Tetrahedron xxx (2015) 1–8

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Identification of new oxidation markers of grape-condensed tannins by UPLC–MS analysis after chemical depolymerization

Laetitia Mouls [a,b,c,*], Hélène Fulcrand [a,b,c]

[a] INRA, UMR1083, F-34060 Montpellier, France
[b] Montpellier SupAgro, UMR1083, F-34060 Montpellier, France
[c] Université Montpellier I, UMR1083, F-34060 Montpellier, France

ARTICLE INFO

Article history:
Received 11 August 2014
Received in revised form 5 January 2015
Accepted 16 January 2015
Available online xxx

Keywords:
Tannin
Oxidation marker
Autoxidation
ULPC–MS
Depolymerization

ABSTRACT

Model solutions of oxidized monomer/dimer mixtures of flavan-3-ol (EC/B2, EGC/B2, ECG/B2, EGCG/B2) were studied after chemical depolymerization by UPLC–MS. New oxidation markers of condensed tannins were identified. This analytical method was applied to a wine sample and most of these oxidized markers were detected. This method will permit following the oxidation state of tannins during the winemaking process and storage in order to improve the quality of the products.

1. Introduction

Wine polyphenols are involved in the colloidal stability of red wines and also contribute to their organoleptic properties, principally color and mouth feel. The major polyphenols responsible for these properties are flavonoids including anthocyanins and proanthocyanidins, which are also called condensed tannins. Anthocyanins are the red pigments of grapes, while proanthocyanidins are polymers, characterized by their high affinity for protein interactions. They are highly concentrated in the solid parts of the grape berry, from which they are partially extracted during maceration. These highly reactive molecules are responsible for the large and expanding diversity of grape phenolics in wine. One important characteristic of polyphenols is that they possess antioxidant properties and accordingly are good scavengers of free radicals, particularly reactive oxygen species (ROS). This role is widely put forward in the health field to prevent diseases related to oxidative stress.[14] However, the *in vivo* effectiveness of polyphenols as free-radical scavengers has not yet been demonstrated scientifically.

In the context of oenology, oxidation can be beneficial to the quality of wines, because it contributes to their suppleness and roundness. A technique called micro-oxygenation was developed 20 years ago to simulate the oxygen exchanges of barrel aging.[4,5] It consists of introducing micro quantities of oxygen (typically 2–5 mL O_2/L*month) in a controlled manner during the winemaking process, usually after fermentation. Conversely, oxygen ingress can take place during the winemaking process, altering the wine quality. For example, some operations such as pressing, cooling, pumping-over, and bottling actually promote oxygen intake.[1]

Nevertheless, advances in oxygen management have been achieved in the last few years by managing oxygen ingress from the production process through bottling and even during the storage of wines. However, oxygen management in the winemaking process still relies on empirical evidence from real-life experience rather than scientific knowledge. The air oxidation (autoxidation) is mediated by metal catalysts, such as iron and copper. Once dissolved in wines, the aerial oxygen is progressively and totally consumed by the wine constituents resulting in both chemical and quality changes.

The effects of oxidation on wine quality are not yet fully understood because the process involves many compounds, including aromatics, polyphenols, ethanol, and sulfites. Their respective contributions as well as their interplay are difficult to decipher. Because a standard value, such as a redox potential, for a chemical cannot be determined in this case, quality assessment of wine requires the comparison of wine samples. The process developed in

* Corresponding author. Tel.: +3 349 961 2454; fax: +3 349 961 2857; e-mail address: laetitia.mouls@supagro.inra.fr (L. Mouls).

http://dx.doi.org/10.1016/j.tet.2015.01.038
0040-4020/

our laboratory[15,16] is based on chemometric processing of chemical and sensory data collected on a relatively large number of wines samples exposed to different levels of oxygen. This approach enables us to rank the wines according to the oxygen transfer rate (OTR). However, the ranking is reliable only for the group of wines tested.

Regarding the chemical changes of polyphenols, pigments appear to be the best markers of oxidation. In a previous study carried out in our laboratory, wines were exposed to different oxygen levels during the winemaking process and post-bottling period. Chemometrics processing of analytical data obtained from both index color measurements and UPLC–DAD-MS profiles showed a good correlation of pigments resistant to sulfite bleaching and OTRs, particularly for pyranoanthocyanins. However, precise chemical analysis of changes in the tannin structures was not performed, unlike with anthocyanins. Only the overall loss of tannins was evaluated and could not be correlated to the oxidation, unlike the catechin monomers. The reactivity of proanthocyanidins has actually been neglected considering the progress achieved in the knowledge of wine pigment structures over the 20 past years. This comes from the polymeric nature of tannins that make their chemical analyses challenging. This difficulty has inhibited a better understanding of tannins' reactivity, including their contribution to the oxidation process.

Despite the realistic difficulty in analyzing the structure of tannins, we decided to investigate their oxidation with the aim to answer the following questions:

- Are the terminal subunits of the tannin chains involved in the same way as the extension subunits?
- What are the roles of intramolecular reactions versus intermolecular reactions? What is the impact on the average degree of polymerization (aDP) of tannins and their physical–chemical properties?
- How does the redox potential of tannin subunits evolve with successive oxidation? Once oxidized, is the subunit more oxidizable compared to a native one?

