IDENTIFICATION AND FORMATION KINETIC STUDY OF PHENOLIC COMPOUNDS-VOLATILE THIOLS ADDUCTS BY ENZYMATIC OXIDATION•

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1. INTRODUCTION

Volatile thiols are powerful molecules which exhibit typical nuances of varietal flavor of wines and can react with non volatile compounds, like the oxidised polyphenols, by a Michael addition reaction having as a result the wine aroma loss (Nikolantonaki *et al.*, 2010). The major phenolic compounds of white grapes and wines are caftaric acid, hydroxycinnamic acids and flavan-3-ols. In the normal wine-making process, *o*-quinones production can occur firstly by the enzymatic oxidation of polyphenols catalysed by grape PPO (EC 1.10.3.1) during crushing and pressing and afterwards by chemical oxidation mechanisms in the presence of oxygen during ageing. Their importance depends on the relative concentration and reactivity of the nucleophile.

The main purpose of this study, which was focused to wine and must matrix, was to elucidate the mechanisms which are implicated in volatile thiols degradation, by the identification and the monitoring of phenolic compounds-volatile thiol adducts formation under enzymatic oxidation conditions. Adducts between the major represented phenolic compounds in white must, caftaric acid, catechin and epicatechin and a key volatile thiol, 3-sulfanylhexanol (3SH), were produced. The role of transition metal ions and SO_2 to these adducts formation was also investigated.

2. METHODS, RESULTS AND DISCUSSION

By using HPLC-ESI-MS, ^{1}H, ^{13}C and 2D NMR (data not published) three adducts of both catechin and epicatechin with 3SH and one adduct of caftaric acid with 3SH were characterized as addition products. In order to better understand the addition mechanism of 3SH to catechin, epicatechin and caftaric acid, the kinetic formation of all adducts characterized (fig. 1) was monitored by HPLC-UV. The addition mechanism involves production of the *o*-quinone from either catechin, epicatechin or caftaric acid by autoxidation catalyzed by PPO followed by 1,4-addition of the 3SH to make a thioether

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with the benzoic ring of the polyphenols. In the presence of PPO (fig. 2), between the three substrates incubated with 3SH in equal concentrations (9 mM), catechin-3SH reaction had the greater adducts yield (10,6 mM; fig. 2, A) followed by caftaric acid–3SH (5 mM; fig. 2, E) and last by epicatechin–3SH at 5 mM (fig. 2, C). Additionally, in agreement with Cheynier *et al.* (1989), after 3 days of incubation, caftaric acid consumption was 68.8 % of its total concentration (fig. 2, F), while consumption of catechin and epicatechin was lower. Even if caftaric acid appeared to be a rich substrate for PPO, contrary to both catechin and epicatechin (fig. 2, F), its autoxidation rate was not correlated with Caf2 production. So, caftaric acid *o*-quinones have poor affinity with 3SH. This result may indicate that each generated caftaric acid *o*-quinone may also react rapidly with another quinone to yield a condensation product through reaction of two semiquinone free radicals (Singleton, 1987). These results put in evidence that under our enzymatic oxidation conditions, between the three substrates, catechin can play an important role in 3SH degradation.

For the three substrates, no lag period was observed at the beginning of the reaction (fig. 2). The addition of 3SH to both catechin and epicatechin followed a linear regression fitting. Caftaric acid-3SH kinetic could be divided into two time periods (fig. 2, E). The first one, from T_0 to T_{1day}, in which Caf2 production rate was high and reached 80 % of its final yield and the phase II, from T_{1day} to T_{3day}, where a reaction rate two times slower than in phase I was present. In both kinetic phases Caf2 formation followed a linear regression fitting.

