# POLYPHENOL OXIDASE ACTIVITY IN SELECTED APPLE CULTIVARS

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## A B S T R A C T

Twenty two scab-resistant apple cultivars, harvested in the 2007 and 2008 seasons, were analysed for phenolic compounds content and polyphenol oxidase (PPO) activity. Tyrosine content was determined in the raw juices of five selected cultivars. The results showed substantial differences in the composition between the investigated apple cultivars, particularly in the phenolic compounds contents. Their total polyphenol content ranged from 161.9 to 882.4 mg/kg f.w., with hydroxycinnamic acids as the main phenolic compounds. The level of PPO activity ranged from 5 to 240 U/g f.w. Almost half of the 22 cultivars show PPO activity below 50 U/g f.w. 'Angold', 'Selena' and 'Gold Milenium' showed the highest PPO activity; between 125-133 U/g f.w. 'Rebella', 'Šampion', 'Topaz', 'Rewena', 'Enterprise' and 'Gerlinde' showed the lowest PPO activity. Statistical analysis showed no correlation between PPO activity and total polyphenol content or hydroxycinnamic acids. No correlation can be confirmed between PPO activity and tyrosine content.

**Key words**: PPO activity, scab-resistant apples, polyphenols, tyrosine

## INTRODUCTION

Catalysis by polyphenol oxidase (PPO) is the main reason for the occurrence of natural and browning reactions, with atmospheric oxygen as the oxidant (Rocha, 2001). Polyphenol oxidase (PPO) catalyses o-hydroxylation of monophenols to give o-diphenols (cresolase activity) and o-diphenols to o-quinones (catecholase activity) (Cestaria et al., 2002). Enzymatic browning causes severe quality defects during handling of light-coloured fruits and vegetables and causes serious problems in the processing technology of low processed fruits and vegetables.

The contribution of a substrate to enzymatic discoloration depends on the concentration of the substrate and on the nature of the other compounds present in the tissue. Catechins, cinnamic acid esters, 3,4-dihydroxy phenylalanine (DOPA), and tyrosine are the most important natural substrates of polyphenol oxidase in fruits and vegetables. In apples, (+) catechin, (-) epicatechin and chlorogenic acid have been identified as substrates for PPO (Rocha, 2001). The catechins were found to be oxidized more rapidly than chlorogenic acid, with (-) epicatechin contributing more than the other compounds to the browning. However, since the concentration of chlorogenic acid in apples is several times higher that of the catechins, its role in browning may be more important (Vamos-Vigyazo, 1981; Rocha, 2001). PPO has generally been recognized to be responsible for enzymatic browning of fruits and vegetables. So far, however, it has not been clearly established whether a relationship between extent of browning, fruit polyphenol content, and enzyme (PPO) activity exists (Carbonaro and Mattera, 2001)

Apples are the most frequently consumed fruits in Poland. Commercial apple production in 2005-2007 amounted to 1.05-2.3 Mt/year (Nosecka, 2008). Apples, like other fruits, are an important part of the human diet. They are a source of sugars, minerals, dietary fibre and various biologically active compounds, such as vitamin C (ascorbic acid) and certain phenolic compounds which are known to act as natural antioxidants (Podsędek et al., 2000). Subclasses of polyphenols in apple are hydroxycinnamates, dihydrochalcones and flavonoids (i.e. flavanols and flavonols) (Schieber et al., 2003).

Scab-resistant cultivars have a heritable immunity to apple scab, a major disease of the fruit and foliage of apples. Apple scab requires several applications of fungicide to control on non- resistant cultivars. The consumption of scab-resistant apples and products made from scab resistant apples, due to their lower levels of potentially toxic residues, may also become appealing to consumers (Kołodziejczyk et al., 2007).

PPO activity and its correlation to polyphenol and tyrosine content in new scab-resistant apple cultivars are not presently known. The aim of this study was to compare the activity of PPO with the concentration of total phenolic compounds and tyrosine.

