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Polyphenol profile, PPO activity and pH variation in relation to colour changes in a series of red-fleshed apple juices

Hélène Février, Jean-Michel Le Quéré, Gildas Le Bail, Sylvain Guyot*

INRA, UR1268 BIA, Team Polyphenol, Reactivity & Processing (PRP), BP 35327, 35653 Le Rheu, France

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ABSTRACT

Recently, rosé ciders and pink apple juices, appeared as a real opportunity to diversify the offer. Juices obtained from red-fleshed apple varieties were used to study the impact of raw material on colour intensity and stability. Juices prevented from oxidation were compared to the corresponding oxidised juices. Juice colours were measured in the CIE Lab space. Polyphenoloxidase activity and pH were measured. Detailed phenolic composition, including anthocyanins, was characterised by reversed phase HPLC coupled to UV-visible and MS detection. Red-fleshed apple varieties revealed great variation regarding red colour intensity of the juice and its enzymatic oxidative degradation. Statistical classification of juices highlighted three behaviours regarding colour and colour degradation; (i) highly coloured juices resistant to colour degradation, (ii) red juices that shifted to orange-yellow colour and (iii) red juices that became pale. No factor could explain alone, nor the classification of juices, nor the oxidation of anthocyanin. Colour degradation must be considered as a multifactorial evolution. Results suggested that PPO activity, pH, concentration of PPO favourite substrates are among the main factors involved, but the amount of the other polyphenols (dihydrochalcones, catechins and procyanindins) must also be taken into account.

1. Introduction

Recently, new and original apple derived products were launched on the market. Among them, rosé ciders and pink juices appear as real opportunity to diversify the offer. Those innovative beverages are produced using red-fleshed varieties that differ from classical varieties by a remarkable amount of anthocyanin pigments present in the flesh (Sadilova, Stintzing, & Carle, 2012). For Rosé French ciders, those varieties are blended with classical cider varieties belonging to bitter, bittersweet, sweet, sharp or sour apples that contain highly variable amounts of polyphenols generally ranging from 1 to 7 g/kg of fresh apple flesh (Sanoner, Guyot, Marnet, Molle, & Drilleau, 1999).

Polyphenols in general possess antioxidant capacities and may partly contribute to protective effects against oxidative stress (Ferrari, Percário, Silva, & da Silva Torres, 2016). Furthermore, apples represent one of the highest phenolic intakes among the daily consumed fruits (Chun et al., 2005). Red-fleshed apples juices might appear as a natural and healthy new product which might encourage customers to increase their daily phenolic consumption. Several polyphenols classes are found heterogeneously in apples tissues. Flavanol monomers, (mainly (−)-epicatechin and (+)-catechin) and procyanindin (catechin oligomers and polymers, also called condensed tannins) are the most abundant. They generally account for more than 80% of total apple polyphenols followed by hydroxycinnamic acids (mainly caffeoylquinic acid), flavonols (mainly quercetin glycosides located in the skin in low concentration), dihydrochalcones (mainly phloridzin and phloretin xyloglucoside). Anthocyanins are found in the skin of red apples, with highly variable concentrations depending on the variety (Guyot & Poupard, 2011; Wojdyło, Oszmiański, & Laskowski, 2008). Anthocyanins are water soluble phenolic molecules very widespread. In nature, they are found under many glycosylated and acylated forms (Kong, Chia, Goh, Chia, & Brouillard, 2003). Regarding the specificity of the raw apple material used to produce rosé juices and ciders, several glycosylated and acylated cyanidins are found but cyanidin-3-galactoside (namely ideain) remains the main anthocyanin represented in red-fleshed apples (Malec et al., 2014; Sadilova et al., 2012).

During conventional French apple processing (especially

* Corresponding author.
E-mail address: Sylvain.Guyot@rennes.inra.fr (S. Guyot).

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crushing and pressing with oxygen exposure), some of the colourless native polyphenols undergo enzymatic browning which confers to ciders and juices their specific yellow to orange colours. The apple polyphenol oxidase (PPO) oxidises specific substrates (mainly caffeoylquinic acid, catechins and dihydrochalcones) into highly reactive *o*-quinones through two mechanisms, namely cresolase (monophenolase) and catecholase activities. *o*-Quinones are rapidly involved in various oxidation-reduction reactions, oxidative coupling reactions or intramolecular rearrangements leading to the formation of many phenolic oxidation products some of them being coloured (Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994). Thus, as a result of those enzymatic oxidations, different hues varying from pale yellow to deep orange can be obtained depending on the polyphenols involved, PPO activity, and other environmental factors (pH, oxygen availability …). For instance, Le Guernevé, Sanoner, Drilleau & Guyot (2004) described a yellow product formed by the oxidation of phloridzin with PPO.

