# Evaluation of antioxidants in currant and gooseberry

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***Abstract***

**Regular fruit consumption helps to strengthen human health by protecting cells from damage by free radicals. Berries of soft fruit contain large quantities of substances with antioxidant activity. The method of Flow Injection Analysis with electrochemical detection for determination of antioxidant activity of soft fruits grown in organic regime (EKO) in comparison to the integrated pest management orchard (IPM) was used. Antioxidant activity was expressed as electrical charge in C (Coulombs) per gram fresh weight of fruits. In total, 49 samples of currant cultivars (red, black, white and pink) and 18 samples of gooseberry cultivars (red and white) were measured. Differences in antioxidant activity between the cultivars were observed, where cultivars with darker fruit reached higher values. The highest antioxidant activity was found in cultivars of black currants, lower in red currants and the lowest in white gooseberries. The highest antioxidant activity was determined among currants for the cultivars 'Triton' from the group of black currants (EKO; value 0.785 C/g), for cultivar 'Jesan' (IPM; value 0.226 C/g) from the group of red currants and for 'Blanka' (EKO; value 0.356 C/g) from the group of white currants. The highest antioxidant activities among gooseberry cultivars were determined in red gooseberries for cultivar 'Karmen' (EKO; value 0.117 C/g) and in white gooseberries for cultivar ´Mucurines´ (EKO; value 0.045 C/g). The higher antioxidant activity was determined both for currants and gooseberries grown in EKO in comparison to IPM.**

**Keywords**: antioxidant activity, flow injection analysis, electrochemical detection, soft berries, human health

## 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 by protecting the body from damage caused by oxidative stress. For this reason, there is a general interest in the identification, quantitative determination and measurement of the antioxidant activity of various substances in fruits. 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. Special position among methods for determining the antioxidant activity of natural substances occupy electrochemical methods that often correlate with various other methods for testing the total antioxidant activity of fruit substances (Paulová at al., 2005; Blasco at al., 2004). The original electrochemical method of measuring total antioxidant activity using flow injection analysis with multi-channel electrochemical detection 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 C (Coulombs). The aim of the study was determination of antioxidants in currant and gooseberry

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cultivars and difference in antioxidant activity of fruits grown in IPM and EKO regimes.

## MATERIALS AND METHODS

### Soft fruits cultivars

Experimental plantings of small fruit were established in 2012 in spacing 3 x 0.8 m in the form of two-stem spindles in the Research and Breeding Institute of Pomology Holovousy Ltd., Czech Republic with organic (EKO) and integrated pest management (IPM) cultivation systems. 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, Berg, Germany) and watered 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 threshold of 30% water volume which is the usual soil moisture for a given type in the specific planting. The soil in the crop belt was covered with a foil to prevent the growth of undesirable vegetation with the exclusion of the application of herbicides. Fruit samples were collected in its optimal harvesting maturity from experimental plantings. All data were statistically processed by an analysis of variance (ANOVA) and Fisher‘s test ($\alpha$=0.05) by STATISTICA software (version 12, Stat Soft).

### Sample preparation

In total 29 currant cultivars in IPM, 20 cultivars in organic regime and nine cultivars of gooseberries both from organic and IPM orchards were analysed. Fruit samples of currants (n=49; red, black, white and pink) and gooseberries (n=18; red and white) were frozen immediately after harvesting and kept at -20 °C prior to analysis. After thawing at room temperature, a representative sample of twenty currant sprigs (fruits without stems) and twenty gooseberries of each cultivar 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 solvent (methanol+ 0.1 % (*v/v*) formic acid) were added, followed by sonication for 30 min in an ultrasonic bath (Bandelin Sonorex, Thermo Fisher Scientific, Inc., Waltham, MA, USA) for completely disintegration of the matrix and antioxidants release. Two replicate samples for extraction were always performed. Subsequent centrifugation (EBA 200, Hettich Zentrifugen, Tuttlingen, Germany) at 5000 RPM for 15 minutes resulted in separation of the liquid phase (supernatant) from the solid sediment. About 1.5 ml of supernatant was decanted from the sample and filtered through a syringe filter (Nylon, 0.22 µm) into a 2 ml vial. The obtained extract was diluted 10 times with a distilled water and stored at 8 °C before analysis.