## MATERIAL AND METHODS

22 scab resistant apple cultivars were evaluated: 'Angold', 'Ariwa', 'Enterprise', 'Free Redstar', 'Freedom', 'Gerlinde', 'Gold Milenium', 'Idared', 'Melfree', 'Novamac', 'Rajka', 'Rebella', 'Regina', 'Reglindis', 'Renora', 'Retina', 'Rewena', 'Rubinola', 'Šampion', 'Sawa' 'Selena', 'Topaz'. The fruits were harvested in 2007 and 2008 at the Experimental Orchard of the Research Institute of Pomology and Floriculture in Dąbrowice. Mean sample was prepared as described by Renard (2005) and ground in liquid nitrogen. The prepared sample was extracted and used for polyphenols and PPO activity determinations.

Extraction was performed as follows: 2 g of fresh sample were mixed with 4 ml of 70% methanol and sonicated for 15 min, then stored 12 h. After centrifugation, the supernatant was collected, and the sediment was re-extracted two times with 3 ml of extraction solvent. Pooled extracts were refilled up to 10 ml.

**HPLC determination of polyphenols**

The determinations were performed using the Dionex HPLC system equipped with a Diode Array Detector (UVD340U Dionex Germering, Germany) and Gemini 5 µ C18 110A column (250 x 4.6 mm), (Phenomenex, Torrance, CA, USA). Phase A was – 0.05% phosphoric acid in water, phase B – 0.05% phosphoric acid in acetonitrile, column temp. 25 °C, flow rate 1.25 ml/min. an elution profile 0-12.5 min 4% B, 12-42.4 min 15% B, 42.4-51.8 min 50% B, 51.8-53.4 50% B 53.4-55.4 4% B. Acetonitrile gradient-grade, methanol gradient-grade and phosphoric acid (HPLC) were purchased from Baker (Deventer, The Netherlands)

Chlorogenic acid, epicatechin, hyperoside, phloridzin and quercetin were used as standards, all from Extrasynthese (Genay, France), to calculate the concentrations of hydroxycinnamic acids, procyanidins, hydroxychalcones and quercetin glycosides and aglycones. Hydroxycinnamic acids, procyanidins and dihydrochalcones were detected at 280 nm while quercetin glycosides and quercetin were detected at 360 nm. The results were quoted as the sum hydroxycinnamic acid, the sum of flavonols, the sum of dihydrochalcones, and the sum of quercetin glycosides. The total polyphenols was quoted as the sum of hydroxycinnamic acids, the sum of flavonol, the sum of dihydrochalcones and quercetin glycosides. The results were expressed in milligrams per kilogram of fresh weight.

PPO activity was determined by adopting the method described by González et al. (1999). Enzyme extraction: 10 g of apple pulp ground in liquid nitrogen was homogenized with 25 ml of 0.05 M phosphate buffer (pH 7) and left for 2 h at 4 °C in the dark. The homogenates were then centrifuged at 4800 g for 5 min on laboratory centrifuge (MPW, Warsaw, Poland). Supernatant was used for the assay. PPO activity was determined at 25 °C by measuring

the rate of increase in absorbance at 420 nm, using the spectrophotometer SP-880 Metertech (Taiwan, R.O.C.). Unless otherwise stated, activity was assayed after incubation for 2 minutes in 3 ml of reaction mixture, consisting of 2.7 ml of 0.1 M catechol in 0.2 M sodium phosphate buffer (pH 5.5) plus 0.3 ml of prepared enzyme. The enzyme activity unit was defined as the change of 0.01 in the absorbance value per minute under the conditions of assay and expressed per gram of fresh mass of fruit sample take for extraction [U/g f.w.].

