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Preparative isolation of apple Flavan-3-ols by pH-zone-refining centrifugal partition chromatography combined with reversed-phase liquid chromatography

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Introduction

Flavan-3-ols (which include procyanidins and condensed tannins) are widespread polyphenols in fruits and edible plants and having numerous properties. They are largely responsible for astrignency and bitterness in cider beverages. To study their sensorial, nutritional and biological features, it is essential to recover pure and native flavan-3-ols fractions. A gentle strategy combining pH-zone-refining centrifugal partition chromatography (pH-ZRPCPC) and preparative reversed-phase liquid chromatography (Prep-RPLC) was developed to purified hundred milligrams of apple flavan-3-ols fraction in a highly purified state.

Optimization of two-phase solvent system for pH-ZRPCPC

The pH-ZRPCPC fractionation was optimized from a crude apple polyphenol extract (named TotPP) obtained from a non-oxygenated juice of the French cultivar Marie Menard (Millet et al. [1]). It contained essentially flavanols monomers and oligomers (46.3%) with an average degree of polymerization close to 3.3, as measured by phloroglucinolysis coupled to reversed-phase hPLC), hydroxycinnamic acid derivatives (35.9 %) and dihydrochalcones (2.6%) (Table 2).

The partition coefficients (K) of the target compounds for a conventional CPC and pH-ZRPCPC (by adding of a base or an acid) were determined for four biphasic solvent systems adapted from OKA [2] composed of ethyl acetate-n-butanol-water with 5:0.5:3:2:5; 2:3:5; and 1:4:5 (v/v) named systems A, B, C and D, respectively. For successful separation using pH-ZRPCPC, it is necessary to respect Kbase << 1 and Kacid >> 1, for an acidic analyte. This allowed to select system B as the best one to discard hydroxyxynamic acid derivatives (HCA) (Table 1).

pH-ZRPCPC + Prep-RPLC procedures

pH-zone-refining CPC was carried out using a FCP200® apparatus (Kromaton Technologies) (Fig. 1). The collected CPC fractions corresponding to flavan-3-ols (FA), dihydrochalcones (DHC) and flavonols (FO) were immediately acidified to avoid autoxidation, and pooled. Prep-RPLC was performed on this intermediate freeze-dried fraction with a Lichrospher 100 RP-18, 12 µm column (Merck, Darmstadt, Germany) and isocratic modes, to recover only flavan-3-ols (Fig. 2).

Analyses of fractions using UPLC-UV/MS

The composition of the collected fraction (called MM-FA) was characterized and compared to that of the crude extract (Fig. 3) showing that it contained only flavan-3-ols (Table 2).

Conclusion

Starting from a crude apple polyphenol extract, our experiments demonstrate the relevance of using CPC combined with a pH-displacement mode to efficiently separate flavan-3-ols from hydroxycinnamic acid derivatives at a preparative scale. The objective was successful through optimisation of solvent systems with suitable conditions for pH-ZRPCPC and an attention paid to avoid autoxidation. Finally, preparative reversed-phase chromatography allowed the separation of the flavan-3-ols with satisfactory purity (82%) and recovery (73%).

REFERENCES