Regarding anthocyanins from apple flesh, these compounds are directly responsible for the pink to red colour of the juice (Malec et al., 2014). However, anthocyanins are present in aqueous media in several molecular species depending on pH dependant thermodynamic and kinetic equilibria (Limón, Gavara, & Pina, 2013). The predominating molecular forms (Fig. 1) for cyanidin-3-glucoside at juice pH values ranging from 3.0 to 4.5 are, in decreasing order, the colourless hemiketal (B), the coloured flavylium cation (AH^+) and the bluish quinoidal base (A), this later being in much lower concentration (Limón et al., 2013). A similar behaviour can reasonably be expected for cyanidin-3-galactoside (ideain), considering that the sugar moiety may have only a little effect on those equilibria (Limón et al., 2013).

Anthocyanins, even those presenting a catechol group such as cyanidin glycosides, are not substrates for PPO. However, anthocyanins with *o*-diphenol B ring (cyanidin-3-galactoside) can be oxidised through coupled oxidation-reduction reactions with enzymatically generated *o*-quinone such as chlorogenoquinone (*i.e.* *o*-quinone of caffeoylquinic acid) (Sarni-Manchado, Cheynier, & Moutounet, 1997). Moreover, non *o*-diphenolic anthocyanins can form adducts with *o*-quinone by non-enzymatic condensation reactions (Sarni, Fulcrand, Souillol, Souquet, & Cheynier, 1995). These reaction pathways involving anthocyanins, colourless polyphenols and PPO are likely responsible for colour changes during the early stages of red-fleshed apple processing.

In this context, the purpose of the study was to explore the variability of a series of red-fleshed apple varieties in terms of phenolic profiles, PPO activity and pH of the juice. In parallel, the colour of those juices prepared either in oxygen-prevented conditions or in conditions favouring enzymatic oxidation was compared. Then, the data are discussed regarding hypotheses that would help to a better understanding of the influence of the raw material (*i.e.* red fleshed apple varieties) on colour expression and stability in the first stages of rosé juices and cider processing.

2. Materials and methods

2.1. Plant material

The apple varieties used in this study corresponded to the collection of redfleshed individuals available in the experimental orchard of IRHS at INRA Angers. Apples were harvested between September 21st and October 2nd in 2015 in the INRA experimental orchard, Domaine de Beaucouzé, Angers, France. Only the varieties for which harvested quantities ranged between 2.8 and 12.3 kg were used.

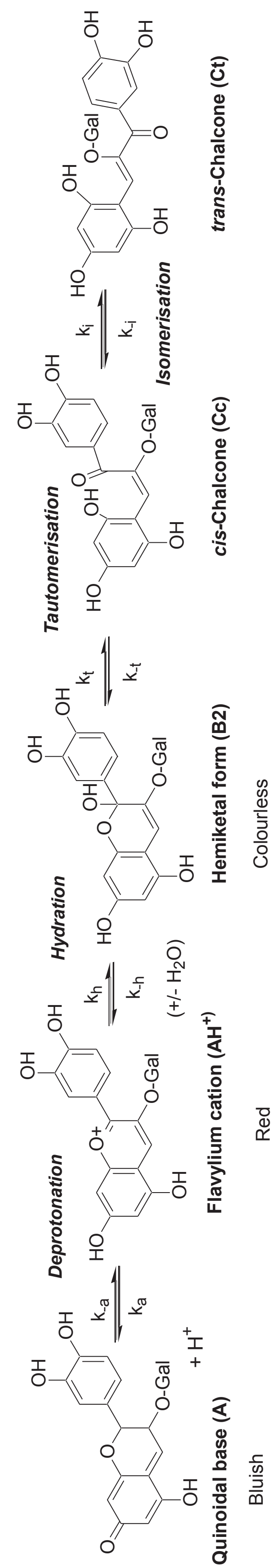

Fig. 1. Chemical reactions and equilibrium determining the different anthocyanin forms and behaviours within the flavylium network (adapted from Limón et al., 2013).