### Antioxidant activity analysis

The total antioxidant activity measured by FIA-ECD (Flow Injection Analysis – Electrochemical Detection) was determined as the charge in µC (micro Coulombs) by integrating the peak area response at four of the working electrodes in series. The electrochemical detector CoulArray consists of coulometric cell with four working porous graphite electrodes and reference hydrogen-palladium electrodes. Solvent mixture of phosphate buffer solution (0.05 mol/l) and acetonitrile (9:1; v:v) with a 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. The working electrode potentials 200, 400, 600 and 800 mV were applied to the dry reference hydrogen-palladium electrode. Antioxidant activity was expressed as electrical charge in C (Coulombs) per gram fresh weight of fruits.

## RESULTS AND DISCUSSION

The results of the total antioxidant activity of currant cultivars measured by FIA-ECD method are shown in Table 1, Figures 1 and 2. Differences between the fruit colored cultivars were observed, darker cultivars reached higher values. The highest antioxidant activity was determined among currants for the cultivars 'Triton' from the group of black currants (EKO; value 0.785 C/g), for cultivar 'Jesan' (IPM; value 0.226 C/g) from the group of red currants and for 'Blanka' (EKO; value 0.356 C/g) from the group of white currants. The lowest antioxidant activity was recorded for cultivar 'Primus' from the white currants group (IPM; value 0.023 C/g). The higher antioxidant activity was determined for currants grown in EKO (Figure 2). In eleven out of twenty cases higher values from the organic plantation were recorded.

Table 1: Comparison of total antioxidant activity of currant cultivars in EKO and IPM

| Cultivar | Fruit color | Value (C/g) | Difference (α=0.05) |
|---|---|---|---|
| ROVADA | Red | 0.033 | a |
| JVT | Red | 0.042 | ab |
| RUBIGO | Red | 0.064 | abd |
| STANCA | Red | 0.075 | ab |
| LOSAN | Red | 0.080 | abcd |
| KOZOLUPSKÝ RANÝ | Red | 0.085 | ab |
| VICTORIA | White | 0.094 | abcd |
| PRIMUS | White | 0.109 | abcd |
| LOSINSKÝ POZDNÍ | Red | 0.111 | abcd |
| DETVAN | Red | 0.116 | abcd |
| HAINEMANNS ROTE SPÄTLESE | Red | 0.132 | abcd |
| TATRAN | Red | 0.147 | abcde |
| JANTAR | White | 0.169 | abcdefgh |
| OLIN | White | 0.170 | abcde |
| JUNNIFER | Red | 0.180 | abcdef |
| OMETA | Black | 0.196 | abcdefg |
| GLOIRE DES SABLONS | Pink | 0.206 | bcdefg |
| JESAN | Red | 0.226 | bcdefgh |
| ORION | White | 0.232 | cefgh |
| BEN LOMOND | Black | 0.253 | cdefghi |
| CERES | Black | 0.277 | efgh |
| BLANKA | White | 0.311 | fghi |
| BEN GAIRN | Black | 0.329 | ghi |
| MORAVIA | Black | 0.365 | hi |
| FOKUS | Black | 0.373 | hi |
| LOTA | Black | 0.414 | ij |
| BEN HOPE | Black | 0.567 | jk |
| DÉMON | Black | 0.588 | k |
| TRITON | Black | 0.644 | k |

Legend: Different letters in rows indicate a statistically significant difference between cultivars (α=0.05)