HPLC-UV monitoring of the incubation solutions of flavan-3-ols with 3SH showed that adducts between catechin-3SH and epicatechin-3SH had similar kinetic rate. 3SH substitution to the B ring of catechin unit was equal for both 5' and 6' position. The 5' position of epicatechin B ring was favored for 3SH addition (E2 2.59 mM) above the 2' position (E1 1.55 mM). The formation rate of Caf2 was slightly higher to that of C2 and double to that of E2. In all flavan-3-ols model solutions, catechin-3SH and epicatechin-3SH, we observed multiple substitutions of 3SH with no difference to the double adducts yield after three days incubation (C3 and E3). The formation of C3 and E3 attested that coupled oxidation reactions took place in our medium. The C3 and E3 formed by reaction of C1 or C2 and E1 and E2 *o*-quinone with a second 3SH molecule. The lack of multiple substitution adducts in the case of caftaric acid suggets that Caf2 was no longer a substrate for PPO as was mentioned for 2-*S*-glutathionylcaftaric acid (GRP) (Singleton *et al.*, 1985).

However, the addition of sulphur dioxide in model medium influenced directly the kinetic rate and yield of all adducts formation (figs. 3 B; D and E). When SO_2 was introduced in the model must with 3SH, we observed the suppression of any phenolic compound consumption.

Consequently, the yield and rate of adducts formation was decreased to a significant extent without, in any case, a complete stop of adduct production. Concerning flavan-3-ol substrates, SO_2 created a lag phase at the beginning of the reactions with equal duration (T_0 to T_{1day}) for both reaction mixtures (catechin-3SH and epicatechin-3SH). After one day incubation, epicatechin-3SH adducts production followed an exponential regression and in the case of catechin-3SH a linear regression fitting. These results let us formulate the hypothesis that epicatechin enters more easily into coupled oxidation mechanisms than

catechin and its *o*-quinones have a great reactivity with 3SH. Epicatechin is oxidized more easily than catechin (Danilewicz, 2007; Nikolantonaki *et al.*, 2010).

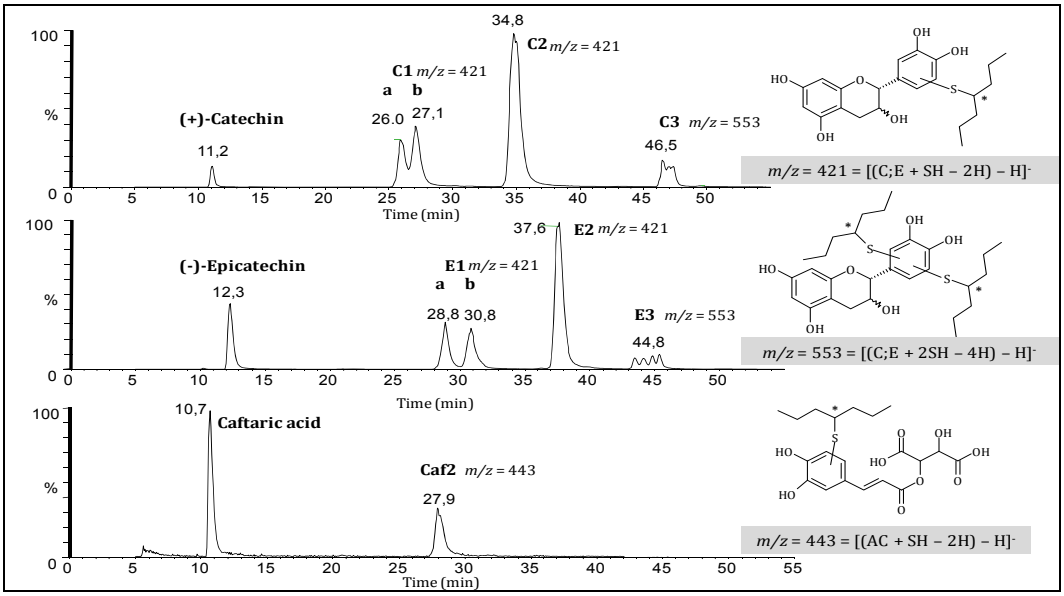

Fig. 1 - LC-MS TIC chromatograms in scan mode (*m/z* 100-100) of catechin-3SH, epicatechin-3SH. Proposed structures for adducts between (+)- catechin and 3-sulfanylhexanol (C1, C2 and C3), (-)-epicatechin and 3-sulfanylhexanol (E1, E2 and E3) and caftaric acid and 3-sulfanylhexanol (Caf2) formed under enzymatic oxidation in acidic conditions.

