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Modulating polyphenolic composition and organoleptic properties of apple juices by manipulating the pressing conditions

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ABSTRACT

Initial crushing and pressing operations have a major influence on the polyphenolic composition of apple juice, therefore, we have tested the impact of variations of this step using three cider apple cultivars of contrasting polyphenolic composition: Guillevic, Kermerrien and Dous Moen. Under inert atmosphere, increased temperature (between 5 °C and 24 °C), increased the extraction of procyanidins from fruit to juice. The crushed apples were also subjected to four conditions of oxidation: preserved from oxidation as above, short contact with air, short contact with air and mixing, long contact with air and mixing. Oxidation decreased the concentrations of native polyphenols in the juices, especially for flavan-3-ols. The golden colour of the juices was initially enhanced with increases in saturation C* and a shift of the hue angle from yellow to orange. However, for the highest oxidation state the colour became paler and more yellow. Bitterness and astringency decreased upon oxidation, probably due to increased retention of oxidised moieties.

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1. Introduction

Apple juice with almost 1.5 million tons of concentrated juice produced mondially each year (USDA-FAS, 2007) is only second to orange juice. It is an important commodity and base for apple and mixed juices. Apples are know as a healthy fruit, and this has often been connected to their polyphenol content (Boyer & Liu, 2004). Extraction of polyphenols from the apple fruit to the juice is very low (Guyot, Marnet, Sanoner, & Drilleau, 2003; Le Bourvellec, Le Quéré, & Renard, 2007; Markowski, Mieszczakowska, & Plocharski, 2009; van der Sluis, Dekker, Skrede, & Jongen, 2002). Better extraction of polyphenols from apple to juice is of nutritional interest but leads to increased risk of haze formation. It is therefore important to be able to control extraction of polyphenols from apple to the juice.

Quantitatively, the main polyphenols in apples are procyanidins, consisting of oligomers and polymers of catechin units, with >95% (-)-epicatechin. Their size differs between the varieties; number average degrees of polymerisation ($\overline{\rm DP}$ n) between 2 and 50 can be observed (Sanoner, Guyot, Marnet, Molle, & Drilleau, 1999). These

procvanidins contribute to the organoleptic properties, especially by their astringency and bitter taste (Lea & Arnold, 1978; Vidal et al., 2003). The second most abundant polyphenols in apple are phenolic acids, namely chlorogenic acid then p-coumaroylquinic acid. These are usually the main polyphenols in the juices and ciders (Alonso-Salces et al., 2004; Mihalev, Schieber, Mollov, & Carle, 2004; Oszmianski & Wojdylo, 2007; van Buren, de Vos, & Pilnik, 1973; van Buren, de Vos, & Pilnik, 1976; van der Sluis et al., 2002; Will, Schulz, Ludwig, Otto, & Dietrich, 2002), though chlorogenic acid is the primary substrate of apple polyphenol oxidase (Goodenough & Lea, 1979; Janovitz-Klapp, Richard, Goupy, & Nicolas, 1990; Le Bourvellec, Le Quéré, Sanoner, Drilleau, & Guyot, 2004b). The third class is that of monomeric flavan-3-ols, both (-)-epicatechin and a minor amount of (+)-catechin. Dihydrochalcones are also present, with high concentrations in the core section and in the pips, while flavonols (quercetin glycosides) and anthocyans are exclusively located in the peel in most varieties.

Most work on influence of process on apple juice has focussed on mash maceration of table apples with pectolytic enzymes to enhance juice yields and facilitate pressing. However, since the pioneering work of de Vos and Pilnik (de Vos & Pilnik, 1973; Pilnik & de Vos, 1970; van Buren et al., 1973; van Buren et al., 1976), it is known that pulp oxidation during enzyming leads to paler juices, with decreased polyphenolic contents and precipitation of an

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amorphous brown material with the cell wall matrix, while increased duration of enzyming may lead to enhanced extraction of flavonol glycosides.

The first step of processing of apple into raw juice is a crushing and a pressing of the fruits, during which polyphenols, polyphenol oxidase (PPO), oxygen and cell walls, initially segregated, come into contact and may react. This is the step where the most changes occur in polyphenolic composition, and extraction can be very limited for some polyphenol classes (Guyot et al., 2003; Hubert, Baron, Le Quéré, & Renard, 2007). Three mechanisms are involved:

- non-covalent interactions with cell walls, which can be modulated by modifying the pressing temperature (Le Bourvellec, Guyot, & Renard, 2004a), contribute primarily to retention of procyanidins in the pomace (Le Bourvellec et al., 2007);
- (2) oxidation: upon destructuration of the apple tissue, PPO oxidises chlorogenic acid to its o-quinones, which can react in various ways notably by formation of adducts with flavan-3-ols or transfer of oxidation to these molecules. With oligo or polymeric flavan-3-ols (procyanidins), this subsequently leads mostly to formation of additional intramolecular bonds (Guyot, Bernillon, Poupard, & Renard, 2008).
- (3) flavonols or anthocyans, located almost exclusively in the apple peel, are physically segregated and only diffuse slowly to the juices.