The method we have chosen to investigate the structural changes of tannins upon oxidation and their reaction mechanisms is based on on-line ultra-performance liquid chromatography–mass spectrometry analyses (UPLC–MS) of the products obtained after chemical depolymerization of oxidized tannins. Indeed, the linkages created by oxidation (biaryl, bi-arylether, and A type-interflavanic linkages) have been shown to be resistant to cleavage under thiolysis conditions,[6] unlike the genuine B-type interflavanic linkages (IFL) connecting the tannin subunits. Therefore, oxidized structures are expected to be released as oligomers, while the non-oxidized ones are still released as monomers. Furthermore, the heterolytic nature of the IFL cleavage enables us to distinguish the extension subunits from the terminal subunits. Indeed, bond-breaking releases the extension units of the polymeric chain as carbocations, which are immediately trapped by a nucleophilic reagent to give the corresponding derivatives. Consequently, this chemical depolymerization approach appeared to be a promising method to answer the two first questions described above.

The approach was primarily developed on the simplest structures, monomer and dimer, then on an extract of apple parenchyma, which harbors tannins solely composed of epicatechin (EC) subunits. Acidic solutions of the compounds were saturated with oxygen by shaking in air and then sealed in bottles. The large air headspace ensured that the solutions remained at or near aerial oxygen saturation. These compounds stood at ambient temperature for several weeks. The structural changes of compounds upon oxidation were monitored by UPLC–MS after chemical depolymerization. In this way, we were able to detect and partially characterize more than 30 oxidation markers from fragmentation mass spectra. Exhaustive fragmentation studies constitute the sole analytical tool to solve the structural characterization of oxidation markers, because they are present in low concentration and some of them are isobaric compounds. The comparison of the oxidation products obtained from monomer, dimer, and polymer solutions permitted the identification of oxidation markers specific to either intermolecular or intramolecular reactions. The presence and number of nucleophile residues within the structure of the oxidation markers was indicative of the origin/nature of the subunits (i.e., extensions vs terminal units) involved.

This same experimental approach was also used in the work reported here, on model solutions containing grape tannin structures. Every solution was composed of the dimer B2 and one of the monomeric subunits of the grape: EC, epicatechin-3-*O*-gallate (ECG) for seed, and epigallocatechin (EGC) for skin (Fig. 1). Even though it is not a constitutive monomer of grape tannins, a solution was prepared with epigallocatechin-3-*O*-gallate (EGCG) in order to compare its reactivity with the others.

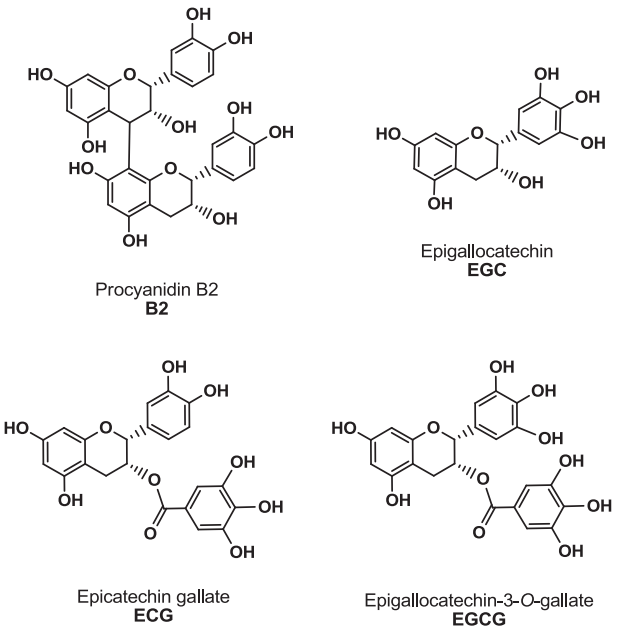

Fig. 1. Dimer and monomers of flavan-3-ol studied.

Lastly, the oxidation markers identified in these model solutions were examined in an experimental white wine that was fermented in the same manner as a red wine in order to be just as rich in tannins.

2. Results and discussion

The autoxidation products formed in the four model solutions (B2/EC, B2/ECG, B2/EGC, B2/EGCG) were monitored over 34 weeks by UPLC–MS analyses after chemical depolymerization by the methyl ester of thioglycolic acid (thioglycolysis). Given the composition of the model solutions, the oxidation markers involving ECG, EGC or EGCG units had to be formed by intermolecular reactions (Fig. 2). This is because, as monomers ECG, EGC, and EGCG can only react with another molecule of themselves or B2. Other products yielded by B2 reactions are either of A-type, resulting from an intramolecular reaction or of dehydrodicatechin type resulting from an intermolecular reaction, as was shown in our previous study.[6] Some of these identified oxidation markers were

Please cite this article in press as: Mouls, L.; Fulcrand, H., Tetrahedron (2015), http://dx.doi.org/10.1016/j.tet.2015.01.038