**HPLC determination of tyrosine**

Tyrosine content was determined in the raw juice of selected scab-resistant apple cultivars: 'Angold', 'Ariva', 'Enterprise', 'Gerlinde' and 'Topaz'. The 100 g of mean apple sample was grounded in a Brown Multiquick blender (Braun GmbH, Kronberg, Germany) under protection of nitrogen. The pulp was manually pressed through a cloth. The obtained juice was immediately 10 times diluted with the HPLC solvent, sonicated, filtered through 0.45 µm PTFE filter (Chromacol Ltd., Herts, UK) and kept frozen until analysed.

Analysis: HPLC determinations were performed using Dionex HPLC (UVD340U, Dionex Germering, Germany) with EC detector: pump P580 (Dionex, Sunnyvale, CA, USA), a 20 µl manual injector, Decade electrochemical detector with flow-through detection cell equipped with glassy carbon electrode and an Ag/AgCl reference one, set at +0.85 V (Antec-Leiden, The Netherlands), Hypersil BDS C18 column (150 x 2 mm, 3 µm Thermo Quest, Hypersil, Runcorn, U.K.). The HPLC solvent delivered with a flow rate of 0.1 ml/min was a water solution of $KH_2PO_4$ 0.1 mol/dm$^3$, phosphoric acid 0.00068 mol/dm$^3$, 1-octanesulfonic acid sodium salt 0.00045 mol/dm$^3$, and methanol 0.72 mol/dm$^3$.

The effect of cultivar on the content of the polyphenolic components and PPO activity was determined using one-way analysis of variance ANOVA, and significant differences between results were determined by Duncan's multiple range test. The differences were considered significant at $p < 0.05$. Calculations were done using Statistica program ver. 7 (StatSoft, Tulsa, USA).

## RESULTS

The results of PPO activity determinations and the contents of main phenolics groups in the researched apple cultivars from two harvest seasons are compared in Table 1.

The total phenolics content (TPH), determined by HPLC, ranged from 161.9 to 882.4 mg/kg f.w. in 2007, and from 210.1 to 675.8 mg/kg f.w. in 2008. The content of hydroxycinnamic acids (HCA) was from 64.2 to 353.1 mg/kg f.w. in 2007, and from 45.6 to 331.6 mg/kg f.w. in 2008.

According to Lee et al. (2003) the content of phenolics in the 6 examined apple cultivars: 'Golden Delicious', 'Cortland', 'Monroe', 'Rhode Island', 'Greening', and 'NY674' was somewhat lower; from 38.22 to

Table 1. PPO activity (U/g f.w.) and polyphenols (hydroxycinnamic acids HCA and total polyphenols TPH) content (mg/kg f.w.) in scab-resistant apple from the 2007 and 2008 seasons

