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ANTIOXIDANT ACTIVITY OF SELECTED APPLE CULTIVARS STUDIED BY ELECTRON PARAMAGNETIC RESONANCE

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The total antioxidant activity of three apple cultivars conventionally farmed (Jonagold, Golden Delicious and Idared) and two cultivars organically farmed (Jonagold and Golden Delicious) were investigated by electron paramagnetic resonance (EPR) spectroscopy. The spin label used for experiments was TEMPO radical (2,2,6,6-tetramethylpiperidine-1-oxyl) with a well-defined EPR spectrum consisting of three equidistant peaks. The results obtained indicated difference in the total antioxidant activity of apples grown in two different ways, conventional and organic, as well as between different cultivars grown in the same way. The Golden Delicious cultivar is richer in antioxidants than the Jonagold and Idared, regardless of the method of farming. The difference between them is higher in conventionally grown apples than in organically farmed apples. Experimental data was fitted with non-linear curve fit for exponential decay of the first order based on the Levenberg-Marquardt method. The high values of the R^2 parameters for all data indicated correctness in the proposition of exponential decay of the first order as a model describing the dynamic properties of spin labels which diminish in the presence of fresh apple juice.

Key words: Antioxidant, apple, conventional farming, electron paramagnetic resonance, organic farming.

INTRODUCTION

It is well-known that different fruits and vegetables contain significant amounts of antioxidant species, especially polyphenols and vitamins (Kaur and Kapoor, 2001; Sun et al., 2002; Willet, 2002; Tzika et al., 2008; Chen et al., 2010; Zhao et al., 2013). Antioxidants are scavengers of free radicals which can cause many medical disorders such as Parkinson's and Alzheimer's diseases, cardiovascular diseases, diabetes, cancer, aging-related disorders etc. Although the human organism has mechanisms to deal with oxygen radicals, like enzyme superoxide dismutase (Davis, 1998; Salvemini and Cuzzocrea, 2003), the intake of foods rich in antioxidants (fruits and vegetables) ensures extra protection from the negative influence of radicals on cells.

The antioxidant capacity of fruits and vegetables can be investigated by several different methods, mostly based on hydrogen atom transfer and electron transfer reactions (Huang et al., 2005; Kulišić-Bilušić et al., 2009; Donovan et al., 1998). Wang et al. (1996) measured the total antioxidant activity of twelve fruits and five commercial fruit juices using an automated oxygen radical absorbance capacity (ORAC) assay with a peroxyl radical generator. The results they obtained indicated that the strawberry had the highest ORAC activity followed by plum and orange. Grape juice had the highest antioxidant activity among the examined commercial fruit juices. Sun et al. (2002) investigated the profiles of total phenolics in common fruits. Total oxyradical scavenging capacity (TOSC) assay measurements showed that the cranberry had the highest total phenolic content followed by apple, red grape and others. Cranberry also had the highest inhibitory effect with regard to HepG2 human liver-cancer cells. Based on those results, the authors proposed a bioactivity index (BI) for dietary cancer prevention as a new alternative biomarker for future epidemiological studies.

Apples are one of the most popular and most consumed fruits worldwide. According to The World Apple and Pear Association (WAPA), worldwide apple production in 2010 totaled 69,569,612 tons (WAPA 2010). Recent works indicate that the organic way of farming has great and unfulfilled potential

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in sustainable fruit growing (Benković-Lačić et al., 2011; Ames and Kuepper, 2004). The presence of apples in many diets is due to their phenolic compound contents. Apple peel is particularly rich in phenolics. The phytochemical content, the antioxidant activity and antiproliferative activity of peel of four apple varieties were studied by Wolfe et al. (2003). They found that the peel of the Rome Beauty apple had the highest total content of phenolics and flavonoids. The Rome Beauty apple peel also showed the highest bioactivity by inhibiting the growth of HepG2 human liver-cancer cells. Khanizadeh et al. (2007) determined the phenolic composition of eleven apple genotypes by HPLC, the total phenolic content (TPC) and antioxidant capacity using ferric reducing antioxidant power (FRAP). Their results confirmed that apple peel had more phenolic compounds than apple flesh. Procyanidins were found to be the predominant group. The antioxidant and antiproliferative activities of Portuguese apple cultivars were studied by Serra et al. (2010). Principal component analysis (PCA) showed that Malápio Fino and Bravo de Esmolfe have the highest antioxidant effect and Reineta Parda is the best cultivar in reducing the proliferative effects of human colon (HT29) and gastric (MKN45) cancer cells.