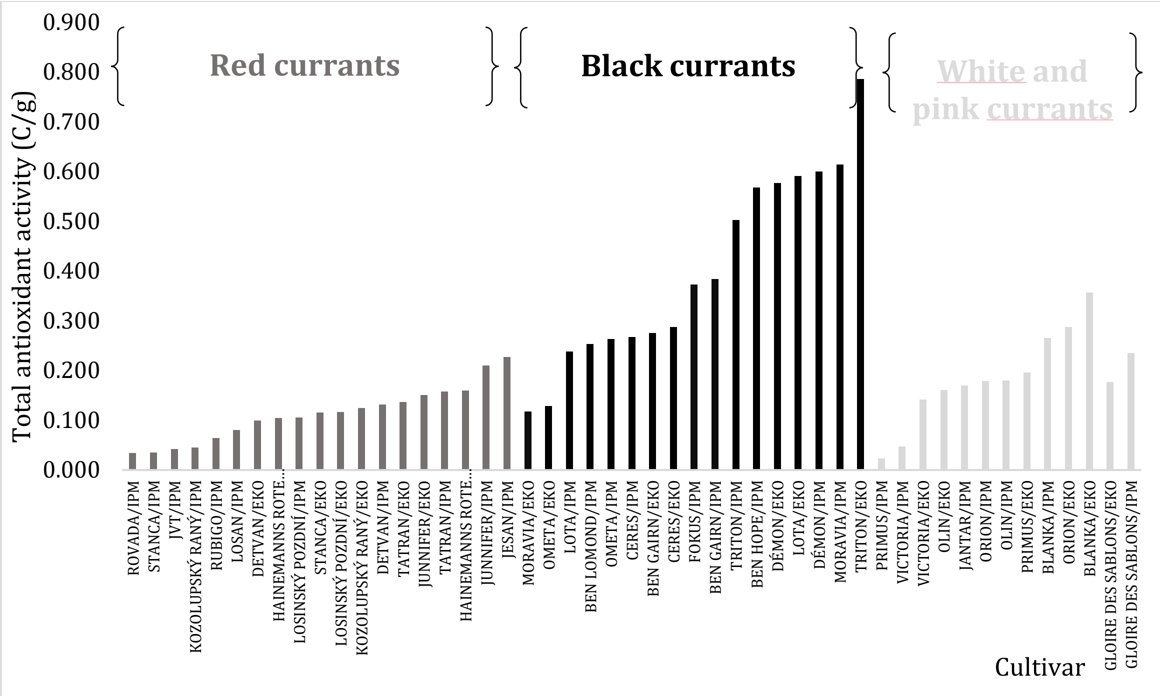


Figure 1. Comparison of total antioxidant activity of currant cultivars measured using the FIA-ECD method.

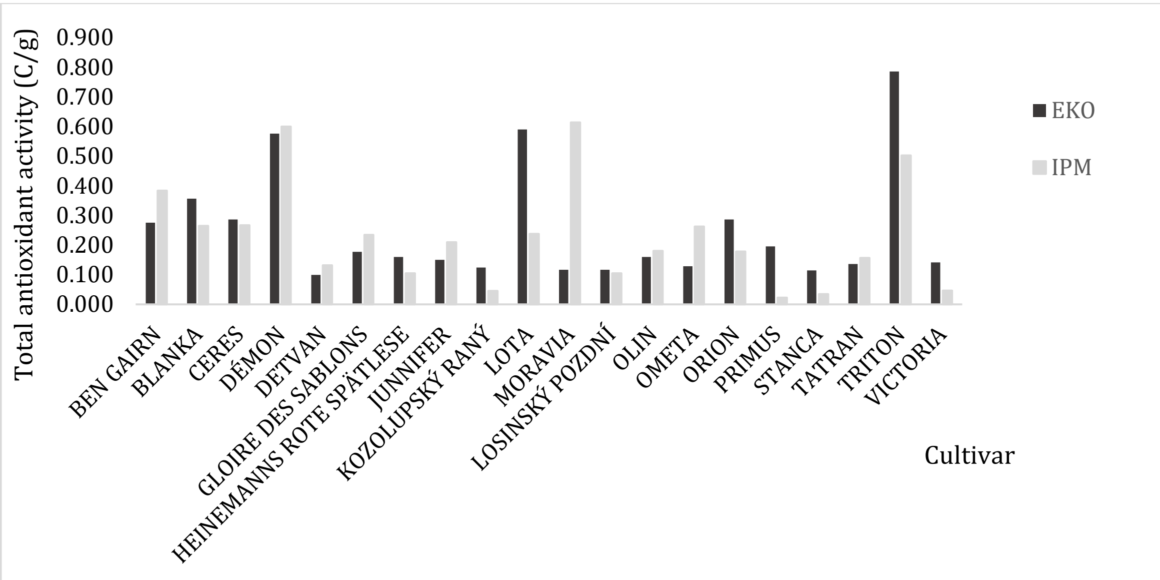


Figure 2. Comparison of total antioxidant activity of currant cultivars grown under EKO and IPM measured using the FIA-ECD method.