| Cultivar | 2007 | | | 2008 | | |
|---|---|---|---|---|---|---|
| | PPO | HCA | TPH | PPO | HCA | TPH |
| Angold | 84.9 fg* | 114.9 d | 368.1 cd | 165.8 g | 92.4 cd | 302.1 c |
| Ariwa | 15.6 ab | 158.1 e | 405.6 cde | 75.0 d | 158.5 ij | 389.9 f |
| Enterprise | 13.1 ab | 279.6 j | 707.2 i | 31.4 b | 331.6 l | 675.8 j |
| Free Redstar | 96.2 g | 240.8 i | 515.7 fg | 201.3 h | 172.0 jk | 428.2 g |
| Freedom | 55.7 e | 156.8 e | 512.80 ef | 95.0 e | 124.9 gh | 422.4 g |
| Gerlinde | 35.8 cd | 93.1 bcd | 161.9 a | 28.7 b | 136.8 h | 443.8 g |
| Gold Milenium | 236.1 h | 90.6 bcd | 230.5 b | 231.7 i | 97.7 de | 254.2 b |
| Idared | 54.4 e | 186.8 g | 382.8 cd | 46.7 c | 151.6 i | 375.7 ef |
| Melfree | 83.7 fg | 156.7 e | 530.3 g | 240.6 i | 134.8 h | 328.2 cd |
| Novamac | 81.6 f | 92.5 bcd | 225.2 b | 91.7 e | 108.8 ef | 251.0 b |
| Rajka | 46.2 de | 96.8 bcd | 452.5 ef | 58.0 cd | 69.1 b | 305.7 c |
| Rebella | 2.3 a | 64.2 a | 358.9 c | 7.4 a | 45.6 a | 301.7 c |
| Regina | 17.1 ab | 210.8 h | 519.4 fg | 30.4 b | 160.3 ij | 569.2 i |
| Reglindis | 22.9 bc | 99.5 cd | 347.5 c | 94.2 e | 72.5 b | 328.9 cd |
| Renora | 58.2 e | 295.2 j | 882.4 j | 85.8 de | 180.4 k | 494.2 h |
| Retina | 37.4 d | 353.1 k | 537.9 g | 74.1 d | 184.8 k | 301.2 c |
| Rewena | 13.7 ab | 115.5 d | 474.3 efg | 33.7 b | 75.1 b | 312.0 cd |
| Rubinola | 38.3 d | 142.7 e | 433.6 de | 27.9 b | 136.8 h | 344.7 de |
| Šampion | 12.9 ab | 72.6 ab | 407.7 cde | 5.2 a | 114.3 fg | 540.8 i |
| Sawa | 6.0 a | 89.4 bc | 636.0 h | 21.5 b | 90.6 cd | 440.6 g |
| Selena | 47.9 de | 141.8 e | 433.2 de | 142.1 f | 83.0 bc | 210.1 a |
| Topaz | 44.1 de | 64.2 a | 460.6 ef | 30.8 b | 79.6 bc | 484.3 h |

*Within the same column, means with different letters are significantly different

91.70 mg/100 g f.w., where mean value was 58.61 mg/100 g f.w. The main components were quercetin glycosides 13.20, procyanidins 9.35, chlorogenic acid 9.02, epicatechin 8.65 and phloretin glycosides at 5.59 mg/100 g f.w., respectively. Markowski et al. (2009) showed

values on a similar level for mash of 'Šampion' and 'Idared' cultivars, which for the 2005 harvest season amounted to 519.7 mg/kg and 352.3 mg/kg, respectively. For the 2004 harvest season the values were somewhat higher, and amounted to 839 mg/kg f.w. for 'Jonagold', 840 mg/kg f.w. for 'Šampion, 910 mg/kg f.w. for 'Idared', and 840 mg/kg f.w. for 'Topaz', respectively.

Biegańska-Marecik and Czapski (2003) determined polyphenols by the Folin-Ciocalteau method, in 10 apple cultivars. The results varied between 31.4 and 222.2 mg/100 g f.w., The mean value was 91.9 mg/kg f.w., while for 'Idared' the mean value was 91.8 9 mg/kg f.w., and for 'Šampion' 77.0 9 mg/kg f.w.

Analysis of polyphenol contents in scab-resistant apples from two harvest seasons proved that 'Enterprise', 'Regina', 'Sawa', and 'Topaz' contained the highest amount of polyphenols, while 'Novamac', and 'Gold Milenium' were characterized by low polyphenol contents. The contribution of individual polyphenols varied between cultivars. 'Renora', 'Retina', and 'Enterprise' cultivars were rich in hydroxycinnamic acids (HCA), while 'Rebella', and 'Topaz' are poor in this group of phenolics (Tab. 1).

Data on Figure 1 show the variability of PPO activity in researched cultivars, the values varied between 5 and 233 U/g f.w. 'Rebella', 'Šampion', 'Topaz', 'Rewena', 'Enterprise', and 'Gerlinde' are cultivars characterized by low PPO activity, whereas 'Novamac', 'Angold', 'Free Redstar', and 'Gold Milenium' were characterized by high PPO activity. According to Podsędek et al. (2000), PPO activity on fresh weight varied between 27 and 312 U/g for 10 examined dessert apple cultivars. 'Šampion' was characterized by the lowest value with 27 U/g f.w., and the value for 'Idared' amounted to 156 U/g f.w.; six times higher than the value for 'Šampion'.

