Polyphenol oxidase and peroxidase activity in apple: dependency on cultivar and fruit processing

O S Hutabarat¹ and H Halbwirth ²

¹Agricultural Engineering department of Hasanuddin University
²Technology University of Vienna

Email: olly_hutabarat@yahoo.com

Abstract. Apples are an important source of polyphenolic and most popular fruits worldwide. Due to their phenolic content, consuming apple in a diet tend to low risk and prevention some chronic diseases. The main enzyme is responsible for quality loss, which are affecting by phenolic degradation, are polyphenol oxidase (PPO) and peroxidase (POX). PPO and POX are correlated to the rate of browning with substrate content and enzyme activity. The objective of this research was to investigate the polyphenol oxidase (PPO) and peroxidase (POX) activity in different apple cultivars. Apples Gravin Goldach, Bay 4152, Sonnen Glanz, Gala Mitslugla apples were obtained from an orchard of Hohere Bundeslehranstalt und das Bundesamt fur Wein und Obstbau in Klosterneuburg, Vienna, Austria, then Apple from each cultivar was cut and separated in two parts, peel with seed (red flesh) and peel without seed (white flesh), then PPO and POX were determined. The results showed that the highest PPO activity is presented by red flesh of Bay 4152 cultivar, whereas the lowest detected in red flesh Gala Mitslugla. PPO activity on apple flesh of red Bay 4152, white Sonnen Glanz, red gravin Goldach and red Gala Mitslugla were 5.01E+04, 4.79+04, 3.17+04 and 1.91E+04 ΔE/S/Kg protein, respectively. PPO activity on apple flesh was not affected by white or red colour. The highest POX activity was found in red flesh of Sonnen Glanz cultivar, whereas the lowest value was presented in white flesh of Gravin Goldach cultivar varied from 1.07E+05 to 7.68E+04 ΔE/S/Kg protein. In all different flesh of apple cultivars showed that the high or low POX activity was not resulted by the red or white flesh coloured. POX is much higher than PPO activity of all cultivars. Result will provide to maintain the phenolic related quality loss and to improve new processing method.

1. Introduction

Apples are an important source of polyphenolic and most popular fruits worldwide. Due to their phenolic content, consuming apple in a diet tend to low risk and prevention some chronic diseases such as cancer, [6] prostate, liver, colon, lung cancers and cardiovascular diseases [3] [6] [7] [10]. Several studies reported that the main phytochemical in apples belong to the group polyphenols which are contribute to the antioxidant activity, namely hydrodynamic acids, flavan 3-ols- anthocyanidins, flavanols and dihydrochalcones. Hydrodynamic acids mainly caffeeoyl quinic acid located in flesh, flavanols mainly quercetin glycosides located in skin and dihydrochalcone mainly phloridzin and phloretin glucoside. [4] [9]. In apple, dihydrochalcones are mainly present in seed followed in core
and peel [1]. Apple seeds is reported rich of phloridzin and contribute to antioxidant. Antioxidant activity in seeds showed higher than peel and flesh [2].

Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. The main enzyme is responsible for quality loss, which are affecting by phenolic degradation, are polyphenol oxidase (PPO) and peroxidase (POX). PPO and POX are correlated to the rate of browning with substrate content and enzyme activity. The main problem in fresh-cut industry is rapid product deterioration due to enzymatic browning which are not only affect flavour and nutrition content, but also reduces visual quality of the product. Despite the negative effects of PPO on plants, PPO have numerous physiological function in plants, such as defence against biotic and abiotic stress, resistance to climatic stress and photosynthesis. PPO utilizes oxygen to oxidize phenolic compounds to quinones that are highly reactive and can combine together and with other compounds to form brown pigments [12].