Electron paramagnetic resonance spectroscopy (EPR) has the unique ability to directly monitor free radicals and antioxidants in natural environments and systems. Tzika et al. (2008) investigated commercially available fruit and vegetable juices with regard to their scavenging activity against the stable nitroxide 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPOL). Their findings indicated that natural antioxidant compounds contained in commercially available juices were not eliminated or inactivated when the juices were kept refrigerated according to the manufacturer's instructions. They also found that freshly prepared orange juice had double the antioxidant activity compared to commercial orange juice. EPR spin trapping was used by Leonard et al. (2002) who proved that the antioxidant activity of fruits and vegetables is dependent on many compounds (flavonoids, carotenoids, organic acids – cinnamic and gallic acid, vitamin E and sulfhydryl compounds), not only on ascorbic acid.

The aim of this work was to compare the total antioxidant activity of three apple cultivars conventionally farmed (Jonagold, Golden Delicious and Idared) and organically farmed (Jonagold and Golden Delicious) using EPR spectroscopy as a precise and sensitive technique to determine free radicals and antioxidants. Results obtained by time-dependent experiments may be used to propose a model describing the dynamic properties of free radicals diminishing in the presence of apple juice.

MATERIALS AND METHODS

APPLE CULTIVARS AND THEIR ORIGINS

Conventionally grown apples (Idared, Jonagold and Golden Delicious) originating from the eastern part of Croatia (the Slavonia region) were treated with pesticides 15 times during the vegetation period of the year 2010. The pesticides were mainly based on insecticides (organophosphorus agents, pyrethroids, carbamates) and fungicides (triazols, phthalimide, anilinopyridines). The apples grown organically (Jonagold and Golden Delicious) also came from the Slavonia region but they were not treated with any pesticides at all (confirmed by organic certificates). All three apple cultivars were picked in mid-September 2010.

SPIN LABEL

2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) was purchased from Sigma Aldrich. Since TEMPO is a nitroxide spin label its EPR spectrum consists of three peaks and can reveal both the qualitative and quantitative properties of the systems examined. The middle peak of nitroxide spin label EPR spectrum is very often one with the largest intensity (magnitude) and the area below it is suitable for quantitative determinations. The relative content of spin label can be monitored either by the double integration of the first derivative EPR spectrum or by measuring the maximum to minimum points intensity of the middle peak (Fig. 1), in comparison to the initial content. Since the first method is much dependable on the signal to noise ratio of spectra it is not suitable for low spin label concentration experiments and the second method was chosen accordingly. All medium peak intensity (MPI) parameters presented in this paper are shown in relation to the initial value and are expressed in percentage (%). MPI decay has been used as a measure for antioxidant activity since the decay is faster when the antioxidant activity in apples is larger and the concentration of antioxidants is higher.

SAMPLE PREPARATION

Four apples (peel and flesh) of each cultivar were randomly taken and mechanically squeezed and their liquid phase (juice) was separated. The TEMPO spin labels were dissolved in acetone to a concentration of $3.6 \cdot 10^{-4}$ mol/l. A sample for EPR measurement was prepared as a properly stirred mixture of 20 µl of spin label and 1 ml of each apple juice.

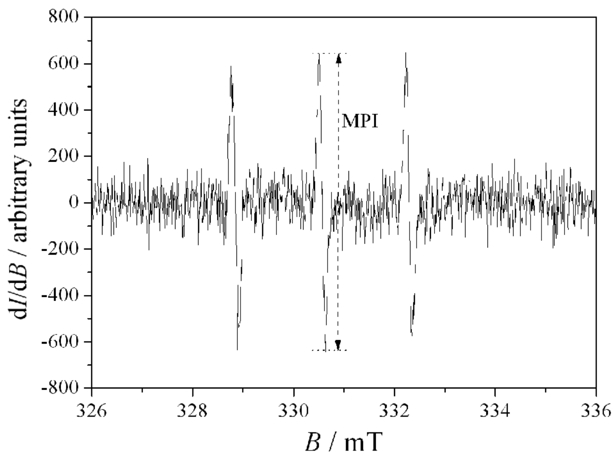

Fig. 1. EPR spectrum of TEMPO in conventionally grown Idared juice. Medium peak intensity (MPI) parameter is denoted.

