ENZYMATIC PREPARATION, PURIFICATION AND ANTIOXIDANT POTENTIAL OF POLYPHENOLIC OXIDATION PRODUCTS FROM APPLE JUICE

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Introduction. Polyphenols contribute to organoleptic properties in apple juice and positive impacts on human health due to their antioxidant activities (1). In the apple processing into juice, some phenolic compounds undergo enzymatic oxidation when there are exposed at the polyphenoloxidase (PPO) in presence of oxygen. Until now, very little is known concerning the oxidation products formed, contribution to the nutritional value, health benefits and organoleptic properties (2).
In the present work, the purpose was to characterize, purify and evaluate the antioxidant potential of the main oxidation products formed in the course of apple processing.

Methods. Enzymatic oxidation using apple PPO was carried out to produce the oxidation products of caffeoylquinic acid (CQA) and mixtures of (-)-epicatechin (EC) and CQA under several conditions. HPLC-ESI-MS was used to analytical exploration. The suitable enzymatic oxidation conditions were scale up to one liter of reaction medium. The polyphenolic fraction was purified using preparative HPLC and the main oxidation products formed were later purified through semipreparative HPLC. Antioxidant potential of the oxidation products purified was tested *in vitro* by different systems (3).

Results. Only two kinds of products were screened: CQA homodimers (m/z 705) and EC-CQA heterodimers (m/z 641) corresponding to the expected products according to the available knowledge (4). Kinetic shows a fast oxidation of the native polyphenols and formation of oxidation products studied (Fig. 1).

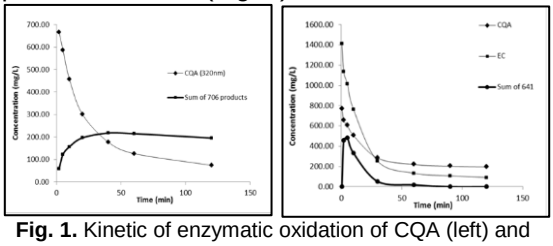

Fig. 1. Kinetic of enzymatic oxidation of CQA (left) and EC-CQA mix (right).

Interestingly, the oxidation products does not resulted in an extensive polymerization of native phenolic compounds, but a multiplicity of small molecules in different oxidation states and isomeric forms was obtained.
Nineteen fractions were obtained by HPLC semi-preparative (Fig. 2), however only fourteen fractions were recovered as pure compounds. The most of the pure compound obtained in this study were present in an oxidized apple juice polyphenolic fraction.

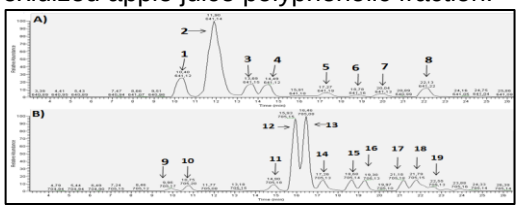

Fig. 2. Extracted m/z 641-ion chromatogram (A) and extracted m/z 705-ion chromatogram (B) of oxidation products from CQA and EC-CQA, respectively.

Nine pure compounds, five heterodimers and four homodimers were used to test the antioxidant potential against the native phenols and trolox equivalents (TE). Homodimers resulted in a smaller TE than native polyphenols. In contrast, the heterodimers had higher TE. Similarly, the IC_{50} for four heterodimers purified was lesser than native polyphenols and trolox compound, resulting in the greater antioxidant potential.

Conclusions. Polyphenol oxidation products obtained by enzymatic synthesis in model solution are close to those present in oxidized apple juice. Some of them (EC-CQA heterodimers) exhibited higher antioxidant potential than their native counterpart.

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