# EVALUATION OF ANTIOXIDANTS IN SOFT FRUITS (CURRANT, GOOSEBERRY)

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## INTRODUCTION:

Fruits are an important source of vitamins, minerals and other nutritionally important substances often with significant antioxidant activity. Fruit consumption has beneficial effects on health via protecting the body from damage caused by oxidative stress. Different methods are used to test the antioxidant activity of natural substances in fruits which can often give different results influenced by the matrix and by the preparation of fruit samples for analysis.
The original electrochemical method of measuring total antioxidant activity using flow injection analysis with multi-channel electrochemical detection using coulometric cell with four working porous graphite electrodes and reference hydrogen-palladium electrodes was used for analysis of antioxidant activity in soft fruits. This method is based on the injection of fruit extract into the carrier phase of the mobile phase, which passes through four series-coupled electrochemical sensors of the coulochemical detector CoulArray to detect and quantify electroactive antioxidants based on the charge in µC (micro Coulombs).

## SOFT FRUITS CULTIVARS:

Fruit samples were collected in its optimal harvesting maturity from experimental plantings with organic and integrated pest management (IPM) cultivation in the Research and Breeding Institute of Pomology Holovousy Ltd. Those plantings located on a gentle southern slope at an altitude of approximately 320 m above sea level were equipped by covering system against rain (company VOEN, Germany) and by the drip irrigation. The plantings were covered after flowering of shrubs and reopened after the fruit harvest. Drip irrigation was switched on automatically when the soil moisture fell below the set limit of 30 % water volume which is the usual soil moisture for a given type in the specific planting. Experimental plantings of small fruit were established in 2012 in spacing 3 x 0.8 m in the form of two-stem spindles. The soil in the crop belt was covered with a foil to prevent soil contamination of the fruit and to prevent the growth of undesirable vegetation with the exclusion of the application of herbicides.

## SAMPLE PREPARATION:

Totally twenty cultivars of currants and nine cultivars of gooseberries both from organic and IPM orchards were analysed. Samples of fruit were frozen immediately after harvesting and kept at -20 °C prior to analysis. After defrosting , a representative sample of twenty currant sprigs and twenty gooseberries were selected for analysis. The berries were homogenized for 10 seconds with a Nutribullet mixer. Homogenized fruit blend (3 g ) was weighed and transferred into 15 ml centrifuge tubes, 5 mL of extraction reagent (methanol+ 0,1 % (*v/v)* formic acid) were added, and followed by sonication for 30 min in an ultrasonic bath to completely disintegrate the matrix and antioxidants release. Subsequent centrifugation at 5000 RPM for 15 minutes resulted in separation of the liquid phase (supernatant) from the solid sediment. The supernatant was decanted from the sample and filtered through a syringe filter (Nylon, 0.22 µm).
Obtained extract was diluted 10 times with an distilled water and stored at 8 °C before analysis.

## ANALYSIS OF FRUIT USING FIA/ECD:

The total antioxidant activity measured by FIA-ECD (Flow Injection Analysis – Electrochemical Detection) was determined as the charge in µC by integrating the peak area response at four of the working electrodes in series. Solvent mixture of phosphate buffer solution ( 0.05 mol/L) and acetonitrile (9:1; v:v) about total pH of 4.7 was utilized as mobile phase. The flow rate of the mobile phase during the measurement was 1 mL/min and the sample injection volume was 10 µL.
Samples of extracts were evaluated according the charge in µC collected on working porous graphite electrodes at following potentials: 200, 400, 600 and 800 mV.
Antioxidant activity was expressed as electrical charge in C (Coulombs) of one gram fresh weight of fruits.

## RESULTS:

The results of the total antioxidant activity of soft fruits measured by FIA-ECD method are shown in Fig. 1. Differences between the fruit colored cultivars were observed. Darker cultivars reached higher values. The highest antioxidant activity of currants was achieved in the cultivar Triton from the group of black currants (value 0,785 C/g). The highest antioxidant activity of gooseberries was achieved in the cultivar Karmen from the group of red gooseberries (value 0,117 C/g).
Carried out analyses showed the higher antioxidant activity of fruits grown under the organic regime (EKO) in comparison to IPM growing system (Fig. 2 and 3). The method of flow analysis with multichannel electrochemical detection was proven in practice as a reliable and very fast method of evaluation of antioxidant activity of fruit extracts.

