Enzymatic synthesis, structures, interactions with saliva proteins and quantification in juices of a series of dehydrodicaffeoylquinic acids, one of the main classes of oxidation products in apple-based beverages

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MAIN CONCLUSION

Dehydrodicaffeoylquinic acids (DDCQAs) are among the main phenolic products resulting from enzymatic oxidation occurring during apple processing. Accounting for dozen milligrams per liters in some apple juices, they present original chemical structures including benzodioxane, dihydronaphtalene, dihydrobenzofuran or dicatechol patterns. Interestingly, those oxidation products exhibited unusual properties regarding their ability to aggregate salivary proteins.

INTRODUCTION

Mainly located in the flesh and in the skin, apples polyphenols show a great diversity of molecules belonging to the classes of hydroxycinnamic acids, catechins, procyanidins oligomers and polymers, dihydrochalcones, flavonols and anthocyanins. When apple are processed into juices and ciders, a great diversity of newly formed polyphenolic molecules is generated by enzymatic oxidation. Some of those oxidation products contribute to the colour of the juice. However, a great majority are colourless and their detailed structures, their real concentrations in the juices or their contribution to sensory, antioxidant and other nutritional properties in the final products are still scarcely known. Previously, we showed that newly-formed products resulting from enzymatic oxidation of 5′-O-caffeoylquinic acid (CQA), also commonly known as chlorogenic acid, still presented high antioxidant activity *in vitro* [1]. However, we have no information regarding their possible involvement in the bitter taste and the sensation of astringency that may be related to the presence of polyphenols in fruit-derived beverages. We remember that, in this particular case, astringency is a consequence of the precipitation of salivary proteins due to the tanning properties of polyphenols. We present here our main recent results related to the oxidation products of CQA focusing on their UV, MS and NMR structural characterisation, their LC-MS quantification in a panel of commercial juices and their capacity to aggregate salivary proteins.

MATERIALS & METHODS

Synthesis, purification and structural analysis: DDCQAs were synthesized from CQA in model solution using a crude apple polyphenoloxidase (PPO) extract. Ten DDCQAs were purified by centrifugal partition chromatography and RP18 HPLC at the semi-preparative scale [2]. Their complete structural elucidation was achieved by MS and 1D and 2D NMR ^{1}H and ^{13}C.

Quantification in apple juices: a new LC-UV-MS method was developped using the purified standards to quantify native polyphenols, the series of DDCQAs and a series of CQA-epicatechin dimers in 45 commercial and experimental apple juices.

Aggregation to salivary proteins (SP): a mixture of soluble DDCQAs was incubated with acidic saliva. After centrifugation, the supernatant was analyse by RP18 HPLC-UV and compared to the initial mixture in order to quantify the remaining SP and DDCQAs [3].

RESULTS & DISCUSSION

A series of ten dehydrodicaffeoylquinic acids (DDCQAs) were synthesized by enzymatic oxidation of a model solution of 5′-O-Caffeoylquinic acid (CQA), using a crude apple polyphenoloxidase extract. Then, they were purified by centrifugal partition chromatography and RP-18 HPLC at the semi-preparative scale [2]. Their complete structural elucidation was achieved by 1D and 2D NMR ^{1}H and ^{13}C revealing original

dihydrobenzofuran, benzodioxan and dihydronaphtalen polyphenolic skeletons. In addition, for the first time a new symmetric structure exhibiting two free catechol groups was identified.

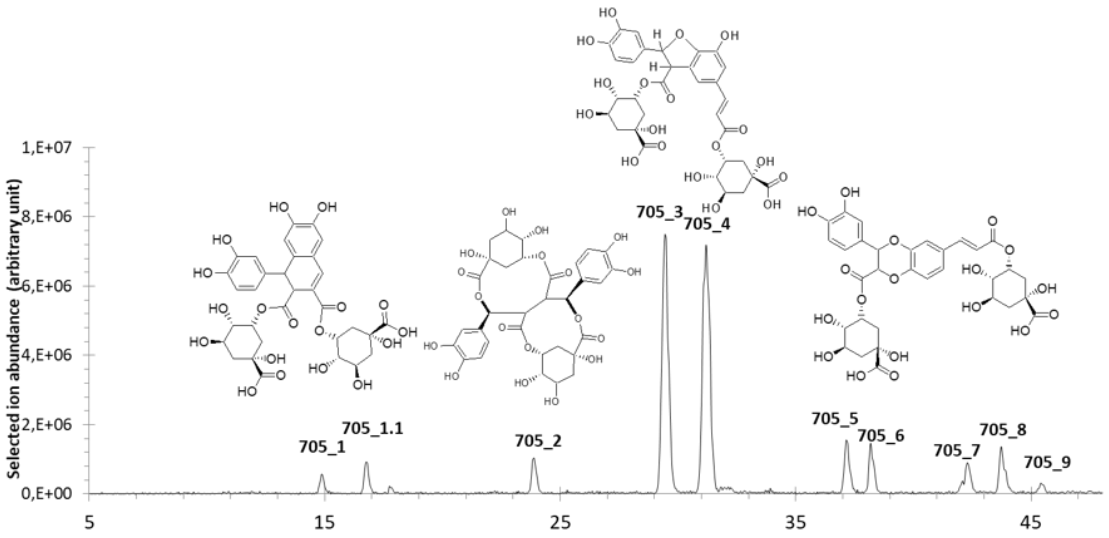

Figure - Extracted ion (m/z) LC-MS chromatogram revealing the series of DDCQAs oxidation products

The capacity of DDCQAs to aggregate salivary proteins (SP) was studied using a precipitation method associated to HPLC analysis of the supernatant [3]. Experiments were conducted in model solutions in order to vary at the same time the concentrations and the ratio of both polyphenols and saliva proteins in the medium. Results showed that interactions between salivary proteins (SP) and DDCQAs are highly impacted by the ratio SP/DDCQAs, and in the tested conditions, we hypothesize that a low number of DDCQAs is necessary to significantly precipitate SP. Moreover, a specific interaction of DDCQAs with cystatins and statherin/PB was observed during these experiments. Some of these observations were confirmed by fluorescence quenching analysis. Surprinsingly, DDCQAs exhibited weak interactions with PRPs (Proline Rich Proteins) but interactions were much more significant with statherins/P-B peptide and cystatins.

Finally, polyphenol oxidation products, including the DDCQAs series completed by some epicatechin-caffeoylquinic acid dimers, were quantified for the first time in real juices by developing a new LC-MS method. Its application to 32 commercial, 13 experimental and 9 craft apple juices revealed that these families of compounds can reach several dozen mg/L apple juice contributing up to 14 % of total polyphenols.

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