The enzymatic oxidation of phenolic compounds can cause blackening or browning of fruits and vegetables either pre-harvest or during post-harvest storage. To enhance the pigmentation of apple processing, some postharvest treatment has been applied, such as cold storage with modified atmosphere, heat treatment, high-field electric pulses, high hydrostatic pressure, etc. Prior to apple processing, it is necessary to determine the polyphenol oxidase and peroxidase activity on apple. The objective of the present study was to investigate the polyphenol oxidase (PPO) and peroxidase (POX) activity in different apple cultivars. Result will provide to maintain the phenolic related quality loss and to improve new processing method.

2. Material and Method

2.1 Plant Material

Apples. Gravin Goldach, Bay 4152, Sonnen Glanz, Gala Mitsulga apples were obtained from an orchad of Hohere Bundeslehranstalt und das Bundesamt fur Wein und Obstbau in Klosterneuburg, Vienna, Austria. Apple from each cultivar was cut and separated using knife in two parts, peel with seed and the others peel without seed. In order to obtain in powder, apple peel with and without seed was prepared using extractor then transferred to conical flask, immediately immersed in liquid nitrogen and stored at -80°C until further use.

2.2 Determination of Peroxidase (POX)

Apple was taken from -80°C, grinded 0.5 g apple powder, 0.25 g quartz sand, and 0.25 g Polyclar AT were homogenized with 3 mL 0.1 M KPI (containing 0.4 % Na-ascorbate, pH 6.0) in a pre-cooled mortar. After centrifuging the homogenate for 10 min at 4°C and 10,000g, 400μl of the supernatant were passed through a gel chromatography column (Sephadex G25 medium, (GE Healthcare, Vienna Austria)) in order to remove low molecular compounds, eluted crude extract was used for peroxidase assay. Crude extract was taken 100 μl added to 1000 μl H₂O₂ - KPi buffer (hydrogen peroxide 30% from Merck and potassium hydrogen phosphate from Kemika) and mixed together with 10 μl of o-dianisidine (Sigma–Aldrich) and quickly put in spectrophotometer (Perkin Elmer, UV/VIS Lambda Bio 20) and time-dependent changes in absorption at 460 nm were determined in comparison with a blank containing only o-dianisidine and H₂O₂–KPi buffer. One unit of POX activity was defined as the change in absorbance of 0.1 per min per mL of enzyme. Activity measurements were carried out in triplicate.

2.3 Determination of Polyphenol oxidase (PPO)

Apple was grinded with 0.5 g apple powder, 0.25 g quartz sand, and 0.25 g Polyclar AT and homogenized with 3mL Tris buffer (containing 0.01 M Tris, 0.2 g EDTA, 0.38 g Dinatrium tetraborat) pH 9.06. After centrifuging the homogenate for 10 min at 4°C and 10,000g, 400μl of supernatant were passed through a gel chromatography column (Sephadex G25 medium, (GE Healthcare, Vienna Austria)) in order to remove low molecular compounds. PPO enzyme activity was measured as
described by Gossinger with slight modification. The reaction mixture contained 400 μl of the supernatant, 340 μl of 0.2 M Pyrocatechol in 100 μl McIlvaine buffer, pH 6.5 and 660 μl of 0.1 McIlvaine buffer. Pyrocatechol oxidation was followed for 12 min with absorbance being recorded at 60 sec intervals at 410 nm, against a blank consisting of pyrocatechol and buffer solution. PPO activity was expressed in units, as the change in absorbance at 410 nm/min/mL of juice or 0.001×A410 min/mL. Activity measurements were carried out in triplicate.

2.4 Result and Discussion

Peroxidase (POX) and Polyphenol oxidase (PPO) activity were observed among cultivars. POX and PPO activity are the most enzyme responsible for quality loss due to phenolic degradation. PPO activity with seed and without seed of Gräfin Goldach, Bay 4152, Sonnen Glanz and Gala Mitslugla are presented in figure 1.