The results presented in this work are similar to previously cited work - mean PPO activity for 'Šampion' cultivar from two harvest seasons was 9 U/g f.w, and for 'Idared' was nearly six times higher; amounting to 50 U/g f.w. Quite different results were published by Biegańska-Marecik and Czapski (2003) about measurements using reference to chlorogenic acid or catechin as well as a differently defined activity unit. The data given by those authors show similar PPO activity on chlorogenic acid for 'Šampion' and Idared cultivars, which amounted to 56.6 UA/100 g and 52.6 UA/100 g respectively, while PPO activity on catechin at 1.4 UA/100 g for 'Idared' is lower, compared to 2.82 UA/100 g for 'Šampion'.

Statistic analysis proved the correlation at the level of 0.78 of PPO activities of apples from two harvest seasons (Tab. 2). After the rejection of cultivars most differing in two harvest seasons ('Melfree', 'Free Redstar', 'Angold', 'Reglindis', and 'Selena'), the correlation coefficient between the two seasons was 0.92. Such results prove, repeatability of

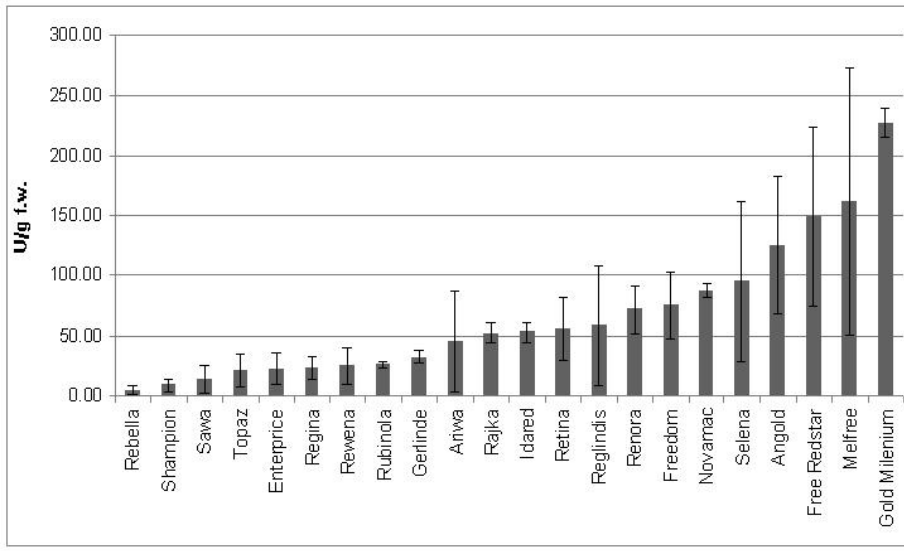


**Figure 1**. Mean of PPO activity in scab-resistant apple from the 2007 and 2008 seasons

Table 2. Correlation factors for PPO activity and polyphenol (hydroxycinnamic acids HCA and total polyphenols TPH) contents for apples from the 2007 and 2008 harvest seasons

| 2007/2008 | Correlation coefficient | Correlation coefficient after rejection of the most differing results |
|---|---|---|
| PPO/PPO | 0.78 | 0.92 |
| TP/TP | 0.50 | 0.80 |
| HCA/HCA | 0.76 | 0.83 |

measured activities for cultivars in the two harvest seasons. Data in Table 2 show the high correlation for hydroxycinnamic acids (HCA) as well. The coefficient was 0.75, and after the rejection of the two most differing cultivars 'Renora' and 'Retina', correlation coefficient for HCA rose to 0.83. Total polyphenols (TP) for two seasons correlated at the level 0.5, and after rejection of the most differing cultivars 'Melfree', 'Gerlinde', 'Renora', 'Retina', 'Selena', and 'Sawa', the factor was 0.79. Statistic analysis showed the correlation between hydroxycinnamic acids, total polyphenols and PPO activity. Results of Podsędek et al. (2000) showed that no correlation was found between specific phenolics groups and PPO activity, nor between phenolic or ascorbic acid

