, a property shared by numerous phytochemicals. Results from various sources have shown that both elderberry fruit and flower are rich in polyphenols. Their antioxidant capacity ranks high when compared to other well known small fruits such as cranberry, mulberry and blueberry, as well as various potent blossom extracts. Using the ORAC method, Wu *et al.* (2004) showed that especially the American elder species (*S. canadensis* L.) had a much higher potential than cranberry and blueberry, two fruits praised for their high antioxidant capacity. In another study (Halvorsen *et al.* 2002), black elder was among the best three small red fruits in

antioxidant potential as measured with the FRAP method. Buřičová and Réblová (2008) reported a medium antiradical (against DPPH•) activity in the aqueous extracts of elder flowers, linden flowers, rosehips, and dead-nettle flowers.

The aim of this paper is to assess and compare the antioxidant properties of several common elderberry products on the Czech market, to describe differences in the composition of reducing compounds in the foods and supplements prepared by different methods, and to find the contribution of particular (kinds of) antioxidants to the overall electrochemical activity (EA).

MATERIAL AND METHODS

Samples. Berries from wild plants grown in the countryside of Central Bohemia were picked at optimum fruit maturity, the stalks were removed, and the fruit was frozen and stored at –18°C. The berries were homogenised in a blender for 2 min after defrosting, squeesed and the juice was separated (sample ***BJ***). Elderberry-apple fruit spread (***BS***) consisted of apple juice concentrate (51.7%) and elderberries (45.2%; Natudis B.V., NL). Tea (***FT***) was prepared from dried flowers (1.5 g, Megafyt, CZ) and boiling water (250 ml) after 10min infusion. Samples of syrups were: ***S1a-c***, traditionally prepared syrups from fresh flowers; the inflorescence with fully opened blossoms was picked and stored at –18°C; 220 g of the flower heads were soaked in cold water (3 l) with citric acid (70 g) for 24 h at 12 ± 1°C; the macerate (sample ***FM***) was strained and mixed with sucrose dissolved in water (1.5 kg in 1 l); ***S2*** (elderberry blossom extract, hibiscus extract, sucrose 55%, Topvet, CZ); ***S3*** (elder blossom extract, sucrose, citric acid, LL Ltd., CZ); ***S4*** (elderberry blossom extract, sucrose, lemon juice, citric acid, TBK Hostětín, CZ); ***S5*** – Yo elder blossom syrup (sucrose, apple concentrate, elder blossom extract, lemon juice, citric acid, aroma, Eckes-Granini, AT). The samples were dissolved or diluted with water – syrups (1:1, v/v), spread (5 g in 10 ml) and juice (1:10, v/v) – and filtered (0.45 mm nylon membrane). Electrochemical activity (EA) of the samples was expressed in g of L-ascorbic acid equivalents (AAE) per kg or l of a sample. All determinations were performed in triplicates at least. The values are expressed together with confidence intervals (P = 0.05).

Antioxidants. The following compounds were used for the evaluation of the sample extracts: L-ascorbic acid; gallic, 4-hydroxybenzoic, protocatechuic, cinnamic, *p*-coumaric, caffeic, ferulic, and chlorogenic acids; (+)-catechin, (–)-epicatechin, ECG, EGC, EGCG; quercetin, isoquercitrin, and rutin (Fluka, CH, or Aldrich, USA).

Analyses. Atlantis C_{18}, 150 × 3.9 mm, 3 μm, with a pre-column, gradient elution (pH 6.5/MeCN/5mM NaCl, diode-array (PDA, 996) & electrochemical detectors (ELD, 2465, all Waters, E_a = +0.8 V) were used for HPLC method. The DPPH assay was accomplished after the method described by Brand-Williams *et al*. (1995).

RESULTS

The most important polyphenols found in elderberry fruit are thought anthocyanins, being responsible for the colour. The HPLC/ELD analysis of fresh juice pressed from black elder fruits revealed only a minor role of the anthocyanins corresponding to 10.2% of the total EA, which amounted to 0.71 g AAE/l (Table 1). The most contributing were cyanidin 3-sambubioside and cyanidin 3-glucoside, which comprised 51% and 40% EA of anthocyanins, respective. The major part of the juice EA was originated from catechins and phenolic acids such as chlorogenic acid; rutin and other flavonols provided 8.1% of the total EA.

Elderberry reaches consumer in a much greater proportion as a processed product than as a fresh fruit. Comparing the beneficial effects of fresh elderberry fruits with that of processed ones, it should be kept in mind that especially anthocyanins stability is affected by numerous factors (Drdák & Daučík 1990). Almost total destruction of anthocyanins as well as chlorogenic acid group occurred during production of the fruit spread containing 45.2% of elderberries, while rutin was more stable and retained on 4.9% of the spread total EA (0.033 g AAE/kg, Table 1). This low AAE value indicated a degradation of most EA compounds (> 90%) during processing provided that the activity of the raw berries was similar to EA of the tested fresh juice.