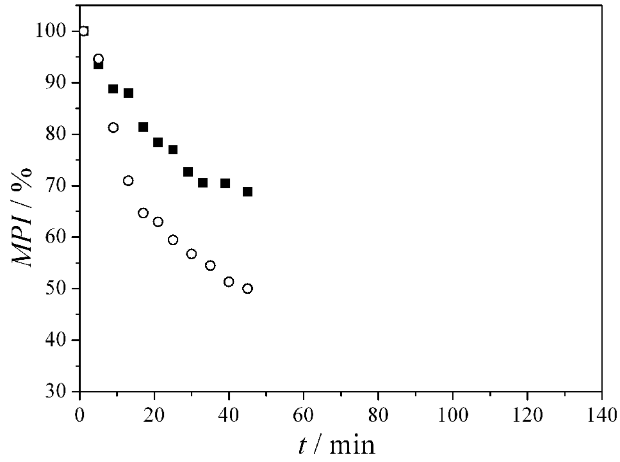

Fig. 2. Time dependence of relative MPI decay in EPR spectra for Jonagold apples farmed conventionally (squares) and organically (circles).

EPR MEASUREMENTS

EPR measurements were done on the Varian E-9 spectrometer (10 GHz). The spectra were recorded with digital acquisition, EW-ESR ware software (Morse, 1987), at room temperature. Sample capillaries were inserted into the standard 4 mm diameter EPR quartz tubes and centered in a TE102 EPR cavity. The instrument was set up at a microwave frequency of 9.29 GHz, the modulation amplitude was 1.0 G, the current was 10.0 mW and the receiver gain was 1250. At each time point, four EPR spectra were recorded and their average values were obtained.

Experimental results obtained both on conventionally and organically grown apples are compared in relation to the initial 100% value.

EXPERIMENTAL DATA FITTING

For each cultivar a non-linear curve fit for exponential decay of the first order ($y = y_0 + A_1 \cdot e^{-x/t1}$) based on the Levenberg-Marquardt method was used. The fitting of experimental data was done with analytical tools provided by Origin software. Parameter R^2 indicates the correlation between the experimental data and the proposed mathematical model.

RESULTS AND DISCUSSION

Jonagold's antioxidant activity with emphasis on the time dependence of antioxidant activities was investigated for apples grown in conventional and organic farming. The results shown in Fig. 2 present the time dependence of MPI decay in EPR spectra, as the measurement for antioxidant activity for Jonagold apples farmed differently.

Antioxidants are well known as sweepers of radicals. A higher concentration of antioxidants in apple juice causes the faster decrease of the TEMPO spin label signal in the sample. Since MPI decay is clearly faster in apples organically farmed, antioxidant activity in those apples is larger and the concentration of antioxidants are higher than in conventionally farmed Jonagold apples. After the first ten minutes, the difference in MPI is approximately 17%. After 25 minutes, antioxidants in conventionally farmed apples reduced the spin label concentration to 76.98% of the initial value, while at the same time antioxidants in organically farmed apples reduced the spin label concentration to 59.45% of the initial value. The observed difference is very significant. These findings indicate greater nutritional opportunities for organically farmed apples since antioxidant activity is an important nutritional parameter in the evaluation of food. This result could be generally comparable with previous findings (Donovan et al., 1998; Tzika et al., 2008).

The antioxidant activity of conventionally and organically farmed Golden Delicious apples was also investigated. The results shown in Fig. 3 present the time dependence of MPI decay in the EPR spectra for differently farmed Golden Delicious apples.

The MPI decay is faster in Golden Delicious apples organically farmed than in Golden Delicious apples conventionally farmed, although the difference is smaller than in the case of Jonagold (approximately 5%). After 30 minutes, antioxidants in conventionally farmed apples reduced the spin label concentration to 51.48% of the initial value, whilst at the same time antioxidants in organically farmed apples reduced the spin label concentration to 44.84% of the initial

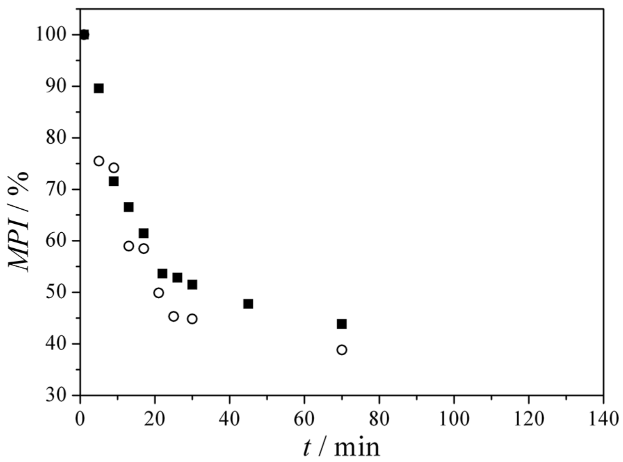

Fig. 3. Time dependence of relative MPI decay in EPR spectra for Golden Delicious apples farmed conventionally (squares) and organically (circles).