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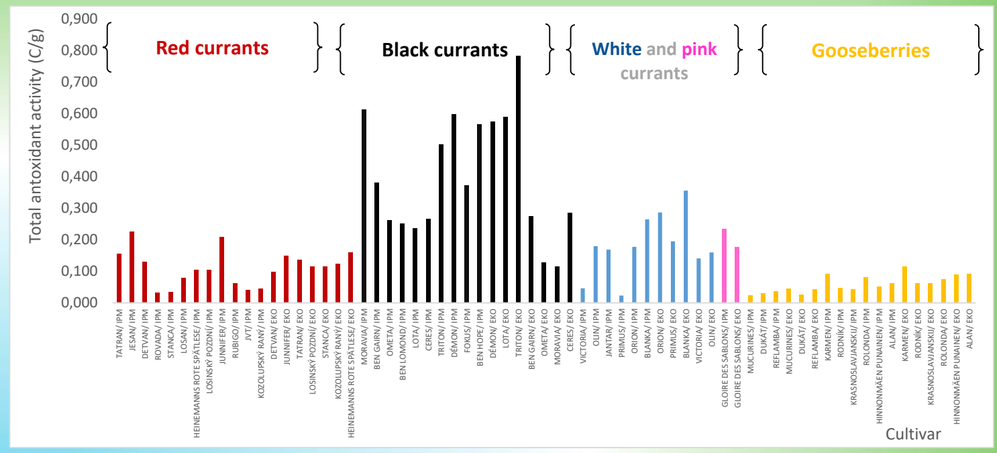


Fig.1 Comparison of total antioxidant activity of currant and gooseberry cultivars measured using the FIA-ECD method and expressed in C/g.

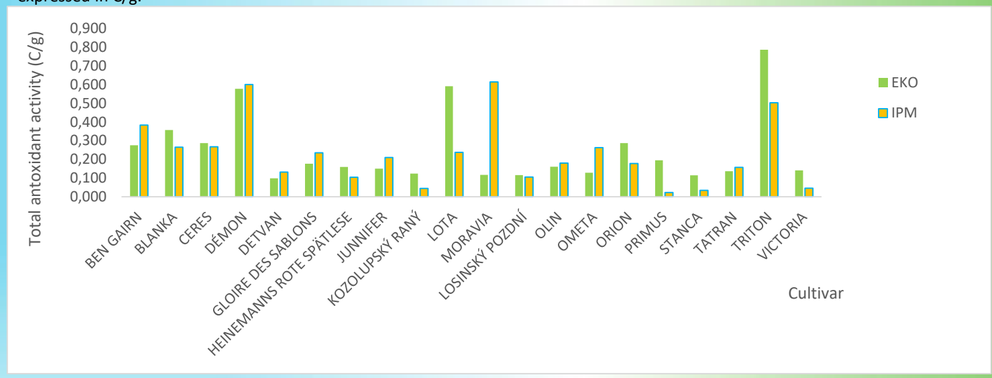


Fig.2: Comparison of total antioxidant activity of currants grown under EKO and IPM measured using the FIA-ECD method and expressed in C/g.

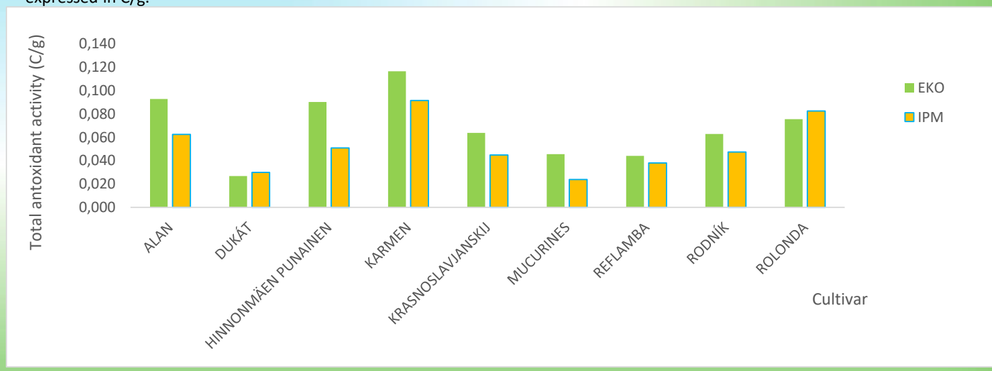


Fig.3: Comparison of total antioxidant activity of gooseberries grown under EKO and IPM measured using the FIA-ECD method and expressed in C/g.