FORMATION IN COFFEE (*Coffea arabica* L.) CHERRIES CULTIVATED BY DIFFERENT AGRONOMIC TECHNIQUES

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INTRODUCTION

Lipoxygenase (LOX) is induced in plants, by biotic stress through a mechanism mediated by methyl jasmonate [1]. The activation of LOX pathway leads to the production of undesired volatile secondary products (mainly aldehydes and terpens), which interfere with the coffee organoleptic profile [2]. Up to date, few evidences have been collected about LOX presence in coffee [3], so this study was undertaken to investigate the possible involvement of the enzyme during oxidative stress in cherries from organic (identified as STRESS) or conventional cultivations (identified as CTRL) in Brazil.

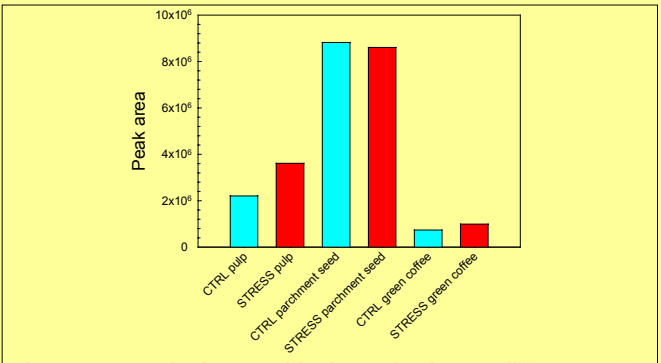


Figure 1. HPLC hydroperoxide determination on different coffee cherry portions cultivated in Brazil and milled after treatment with liquid N_2. Acid extraction (pH 2) was performed using hexane as solvent. Samples were then dried with N_2 flux and resuspended in mobile phase (acetonitrile, water and acetic acid, 80:20:0,1). Linoleic acid hydroperoxide was used as an internal standard.

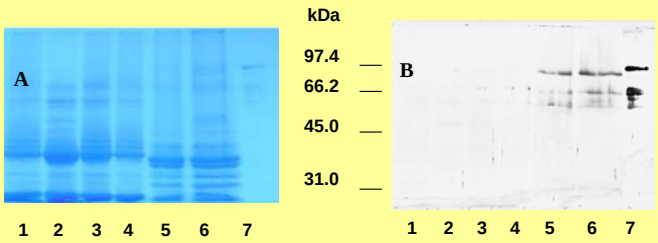


Figure 2. SDS-PAGE and Western-Blot of coffee protein obtained from different parts of the fruit. Panel A shows protein pattern in cold acetone extracts. Each lane was loaded with 30 µg of protein. Panel B indicates cross-reactivity with pea LOX Ab.

1: CTRL seeds
2: STRESS seeds
3: CTRL parchment seeds
4: STRESS parchment seeds
5: CTRL pulp
6: STRESS pulp
7: soybean LOX

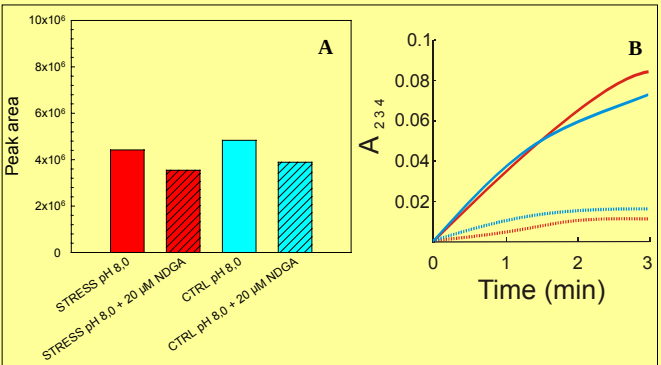


Figure 3. Lipoxygenase activity measured by hydroperoxide (HPLC) determination (panel A) and by conjugated diene formation (panel B) in coffee pulp "light fraction" membranes (microsomes). Procedures for hydroperoxide extraction and HPLC evaluation were as in Fig. 1. Conjugated diene formation was measured as absorbance increase at 234 nm. Red lines or columns= STRESS; cyan lines or columns = CTRL. Solid lines = FFA; dashed lines = FFA + 20 µM NDGA.

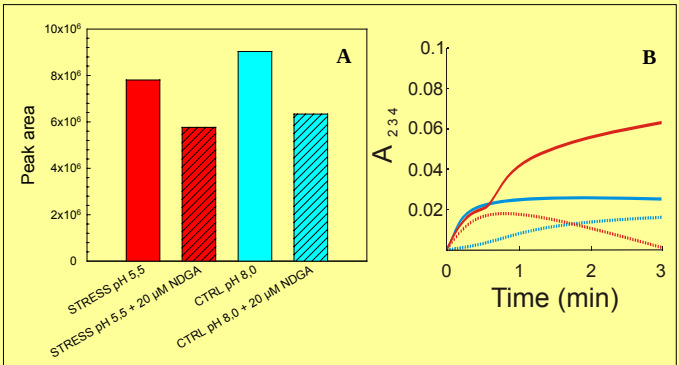
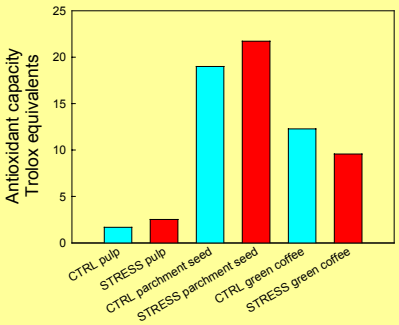


Figure 4. Lipoxygenase activity measured by hydroperoxide (HPLC) determination (panel A) and by conjugated diene formation (panel B) in coffee pulp "heavy fraction" membranes (mitochondria). Procedures for hydroperoxide extraction and HPLC evaluation were the same as in Fig. 1. Conjugated diene formation was measured as absorbance increase at 234 nm. Red lines or columns= STRESS; cyan lines or columns = CTRL. Solid lines = FFA; dashed lines = FFA + 20 µM NDGA.

Figure 5. Antioxidant activities of aqueous extracts (pH 7.5) determined by crocin kinetic competition on different portions of coffee cherries, treated with liquid nitrogen. Peroxy radicals are generated *in situ* by diazocompound decomposition. The bleaching of crocin is directly correlated to radical production and happens with a constant speed. When part of the peroxyl radicals are quenched by other antioxidants the bleaching rate is lower, and it is correlated to the concentration of the other antioxidants. The ratio between the bleaching speed of crocin without or with other antioxidants yields the antioxidant capacity of the sample.

The slope of a known antioxidant, such as Trolox C, can be calculated. Other substances, even complex mixtures, such as coffe extracts, can be measured, and their values related to that of Trolox C. The result will be an equivalent millimolar concentration of Trolox C.



CONCLUSIONS

Above exposed data demonstrate, for the first time, the presence of LOX in coffee cherries, whose activity is associated to membrane fractions. Furthermore, the expression of different isoenzymes, characterized by different pH optima, seems to be related to the cultivation techniques. In particular, organic methods cause an oxidative stress in coffee fruits, leading to the synthesis of an acidic LOX activity in fruit pulp, detectable both as hydroperoxide production and conjugated diene formation. Instead pulp fruit from conventional cultivations show a comparable LOX activity, but with an higher pH optimum. Acidic LOX activity could represent a typical physiological response against biotic stress. These results could represent a starting point to better understand the evolution of undesired volatile compounds linked to the LOX pathway, which may affect coffee quality and flavours.

REFERENCES

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