PPO activity of apple without seed was higher than with seed, except for Gravin Goldach. Gravin Goldach was the cultivar with the highest PPO in seed than without seed which was 4.23E+04, 3.17E+04 ΔE/S/Kg protein, respectively. Furthermore, for both Bay 4152 and Sonnen Glanz cultivar, PPO activity was slightly higher in without seed than with seed which were 5.01E+04, 4.60E+04, 4.79E+04, 4.08E+04 ΔE/S/Kg protein, respectively. Highest activity presented by Bay 4152 followed by Sonnen Glanz and Gala Mitslugla cultivar. All cultivars with and without seed did not show significant differences for PPO activity, except for Gala Mitslugla. PPO activity of Gala Mitslugla without seed 2.0544-fold higher than with seed. Lowest activity was detected in Gala Mitslugla cultivar with seed. PPO activity was low in varieties showing less sensitivity to oxidation.

![Figure 1. PPO activity on different cultivars](image-url)
Figure 2. PPO activity on apple flesh

PPO activity on apple flesh were determined. PPO activity on apple flesh in different cultivar can be seen in Figure 2. PPO activity on red flesh apple bay 4152 cultivar was slightly higher than white flesh of Sonnen Glanz but as not as lowest than the red flesh Grafin Goldach and red flesh Gala Mitslugla. The highest and the lowest PPO activity on apple flesh is demonstrated by red flesh apple. PPO activity on apple flesh was not affected by white or red colour. Comparison between the lowest and highest PPO activity on the same red flesh was 2.623 folds. However, comparison between the lowest and highest PPO activity on the different coloured flesh was considerably higher although less than the PPO activity on the same coloured flesh. The highest activity on flesh is presented by red flesh Bay 4152 cultivar, whereas the lowest detected in red flesh Gala Mitslugla. PPO activity on apple flesh of red Bay 4152, white Sonnen Glanz, red gravin Goldach and red Gala Mitslugla were 5.01E+04, 4.79+04, 3.17+04 and 1.91E+04 ΔE/S/Kg protein, respectively.

Figure 3. POX activity on different cultivar
Figure 3. shows POX activity on different apple cultivars with and without seed. Gravin Goldach, Bay 4152, Sonnen Glanz and Gala Mitslugla apple cultivars with and without seed were tested for enzymatic activity of POX. POX activity in apple cultivars without seed was higher than the with seed in the most of sample. Contrary to PPO activity, POX activity of Gravin Goldach without seed was higher than with seed. Furthermore, POX activity of Gala Mitslugla with and without seed showed quiet similar, as not as PPO activity, which was 8.20E+04, 7.68E+04 ΔE/S/Kg protein, respectively. In the same cultivar no significant differences POX activity for both with and without seed, particularly in Bay 4152 cultivar, POX activity was found 1.01E+05 and 1.03E+05 ΔE/S/Kg protein, respectively. However, Sonnen Glanz cultivar without seed was detected 1.22 higher than with seed which was 1.07E+05, 8.73E+04, ΔE/S/Kg protein, respectively. The highest POX activity was found in Sonnen Glanz without seed, whereas the lowest value was presented in Gravin Goldach with seed varied from 1.07E+05 to 7.68E+04 ΔE/S/Kg protein.

Figure 4. POX activity on apple flesh

POX activity in apple flesh is available in Figure 4. Highest activity was achieved by white flesh Sonnen glanz cultivar and the lowest presented by Red flesh Gravin Goldach. For both cultivars Grafin Goldach and Gala Mitslugla with the same red flesh showed almost the same activity but compare to Bay 4152 the activity quiet less. POX activity between apple flesh of red bay 4152 and white flesh Sonnen Glanz was quiet similar, namely 1.01E+05 ΔE/S/Kg protein and 1.07E+05 ΔE/S/Kg protein, respectively. Also, the POX activity was quiet the same for both red flesh Grafin goldach and red flesh Gala mitslugla cultivars, 8.40E+04, 8.20E+04 ΔE/S/Kg protein, respectively. In all different flesh of apple cultivars showed that the high or low POX activity was not resulted by the red or white flesh coloured.
As is apparent from figure 5, POX activity differed significantly than PPO activity among the four cultivars. It was highlighted that POX activity was 1.82-3.79 fold higher than that PPO activity. Gala Mitslugla with seed is recorded the extremely high differences POX compared to PPO activity, whereas the lowest value was detected in Gravin Goldach cultivar with seed. The highest and the lowest were presented by cultivar with seed, indicating PPO and POX-dependent difference in cultivar. POX activity of Bay 4152 and Sonnen Glanz with and without seed were observed higher 2.03-2.23-fold than PPO.