The same mechanisms may also explain the polyphenolic composition of other juices obtained by pressing, notably for other fruits from the Rosaceae family, which contain the same classes of proanthocyanidins, monomeric flavan-3-ols, phenolic acids and flavonols.

Cider apples are a particularly apt system to study extraction of polyphenols from fruit to juice as they present high concentrations of polyphenols with a wide variability in the ratios of the different classes, in the size of procyanidins and in polyphenol oxidase activity. In this study we investigated the potential for modulating the polyphenol composition in juice from three contrasted cider apple varieties, by varying the temperature at pressing under stringent exclusion of oxygen, or by allowing the crushed apple mash to oxidise to varying amounts. Impact of these treatments was assessed analytically and, for oxidation of the mash, organoleptically.

2. Materials and methods

2.1. Reagents

Acetonitrile, HPLC gradient grade, was purchased from Fisher Bioblock Scientific (Illkirch, France). (+)-Catechin, (-)-epicatechin, phloridzin, chlorogenic acid and quercetin were obtained from Sigma Aldrich Co (St. Louis, MO). Procyanidin B2 and *p*-coumaroylquinic acid were obtained as described by Contreras-Dominguez et al. (2006) and Benoit et al. (2006), respectively. Epicatechin benzylthioether was a gift from J.-M. Souquet (INRA, UMR SPO, Montpellier, France). All others reagents were analytical grade. Ultrapure water was obtained using Milli-Q water system (Millipore, Bedford, MA).

2.2. Plant material

Apple fruits of the Kermerrien, Guillevic and Dous Moen varieties were harvested at commercial maturity during the 2003 and 2006 seasons in the experimental orchard of the Institut Français des Productions Cidricoles (Sées, France).

The three apple varieties had been chosen from previous work (Le Bourvellec et al., 2004; Sanoner et al., 1999b) to represent, respectively:

- one cultivar with procyanidins of a high degree of polymerisation (Guillevic), which belongs in the "sharp" class of cider apples (Lea & Drilleau, 2003);
- one cultivar with high concentrations of procyanidins of a low degree of polymerisation (Kermerrien), belonging to the "bitter" taste class;
- one cultivar with high concentrations of phenolic acids and procyanidins (Dous Moen), and with high polyphenoloxidase activity, of the "bitter-sweet" class.
 - This choice allowed us to vary both the affinity for cell wall remnants by variation of the procyanidin degree of polymerisation, and the propensity to oxidation by the polyphenoloxidase activity and the abundance of its primary substrate, chlorogenic acid.

Aliquots of the apples $(3 \times 10 \text{ fruits})$ were collected prior to pressing and sampled as described in Renard (2005) then freezedried and ball-milled for analysis of polyphenol composition.

2.3. Juice extraction

All juice extractions were carried out in duplicate.

For impact of temperature, apples were pressed at four temperatures: 4, 11, 18 and 25 °C in a Speidel-90 "hydropress" pneumatic press (Speidel Tank- und Behaelterbau GmbH, Ofterdingen, Germany), modified by addition of an inox steel tube around the press to allow inerting by a heavier-than-air gas. Apples (ca. 20 kg/pressing) were left at the chosen temperature for 24 h for temperature equilibration. The press was stored overnight in a room at the chosen temperature and flushed with water (equilibrated at the chosen temperature) prior to pressing. Inerting was carried out by flushing the water out of the press body with $\rm CO_2$; the press was connected to the exit of the grinder by plastic tubing and crushing started when a burning match inserted in the fruit entrance of the grinder could not maintain combustion. A standing time of 20 min was observed between crushing and pressing of the apples to allow juice diffusion and procyanidin adsorption.

For oxidation, the apples were pressed as above, at 11 °C. Four oxidation conditions were used, labelled OX0 to OX3 in increasing oxidation order: OX0 (inert atmosphere); OX1 (contact with air during crushing); OX2 (short stirring of the mash in air) and OX3 (long stirring of the mash in air). Pressing was carried out under inert atmosphere for the OX0 condition, as described above. For the OX1 condition, the press was not under inert conditions so apples were crushed in air and left to stand for 20 min. For the OX2 condition, the crushed apples were dropped in a drum, which was closed and agitated "head-over-tail" for 20 min at room temperature (11–15 °C) prior to pressing. For the OX3 condition, the incubation was prolonged to 4 h.

Yields were determined as the difference between the initial weight of apple and the weight of wet pomace. For polyphenol analyses, aliquots of unclarified juices (0.5 ml) were sampled in tubes spiked with NaF (final NaF concentration: 1 g/l) and freezedried. For determination of the polyphenoloxidase activity, an aliquot of OXO unclarified juice (i.e. juice prepared in inert conditions) was transferred to an airtight container previously blanketed with carbon dioxide.

For all other analyses, juices were clarified using Rapidase C80L (115,000 viscosimetric unit/ml, DSM Food, Séclin, France) at 0.1 ml/l of juice, followed by incubation for 18 h and 10 °C then microfiltration of the clear supernatant on a polyvinylidine difluoride membrane, 0.45 mm (Millipore Pellicon, Millipore Corp.) followed by freezing and conservation at $-20\,^{\circ}\text{C}$.

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