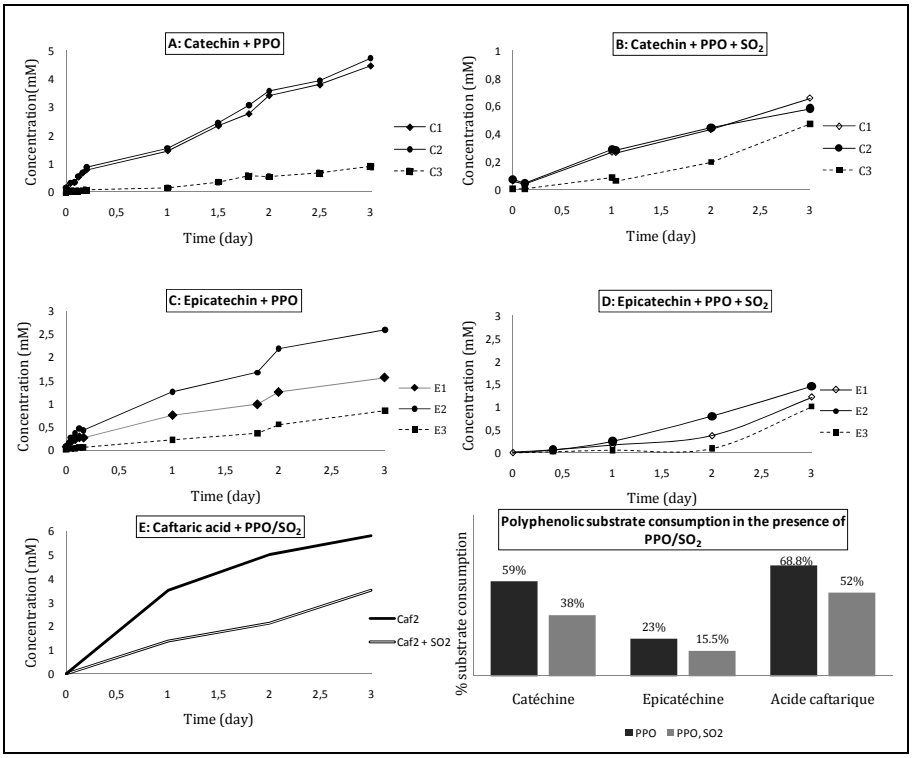

Fig. 2 - Kinetic rate for the formation of adducts of catechin (C1, C2, C3) and epicatechin (E1, E2, E3) and caftaric acid with 3-sulfanylhexanol (Caf2) under enzymatic oxidation conditions in the presence or not of SO_2 (30 mg/L). Polyphenolic substrate consumption at the end of the kinetic study.

Finally, caftaric acid-3SH mixture incubated with SO_2 followed the same kinetic profile as in the absence of sulphur dioxide with the presence of two different time periods. The final yield of Caf2 production was twice lower than in the lack of SO_2 and this reduction was proportional to caftaric acid consumption.

Abstract

By using HPLC-ESI-MS, ^{1}H, ^{13}C and 2D NMR, new addition products between catechin, epicatechin, caftaric acid and 3SH were characterized. Caftaric acid formed more rapidly adducts with 3SH than catechin and epicatechin in the absence of other nucleophiles. Epicatechin was two times more reactive than catechin with 3SH. Sulphur dioxide decreased the yield and rate of adducts formation to a significant extent without in any case stopping adducts production. In the grape juice, the 3SH exists in a limited proportion under a free volatile odoriferous compound and mainly under as cysteine and glutathione conjugates, and so the abundance of such adducts is likely limited (Peyrot des Gachons *et al.*, 2002). This identification and these kinetic results are assumed to occur in wine during ageing as the proportion of volatile 3SH is more important and its contribution crucial to wine aroma.

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