As can be seen from figure 6. POX activity differed strikingly than PPO activity in apple flesh among cultivar. POX activity in red flesh gala Mitslugla was detected 4.29 higher than PPO. It can be inferred that apple Gala Mitslugla cultivar had higher comparison between POX and PPO in flesh. POX of white flesh Sonnen Glanz was 2.23 higher than PPO followed by red flesh Bay 4152, whereas red flesh Grafin Goldach had the second highest comparison, it was 2.65 times.
3. Discussion
POX and PPO are the main enzymes responsible for quality loss due to phenolic degradation. PPO and POX activity was measured of four different cultivars. The highest PPO activity was presented by Bay 4152 cultivar without seed (white flesh) whereas the lowest followed by Gala Mitslugla with seed. Cultivar with high POX activity is involved in resistant to apple deseases [11]. POX activity in apple cultivars without seed was higher than the with seed in the most of samples. Moreover, in the same cultivar no significant differences POX activity for both with and without seed. PPO activity of Bay 4152 cultivar without seed was highest, whereas the lowest was recorded on Gala Mitslugla with seed. Gravin Goldach cultivar with seed. High PPO activity on Bay 4152 cultivar can be related to the accumulation of reactive oxygen species (ROS) and overall redox potential values [8]. A comparable activity of both enzyme, PPO and POX, showed POX is extremely higher than PPO activity.

Highest enzymatic of PPO in flesh has been demonstrated on red flesh Bay 4152 cultivars, whereas the lowest was recorded on Gala Mitslugla. White flesh Sonnen Glanz cultivar was higher PPO activity compared to red flesh Gravin Goldach and red flesh Gala Mitslugla. However, contrast to Gala Mitslugla, the lowest PPO activity presented by this cultivar. In addition, low PPO activity may also contribute to the stability. Higher enzyme activity of PPO is equal high total phenolic content as well, due to polyphenolic are the main substrate for PPO. POX is a secondary reaction of the oxidation of phenolic substrate using hydrogen peroxide as an oxidation agent. Lower and higher POX activity are not strong correlation with phenolic content.

The apple polyphenol oxidase specific substrates into highly reactive o-quinones. Hydroxylation in position 3 is the first step in the oxidation of phloretin by PPO, which results in the formation of highly reactive quinoid structures that can interfere with cell invading pathogen. Several authors have previously described the correlation between PPO and POX with resistant to pest and deseases [8]. PPO contributes to lignifications and pigmentation and together with POX it consumes oxygen and produce quinones, which may reduce plant digestibility for the pest. Hutabarat at all demonstrated that increase or decrease in POX and PPO activities in apples is contribute to apple resistance to Erwinia and reduce susceptibility to apple scab and fire blight. [5] Despite Gravin Goldach showed highest PPO activity and Sonnen Glanz cultivar presented highest POX activity, but it was not followed with high together for both. Therefore, we highly recommended Bay 4152 cultivar is potential for molecular breading due to high PPO and POX activity and Gala Mitslugla cultivar for fruit processing.

4. Conclusion
PPO and POX activity of four cultivars were determined. The results indicate high PPO activity is not followed by POX activity. Additionally, the activity of PPO is extremely higher than POX. Surprisingly, despite a comparison of the isoenzyme pattern obtained with o-dianisidine and DOPA clearly showed distinct differences but is not confirm the higher PPO activity as compared to POX. However, no significant differences between PPO and POX activity of four cultivars with and without seed. The first report on high Polyphenol oxidase and peroxidase activity of Bay 4152 cultivar suggests a high potential cultivar for molecular breeding and Gala Mitslugla cultivar for fruit processing.

5. References
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