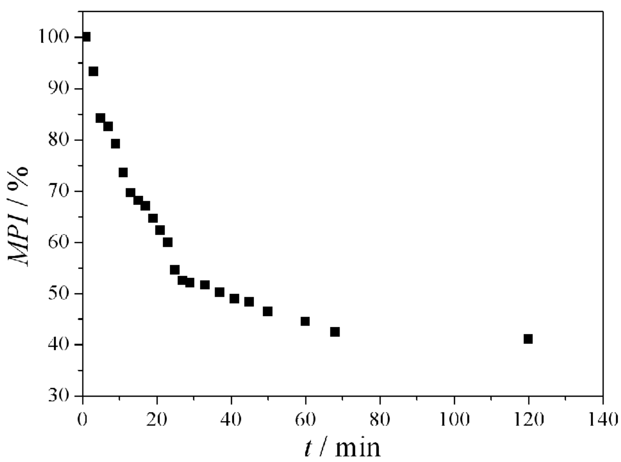

Fig. 4. Time dependence of relative MPI decay in EPR spectra for Idared apples conventionally farmed.

value. It indicated that the antioxidant activity in organically farmed Golden Delicious apples is larger and the concentration of antioxidants is higher. In comparison to results obtained with the Jonagold variety, these results suggest a higher antioxidant activity which provides even greater nutritional opportunities for the Golden Delicious variety, especially for organically farmed apples. These findings are also correlated with previous findings by different authors (Wolfe et al., 2003; Serra et al., 2010).

The exponential decay of MPI in EPR spectra of the TEMPO spin label in conventionally farmed Idared apples, as a measure of antioxidant activity, is shown in Figure 4.

The MPI decay is qualitatively and quantitatively similar for Golden Delicious (Fig. 3) and Idared (Fig. 4) apples conventionally farmed which testifies to their similar antioxidant activity and the concentration of antioxidants. After 30 minutes antioxidants in conventionally farmed Idared apples reduced their spin label concentration to 52.10% of the initial value (in comparison to 51.48% for Golden Delicious apples) and after 45 minutes the spin label concentration decreased to 48.29% of the initial value (in comparison to 47.75% for Golden Delicious apples).

The results of fitting (Tab. 1) clearly demonstrate that the t_1 parameter, describing the time scale of decay, is the largest for Jonagold (t_1 = 25.12 min) while Idared and Golden Delicious have smaller values (18.59 min and 12.60 min, respectively). Although y_0 and A_1 parameters differ, it means that conventionally farmed Jonagold apples have less antioxidant activity than Idared and Golden Delicious grown in the same way.

The results of fitting presented in Table 1 clearly demonstrate that the t_1 parameter has a larger value for Jonagold (t_1 = 26.48 min) than for Golden Delicious (12.35 min), although y_0 and A_1 parameters slightly differ. It means that organically farmed Jonagold apples have a smaller antioxidant activity than Golden Delicious grown in the same way.

The high values of R^2 parameters indicate a very good correlation between the experimental data and the proposed mathematical model of exponential decay of the first order.

TABLE 1. Fitting parameters obtained by non-linear curve fit for exponential decay of first order based on Levenberg-Marquardt method.

Cultivar/parameter	y_0	A_1	t_1/min	R^2
Jonagold – conventionally farmed	61.31±3.39	40.19±2.91	25.12 ±4.55	0.98677
Golden Delicious – conventionally farmed	44.78±1.65	61.05±2.42	12.60 ±1.20	0.98917
Idared – conventionally farmed	42.30±3.06	65.28±3.83	18.59 ±2.67	0.97667
Jonagold – organically farmed	33.69±3.66	68.06±3.94	26.48 ±3.60	0.97398
Golden Delicious – organically farmed	38.72±3.33	64.09±4.39	12.35±2.04	0.97268

CONCLUSION

The reported study contains EPR investigations of antioxidant activity in three apple cultivars conventionally grown (Jonagold, Golden Delicious and Idared) and two cultivars organically farmed (Jonagold and Golden Delicious). The results clearly indicate the difference between the two methods of farming, conventional and organic, as well as between cultivars grown in the same way. The Golden Delicious cultivar is richer in antioxidants than the Jonagold and Idared, regardless of the method of farming. The difference between them is higher in conventionally grown apples than in organically farmed. High values of R^2 parameters for all experimental data indicate correctness in the proposition of exponential decay of the first order as a mathematical model for the explanation of the dynamic properties of antioxidants.

AUTHORS' CONTRIBUTIONS

KM, BB, TBL idea and work design; TBL sample preparation; KM, BB EPR experiments; KM, BB data analysis; TBL table and figures preparation; KM draft manuscript preparation; BB, TBL critical revision of manuscript. All authors declare that there are no conflicts of interest.

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