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Utilization of high performance liquid chromatography for determination of antioxidant capacity

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Abstract

In this study, we are interested in possibility of determination and expression of biological value of twenty perspective genotypes of apricot (*Prunus armeniaca* L.). Work focuses on verification and validation of HPLC technique with electrochemical detector (ED) for determination of total antioxidant capacity (TAC), which may represent suitable method for comparison of biological value of fruits.

Keywords: Total antioxidant capacity, HPLC, *Prunus armeniaca* L.,

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Introduction

Due to chemical variability of compounds with antioxidant capacity present in fruits, content of individual compounds is usually not known at all. Detection of biologically active compounds in biological matrix is usually very complicated, determined quantitative values are usually different and present compound/compounds responsible for antioxidant properties are not identified both using effective separation analytical methods and instruments. In addition, levels of individual antioxidants present in fruits need not necessarily correspond to total antioxidant capacity/activity (Adam et al. 2007). Main problem in this field is represented by absence of ideal analytical methods and techniques. Due to fact that antioxidants very easily participate in redox reactions, they are very suitable for electrochemical detection. Electrochemical determination of total antioxidant capacity is one of the possibilities how to express biological and nutrition value of fruits (Gazdik et al. 2008).

Materials and methods

Total antioxidant capacity was monitored using HPLC technique with coulometric detection, which represents one of the most sensitive detection techniques (Beklova et al. 2008). Concurrently, HPLC profile of fifteen flavonoids was detected.

HPLC-ED system consisted of two chromatographic pumps, Model 582 ESA (ESA Inc., Chelmsford, MA) (working range 0.001-9.999 ml min^{-1}) and reversed chromatographic column Zorbax SB C18 (150 × 4.6; size of particles 5 μm, Agilent Technologies, USA). For UV, UV detector Shimadzu (Model 528, ESA, USA) was used. For electrochemical detection, twelve-channel detector CoulArray (ESA, USA) with flow analytical cell (Model 6210, ESA, USA) was used. Flow cell contains planar glassy carbon electrode, hydrogen-palladium electrode as reference electrode and carbon electrode as auxiliary electrode. Sample was injected automatically by the use of autosampler (Model 542, ESA, USA), which has incorporated thermostat place for column. Column was thermostated to 30°C. Output data were processed by application CSW 32 software (Version 1.2.4, Data Apex, Czech Republic).

Real samples were prepared by graining of apricots in mortar with mixture of 50% methanol in water (v/v). After that homogenate was vortexed at 20 minutes under ambient temperature. Supernatant was obtained by centrifugation under 14.000 rpm, 10 minutes and then directly injected to HPLC.

Chromatographic conditions were optimized: volume of injection of standard mixtures and real samples 30 µl, mobile phase A consisted of formic acid (0.2 %, v/v), mobile phase B was acetonitrile. Profile of gradient was linearly increased from 12 to 22 % for B (v/v) (0 - 20 min), to 50 % B (20 – 25 min), to 55 % (25 – 30 min). Flow rate was 0.8 $\mu l.min^{-1}$. Electrochemical detector scanned responses at potentials -80, 0, 80, 160, 240, 320, 400, 480, 560, 640, 720 and 800 mV. Resulting detection was expressed in microcoulombs. Mathematical and statistical analysis of experimental data was carried out in package MATLAB®, Version 7.9.0.529 (R2009b).

Results and discussion

For determination of total antioxidant capacity, HPLC-ED technique with gradient elution was used for determination of flavonoids in plant samples. Based on differences of signal areas, comparative series of determined fruits, where differences in contents of antioxidant flavonoids are expressed as relative % and interpreted as relative antioxidant capacity, were investigated.

Lignite is able to absorb high amount of water, in mined state contains 50% at least. This ability is reversible during processes of drying and hydratation. Natural mineral zeolite is additive soil substance of volcanic origin (tetragonal sodium aluminosilicate), which contains approximately 70 % of silicon oxide. Zeolite is highly porous with ability of water and ions absorption with subsequent releasing based on ions exchange (Diopan et al. 2008).

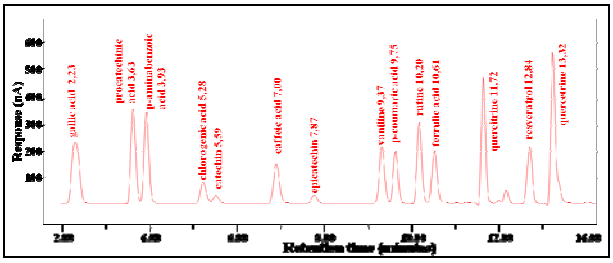

Figure 1: Chromatogram of cultivars LE-9299. HPLC profile of fifteen flavonoids

We also determined total antioxidant capacity of fruits of individual apricot cultivars related to content of major represented antioxidant compounds; quantification of present antioxidants with their participation on TAC was also possible.

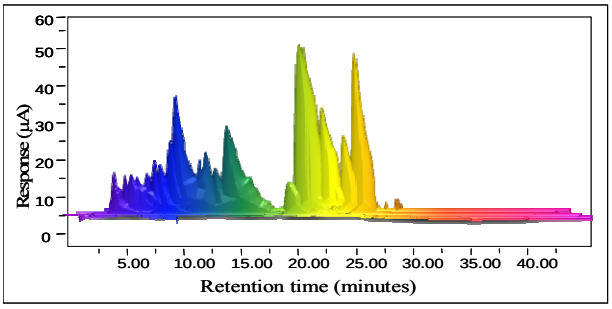

Figure 2: Output of CoulArray detector with twelve working electrodes in the case of cultivars LE-9299Conclusion

Our results (Fig 1 & Fig 2) demonstrate variation of antioxidant capacity in dependence on type of phenolic compounds present in fruits as well as variation between individual types of phenolic compounds – some types of phenolics demonstrate higher antioxidant in comparison with others (Diopan et al. 2008). It is supposed that on protective effect participates possibility of plant polyphenolics to scavenge reactive oxygen radicals, which are able to generate highly reactive hydroxyl radicals (Adam et al. 2007; Beklova et al. 2008). Due to direct connection between antioxidant activity/capacity and ability of compound to be oxidized/reduced, it means due to ability to provide signal in electrochemical detection (Gillman et al. 1995), connection of selective and very sensitive electrochemical detection with high performance liquid chromatography (HPLC-ED) enabling simultaneous separation of many constituents in one run represents ideal analytical tool for solving of these very difficult questions.

Conclusion

We developed suitable separation procedure and technique for determination of total antioxidant capacity in newly cultivated apricot cultivars. HPLC/ED enabled monitoring of reaction kinetics at applied potential, evaluation of structures of antioxidant complexes and their total antioxidant capacity.

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