contents and PPO activity. The results of researchers from China were different (Song et al., 2007). They described four cider apple cultivars, namely: 'Marie Menard', 'Frequin Rouge', 'Kermerrien', and 'Douce Coetligne', and 6 juice apple cultivars, namely, 'Judeline', 'Judaine', 'Judestar', 'Juliana', 'Jurella', and 'Granny Smith'. They found that PPO activities in apples had positive correlations with the total polyphenols, proanthocyanidins, chlorogenic acid, and catechin contents, the coefficients were from 0.82 to 0.89.

Other results, showing the inhibitory influence of flavanols on PPO activity are known as well. Proanthocyanidins, with a degree of polymerisation of 80, were inhibitors at a concentration of 0.02 g/l. Other very active inhibitors were oxidation products of coffeoylquinic acid, also at very low concentrations (Mayer, 2006; Le Bourvellec et al., 2004). Xie et al. (2003) investigated the inhibitory effects of some flavonoids on the activity of mushroom tyrosinase and their results showed that flavonols: quercetin, galangin, fisetin, 3,7,40-trihydroxyflavone, and morin are competitive inhibitors of PPO, and their inhibition constants were 29, 58, 75, 154, and 410 μM, respectively. The inhibition constant of phloridzin dehydrate was 64.3 μM, which is similar to those of galangin and fisetin. Presumably, their inhibition comes from their ability to chelate copper in the active centre of the enzyme. The chelation reaction was reversible. The difference in tyrosinase inhibitory activity of flavonoids can be explained by the presence of an intramolecular hydrogen bond between hydroxyl groups which interferes with the chelation of copper in the enzyme involving the hydroxyl and carbonyl groups (Wang, 2007).

Many literature references can be found, nevertheless there is no direct proof of the influence of phenolics and PPO activity on enzymatic browning. Some authors maintain that the rate of enzymatic browning depends mostly on PPO activity, while others link enzymatic browning to polyphenol content. (Mayer, 2006; Biegańska-Marecik and Czapski, 2003). Results by Biegańska-Marecik and Czapski (2003) indicate that polyphenol content has more of an influence on enzymatic browning than PPO activity. Some researchers maintain that enzymatic browning depends on polyphenol groups (Biegańska-Marecik and Czapski, 2003; Podsędek et al., 2000). In our research, no correlation between PPO activity and total polyphenols, nor between PPO activity and hydroxycinnamic acids was found. The correlation coefficients were below 0.4 and 0.1, respectively.

Reference data indicate a significant influence of tyrosine content on enzymatic browning (Stark et al., 1985; Friedman, 1997). Research by Stark (1985) considered the influence of polyphenols and tyrosine on enzymatic browning of potato. The results showed that enzymatic discoloration for combined data from