Analysis of gooseberries both from IPM and organic plantations showed the same trend compared to that found in currant cultivars (Table 2). Higher antioxidant activity was determined in red fruit cultivars in comparison to white fruit cultivars. The highest antioxidant activity was recorded for cultivars 'Karmen' (EKO; value 0.117 C/g) from the red gooseberry group and 'Mucurines' (EKO; value 0.045 C/g) in the group of white gooseberries (Figure 3). The lowest antioxidant activity was recorded for cultivar 'Mucurines' from the white gooseberry group (IPM; value 0.024

C/g). Also results of analyses showed higher antioxidant activity of gooseberry fruits grown in organic regime. In seven cases of nine gooseberry cultivars from the organic plantation had higher value of antioxidant activity of fruits than cultivars from IPM regime (Figure 4).

Table 2: Comparison of total antioxidant activity of gooseberries in IPM and EKO regime

| Cultivar | Fruit color | Value (C/g) | Difference (α=0.05) | Cultivar | Fruit color | Value (C/g) | Difference (α=0.05) |
|---|---|---|---|---|---|---|---|
| DUKÁT | White | 0.028 | b | HINNONMÄEN PUNAINEN | Red | 0.071 | cd |
| MUCURINES | White | 0.035 | ab | ALAN | Red | 0.078 | d |
| REFLAMBA | White | 0.041 | ab | ROLONDA | Red | 0.079 | d |
| KRASNOSLAVJANSKIJ | Red | 0.054 | ac | KARMEN | Red | 0.104 | e |
| RODNIK | Red | 0.055 | ac | | | | |

Legend: Different letters in rows indicate a statistically significant difference between cultivars (α=0.05)

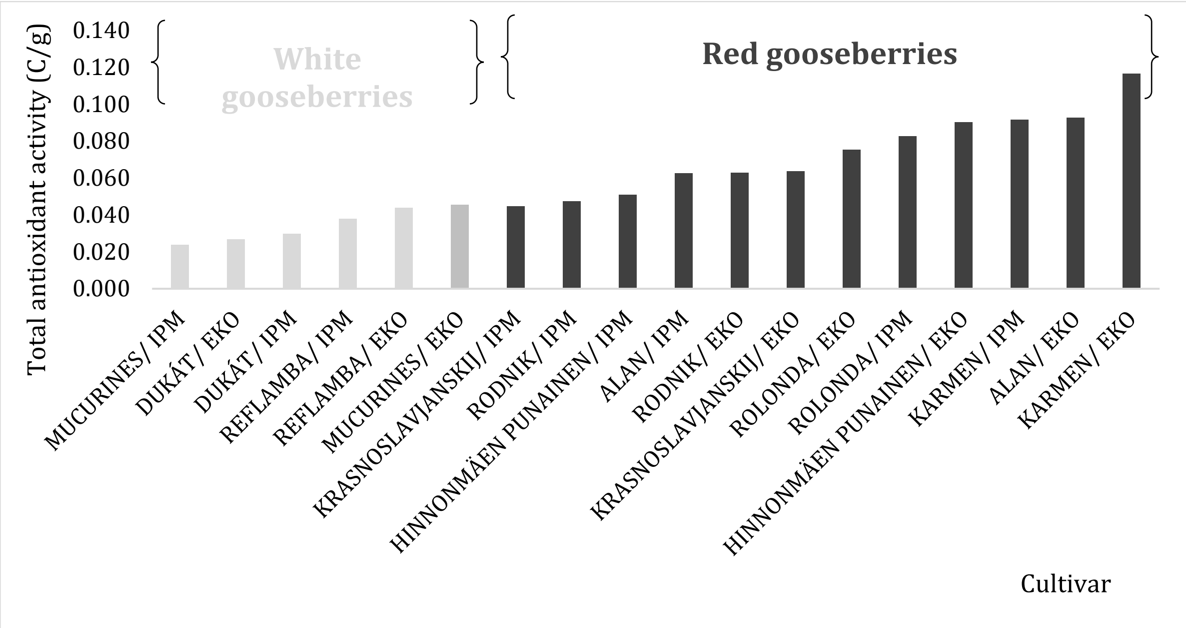


Figure 3. Comparison of total antioxidant activity of gooseberries measured using the FIA-ECD method.