Elder blossoms are distinguished by their intensive, pleasant odor and serve as a basis for industrially produced soft drinks and as extracts to increase the nutritional value of different foods and diets. Although products such as cough syrups and teas made from elder flower are usually safe taken in moderation (Mills & Bone 2005), after the Czech food regulations (Ministry of Ag-

riculture 1997), elder flowers and berries can be used for the preparation of infusions and teas in mixtures with other plant materials up to 30% (m/m). The consumption of tea infusion of elder flowers is among the most used ways of the elder flower antioxidants' intake. Its EA was found to be equivalent to 21 g AAE/kg of dry flowers (Table 1). The content of rutin found in the infusion was 10.9 ± 0.4 g/kg of dry flowers weight, which is in good agreement with literature (Dawidowicz *et al.* 2006). Rutin was responsible by 63 ± 2 % and the other flavonols by 19 ± 1% for the infusion colour (Abs_{420nm}). While the EA in infusions was derived mainly from rutin (27 ± 1%), other flavonols and their glycosides (9.5 ± 0.5%), derivatives of caffeic (chlorogenic) acid and flavan-3-ols, quite different distribution of the active compounds was found within the syrups studied.

Based on dry flowers weight, the acidified 24 h macerate (FM) from fresh flowers resembling traditional preparation of elder blossom syrup revealed only 42% EA comparing to the infusion from dried flowers. Phenolic acids such as protocatechuic acid and derivatives of caffeic acid together with catechins were the major EA compounds. The addition of citric acid adjusting the pH value to 3.1 affected the polyphenoloxidase (PPO) activity as well as subsequent browning. There may be further decrease in PPO activity below pH 4 due to less tight binding of copper in the enzyme, permitting chelators such as citric acid to remove the copper. Lower pH has an effect on the destruction of flavonols and their glycosides during the 24 h maceration and storage, not on the extraction efficiency. Different extraction temperatures (20°C *vs.* 100°C) may influence the yield, but not the composition (data not shown).

EA of the studied syrups depends on the technology used and the content of elder flower and other ingredients. Traditionally prepared syrups from fresh flowers (S1a-c) possessed 0.033–0.045 g AAE/l of syrup and were very pale. Commercially available syrups are prepared mostly with the use of extracts from dry flowers and/or elder blossom aroma. Syrup S2, sold as a food supplement with the declared action against cold and influenza, is more concentrated than syrups intended for a beverage preparation. Its EA (0.090 g AAE/l) was significantly higher than that of the other syrups. L-Ascorbic acid, which was only minor or not detected in the other syrups, was the cause of 57% of EA in S2; rutin participated by 7.5%. Commercially produced S5 with the addition of apple concentrate and elder blossom aroma did not contain detectable amounts of rutin and other flavonols. Close correlation (r^2 = 0.98) between EA and colour (Abs_{420nm}) was found only for the products prepared from dry flowers, where flavonols were present in significant amounts.

The expected antioxidant activity associated with electrochemical characteristic was compared with a free-radical scavenging capacity. Using DPPH·

Table 1. Electrochemical activity (EA; +0.8 V) of elder flower and berry products with contributions of flavonols and anthocyanins

Samples	Total EA (g AAE per kg or l)	Flavonols' EA (%)	Anthocyanins' EA (%)
Syrup S1a	0.045 ± 0.007	< 0.5	–
S1b	0.040 ± 0.006	< 0.5	–
S1c	0.033 ± 0.007	< 0.5	–
S2	0.090 ± 0.003	8.6 ± 0.4	–
S3	0.054 ± 0.004	10.3 ± 0.3	–
S4	0.041 ± 0.004	6.5 ± 0.3	–
S5	0.045 ± 0.003	< 0.6	–
Tea FT	21 ± 2*	37 ± 2	–
Macerate FM	8.8 ± 0.8*	< 1	–
Juice BJ	0.71 ± 0.06	8.1 ± 0.3	10.2 ± 0.8
Spread BS	0.033 ± 0.003	4.9 ± 0.3	< 0.5

*based on dry flowers' weight

assay, elderberry juice possessed 0.3% and tea infusion, based on dry flowers' weight, 5.2% antiradical activity of L-ascorbic acid. Our results revealed that various forms and preparations comprise often quite different mixtures of active compounds depending on the treatment conditions, while the total EA levels need not vary so much. The data confirmed that both elder flower and fruit can serve as a good source of bioactive polyphenols in human diet and all the studied spectrum of black elder products can be regarded as good candidates for nutritional supplement formulations.

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