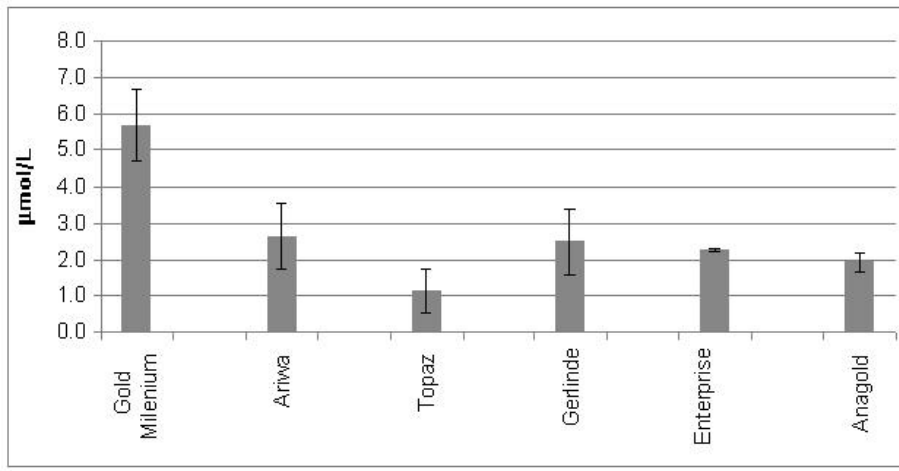


**Figure 2**. The content of tyrosine in juice from selected scab-resistant apple cultivars

the 1983 and 1984 growing seasons was highly correlated with total phenol (r = 0.89) and tyrosine (r = 0.85) concentrations.

Figure 2 shows the content of tyrosine in juice from selected scab-resistant apple.

The content of tyrosine in studied apple cultivars was diversified and varied between 1.1 and 5.7 µmol/l. The lowest tyrosine content was in 'Topaz' juice, while the highest in 'Gold Milenium'. Compared to A.I.J.N. Code of Practice, the maximal tyrosine content in apple juices is 60 µmol/l. The levels registered in our examined juices were significantly lower, reaching less than 6 µmol/l at highest. PPO activity for the examined cultivars did not correlate with tyrosine content, correlation coefficient was below 0.2.

## CONCLUSIONS

The mean PPO activity in 22 researched scab-resistant apple cultivars varied between 5-240 U/g f.w. Almost 40% of investigated cultivars from two harvest seasons were characterized by repeatability of PPO activity, with a standard deviation below 30%. Almost half of the 22 cultivars were characterized by PPO activity below 50 U/g f.w. On the other hand, almost 15% of the cultivars, i.e.: 'Angold', 'Selena' and 'Gold Milenium' showed the hightest PPO activity between 140-250 U/g f.w. Polyphenol content in researched apple cultivars varied 160-900 mg/kg f.w. Statistical analysis indicated that the correlation between PPO activity and sum of polyphenols and polyphenol groups in the

researched cultivars does not prove the interdependence between those values. For the investigated cultivars a satisfactory correlation between PPO activity and tyrosine content was not confirmed.

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# AKTYWNOŚĆ OKSYDAZY POLIFENOLOWEJ WYBRANYCH ODMIAN JABŁEK

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## S T R E S Z C Z E N I E

Oznaczono zawartość polifenoli i aktywność oksydazy polifenolowej (PPO) w dwudziestu dwóch odmianach jabłek parchoodpornych z sezonów 2007 i 2008. W sokach surowych pięciu wybranych odmian oznaczono zawartość tyrozyny. Wykazano zasadnicze różnice składu badanych odmian, zwłaszcza dotyczące zawartości substancji polifenolowych. Zawartość polifenoli ogółem wynosiła od 161,9 do 882,4 mg/kg ś.m., gdzie głównymi składnikami polifenolowymi były kwasy hydroksycynamonowe. Aktywność PPO wynosiła od 5 U/g ś.m do 240 U/g ś.m. Ponad połowa z 22 odmian wykazywała aktywność PPO poniżej 50 U/g ś.m. Odmiany 'Angold', 'Selena' i 'Gold Milenium' wykazywały najwyższą aktywność PPO pomiędzy 125-133 U/g ś.m., a 'Rebella', 'Szampion', 'Topaz', 'Rewena', 'Enterprise' i 'Gerlinde' najniższą aktywność PPO. Statystyczna analiza danych nie wykazała korelacji pomiędzy aktywnością PPO a zawartością polifenoli ogółem lub zawartością kwasów hydroksycynamonowych. Nie potwierdzono korelacji pomiędzy aktywnością PPO a zawartością tyrozyny.

**Słowa kluczowe**: aktywność PPO, jabłka parchoodporne, polifenole, tyrozyna