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## S12. Enzyme activity profile of peroxidases and polifenoloxidases of *Malus domestica* Borkh varieties from Portuguese orchards during cold storage

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#### Abstract

The region of Beira Alta, Portugal distinguishes itself as a favorable area for apple growing that produces fruits with great economic importance. The polyphenoloxidase (EC 1.14.18.1, PPO) a copper-enzyme located in plastids, catalyzes the oxidation of phenols to quinones present in the vacuoles, generating brown coloration products. In general, the enzymatic browning occurs when phenolic compounds are released by the vacuoles and contact with PPO in the presence of oxygen. In other hand, the heme-enzymes peroxidases (EC 1.11.1.7, POD) belong to oxidoreductases involved in capture of hydrogen peroxide generated by cell activity and protection against pathogens. Despite the features previous described, POD and PPO, contributes to deterioration of fruits color, resulting in adverse changes in flavor, contributing to the decrease of its useful life and consequent market value, being good markers of ability to preservation. So, the aim of this study was to assess the profile of PPO and POD enzyme activity of three varieties of Malus domestica Borkh, during cold storage. Apples of Golden Delicious, Starting and Bravo de Esmolfe varieties were randomly harvested from orchards in the region of Celorico da Beira during harvest season and stored at 4 °C for 83 days. The fruits were washed in tap water, individually weighted, peeled and stoned, cut in small portions and crushed in blender to obtain the juice. Then, the juice was centrifuged at 18000 gduring 40 min at 4 °C. Aliquots of supernatant were stored in 0.12 M phosphate buffer pH 6.5 at -20 °C, for further determination of PPO (Valero, 1991) and POD (Cano, 1998) activities. The results show that no significant differences were detected in POD activity of three varieties of M. domestica, 2 days after collection (p < 0.05). However, this enzyme activity increased significantly in Starking apples (5x, p < 0.05) throughout 83 days of storage, being this variety that was most affected along time, probably due an increase of intracellular peroxides. In other hand, PPO activity was not detected in Bravo de Esmolfe and Golden Delicious in the 2 days post-harvest period. This catalytic activity was only detected in Bravo Esmolfe and Golden Delicious varieties at 20 and 55 days post-harvest period, values which remained without significant changes until 83 days of storage. In contrast, apples of Starking variety exhibit a significant increase of PPO activity throughout the storage period of 83 days. A Pearson analysis revealed a correlated increase of POD and PPO activities along time (r = 0.884, p < 0.05). So it can infer that Starking variety has the worst characteristics of cold preservation for long time periods.

#### References

Valero, E; Varon R; Garcia-Carmona, F (1991) *Biochem. J.* 277, 869-874. Cano, M P; Hernández, A (1998) *J. Agric. Food Chem.*, 46, 266-270.

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