

Lipoxygenase Activity and Hydroperoxide Formation in Coffee (*Coffea arabica* L.) Cherries Cultivated by Different Agronomic Techniques

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SUUMARY

It is widely accepted that biotic stress induces acidic lipoxygenase (LOX) activity mainly due to the effects of methyl jasmonate (Buzi et al., 2004). The activation of LOX pathway leads to the production of a large amount of oxidation products, which may affect the coffee in organoleptic profile (Kohlmann et al., 1999). Hitherto the presence of LOX in coffee (*Coffea arabica* L.) plants is still scarcely examined (Rojas et al., 1993), therefore the aim of this study was to investigate the possible involvement of this enzyme in oxidative stress of cherries from plants cultivated in organic and conventional manners. Primary and secondary oxidation products were evaluated in three separate cherry fractions: pulp, parchment seed and green coffee. Hydroperoxides, measured by HPLC, were highest in parchment seed and lowest in green coffee, but no significant difference was found between the samples obtained from the two cultivations. However, the pulp fraction from organic cultivated plants had a larger amount of primary peroxidation products, when compared to that from conventionally cultivated plants. The presence of LOX was then examined in all cherry fractions by immunochemical analysis. Only the pulp fraction showed an 84 kDa protein that exhibited a cross-reactivity against a pea LOX antibody. Subsequently, the presence of LOX was confirmed in heavy (28000 g) and light (100000 g) membrane pulp fractions by an enzymatic assay, immunochemical method and hydroperoxide formation. In both organic and conventional cultivation systems, the light membrane showed the highest LOX activity, measured as conjugated diene formation, at pH 8.0. Instead, the heavy membrane fraction highlighted a different pH optimum, which was found to be 5.5 in pulps from organic cultivations and 8.0 in the conventional ones, respectively. All the activities reported were inhibited by nordihydroguaiaretic acid (NDGA) and caffeic acid, well known LOX inhibitors. The NDGA-sensitive hydroperoxide production was simultaneously confirmed by RP-HPLC analysis. Oxidative stress was also detected by evaluating the antioxidant activity in all fractions. A lipophilic extraction, performed using dioxane:water (95:5, v/v), revealed that parchment seeds possessed the lowest antioxidant capacity. On the other hand, a significant difference in the antioxidative capacity was detected in parchment seeds from organic and conventionally cultivated plants. Taken together, these results show, for the first time, the presence of LOX in coffee cherries, whose activity was associated to membrane fractions. Furthermore, the cultivation techniques seem to induce the activation of different isoenzymes. In particular, the organic ones were subjected to an oxidative stress in coffee fruits leading to the expression of an acidic LOX activity, detectable in the pulp fraction.