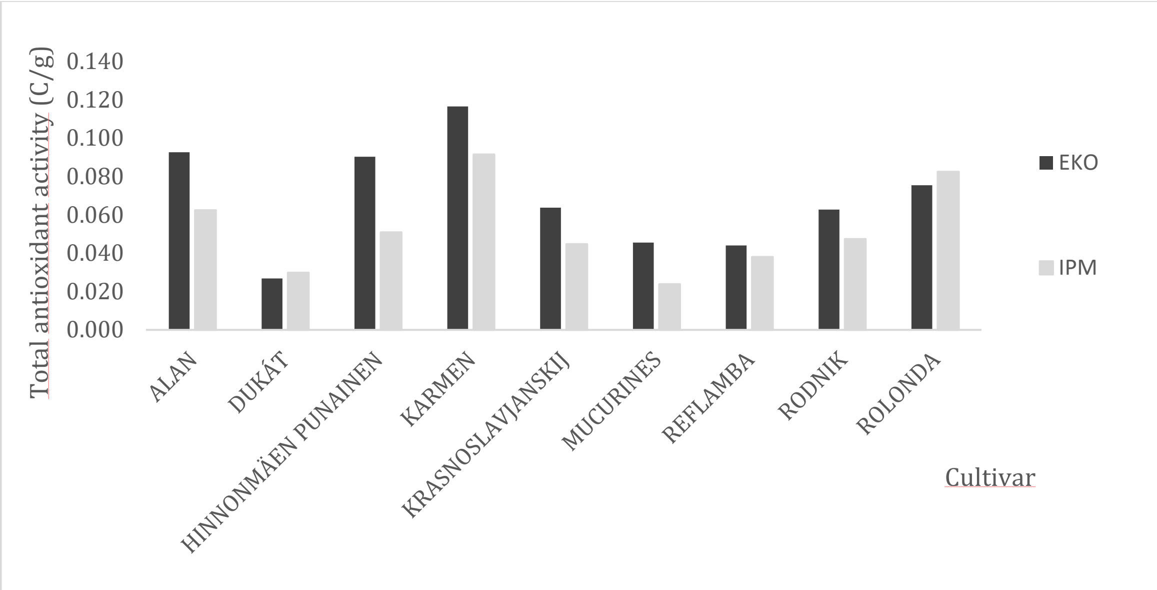


Figure 4. Comparison of total antioxidant activity of gooseberry cultivars grown under EKO and IPM measured using the FIA-ECD method.

The higher antioxidant activity determined for organic fruit samples is in accordance with results reported in the literature (Benbrook, 2020; Kazimierczak, 2008). Substances with antioxidant activity are among the so-called secondary plant metabolites. Their synthesis responds to environmental conditions (climate, soil type, weather, pest infestation or pest control). It is assumed that fruits grown under the organic regime are forced to be more self-protected against pests and therefore contain more antioxidants than chemical-protected fruits.

## CONCLUSION

The method of flow analysis with multichannel electrochemical detection was proven in practice as a reliable and very fast method for evaluation of antioxidant activity of fruit extracts. The FIA-ECD method allows automatic determination of antioxidant activity of up to 50 samples per hour and allows to observe differences between fruit cultivars. The content of antioxidant substances in evaluated fruit cultivars described by the total antioxidant activity was significant at level $\alpha$=0.05. It is noteworthy that carried out analyses showed the higher antioxidant activity of fruits grown under the organic regime in comparison to IPM growing system. In eleven currant cultivars out of twenty, higher antioxidant activity was recorded in the organic plantation compared to IPM. Also, in seven cases of nine, gooseberry cultivars from the organic plantation had higher value of antioxidant activity of fruits than cultivars from IPM regime. However, this information should not be regarded as dogmatic as the synthesis of antioxidants are influenced by several other factors. Differences between EKO and IPM regime are not statistically significant at $\alpha$=0.05 and lay within the cultivar-specific standard deviation.

## ACKNOWLEDGEMENTS

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