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Influence of fruit juice processing on anthocyanin stability

Fabian Weber*, Lena Rebecca Larsen

Institute of Nutritional and Food Sciences, Molecular Food Technology, University of Bonn, Römerstrasse 164, D-53117 Bonn, Germany

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A B S T R A C T

Anthocyanins are quality determining compounds in red fruits and their corresponding juices. The fate of anthocyanins during production of juices is determined by countless factors and all of these need to be taken into consideration to optimize juice processes. Storage and pre-press procedures like freezing and thawing may influence the fruit's integrity and accordingly affects the extraction of valuable components during the subsequent mashing and pressing. Conventional thermal or novel non-thermal treatments to ensure microbial safety have both positive and negative effects on the anthocyanins. By inactivation of oxidizing enzymes, profiles and quantities of anthocyanins may be maintained, but more severe conditions may have adverse effects. To improve juice extraction and to increase yield, enzyme-assisted degradation of the cell walls is conducted. The applied enzyme preparations contain numerous side activities which also may degrade anthocyanins. Clarification and concentration will further reduce the final anthocyanin concentrations. Many studies have been published regarding evaluating individual fruits or single processing steps but, obviously, these results are not necessarily transferable. Accordingly, this review aims to summarize all relating studies comprehensively to the fate of anthocyanins during juice processing giving an overview of underlying mechanisms as well as the chemical and analytical background.

1. Introduction

The consumption of plant-derived food is a central part of the human diet. Besides macronutrients, fruits and vegetables contain various secondary plant metabolites which are associated with numerous positive health benefits (Dillard & German, 2000; He & Giusti, 2010). Since consumers show an increasing awareness of health issues, these compounds are getting into the focus not only of scientific research but also of marketing considerations (Schonfeldt & Gibson, 2010). The food industry offers many different fruit and vegetable products which may be introduced into one's diet to increase the consumption of secondary plant metabolites. Fruit juices are recently labeled as functional food as they contain high amounts of secondary plant metabolites and have a traditional high proportion in the human diet (Seeram, 2008). Especially red juices made from berries are gaining in popularity due to their high concentration of secondary plant metabolites, specifically polyphenols, and also because of their appealing taste and color. This color is evoked by anthocyanins, a sub-group of polyphenols. Besides their coloring properties, anthocyanins have been shown to possess various beneficial biological effects (He & Giusti, 2010). Therefore, the content of anthocyanins in juices is not only a matter of sensory quality but also concerning of potential health effects.

Processing of fruits and vegetables inevitably includes steps with detrimental conditions for anthocyanins (de Pascual-Teresa & Sanchez-Ballesta, 2008). The production of red juices starts with the harvesting of berries, either manually or automated. After sorting and washing, the berries are processed either into a juice by various procedures or into purees for other usages in the food industry. The production of juices necessitates the disintegration of fruit cells to release the liquid. Several enzyme preparations are commonly applied liberating the cell-bound water and increasing the extraction of the fruit. These are cell wall degrading enzymes such as pectinases or cellulases (Landbo & Meyer, 2001). The treated mash will then be pressed to separate the liquid and the pomace. Different thermal treatments to inactivate fruit borne and added enzymes and to ensure microbial safety are included during processing (Patras, Brunton, O'Donnell, & Tiwari, 2010). In the last decade, novel technologies like high-pressure treatment, pulsed electric fields or ultrasound have been introduced into juice processing (Knorr et al., 2011). These techniques are mainly applied to reduce heat loads. They have been shown to inactivate microorganisms and enzymes, but at the same time, they facilitate the extraction of the fruits by destroying cells. Before the final bottling step, the juice can be clarified and filtered to achieve a longer shelf life regarding cloudiness. The produced juices may also be concentrated, which normally includes additional thermal treatments.

As a consequence of virtually all of these processing steps, the

* Corresponding author.
E-mail address: fabian.weber@uni-bonn.de (F. Weber).

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genuine amounts and profiles of anthocyanins are altered. This may result in lower concentrations which might lead to a loss of color and can also be regarded as a loss of potential health benefits. A decrease in measured anthocyanin concentration is not necessarily accompanied by a loss of these compounds (Howard, Prior, Liyanage, & Lay, 2012). They might be derivatized or complexed by other compounds and thus defying analytical detection. More recently, the natural profile of secondary plant metabolites is also considered as a quality related factor. On the one hand, their relative amounts are used as authentication markers (Fügel, Carle, & Schieber, 2005) and on the other hand studies demonstrated different biological properties of various polyphenols (Smeriglio, Barreca, Bellocco, & Trombetta, 2016; Wen & Walle, 2006). Besides enhancing overall concentrations, preserving natural profiles of anthocyanins should be considered as a objective for process optimization. However, a deeper knowledge is needed to understand the changes of anthocyanin concentrations and profiles during juice processing to improve processing steps and eventually the resulting products.

This review summarizes the effects of the mentioned processing steps on the quantity and profile of anthocyanins during the production of berry juices. Table 1 gives an overview on the processing induced changes of anthocyanin content and profiles of selected fruits. Effects of harvesting and post-bottling effects are excluded since they are not considered part of juice processing.

2. Stability of anthocyanins

The chemistry of anthocyanins has been reviewed extensively (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009) and will only be delineated in short. Anthocyanins are part of the flavonoid sub-group of the polyphenols and bear an oxidized C-ring. Their pH dependent structure induces different colors ranging from the red colored flavylium cation in an acidic environment to the blueish quinoidal form at higher pH values. Anthocyanins can be further differentiated by the substitution pattern, particularly at the B-ring, and by the degree and nature of the glycosylation of the anthocyanidin aglycone, predominately at positions 3 and 5. Esterification with various aliphatic or aromatic acids can modify the attached sugar moieties (Andersen & Jordheim, 2014).

Several factors have been demonstrated to affect the stability of anthocyanins. Besides the structure related influences, several exogenous parameters like temperature, light, and oxygen, especially the pH and the presence of copigments are the most determining factors of anthocyanin stability (Markakis, 1982; Patras et al., 2010). Not only the anthocyanin structure includes several reactive positions but also the surrounding matrix in the berry or juice holds numerous reactions partners (Andersen & Jordheim, 2014). This leads to a manifold of possible reactions during juice processing. The term anthocyanin stability can be differentiated into three general routes of anthocyanin modification: polymerization, cleavage, and derivatization. Whereas cleavage of the anthocyanin leads to colorless compounds, polymerization is accompanied by browning. Derivatization reactions might result in various colored molecules.

Driven by the pH value, the acid-base equilibrium and the hydration of the C-ring are two reversible structure modifications inherent to anthocyanins. These equilibria result primarily in color changes but might lead to secondary reactions. The predominating flavylium cation form of the anthocyanidins has been demonstrated to be less stable than the corresponding glycosides (He & Giusti, 2010). Thus, hydrolysis of the glycosidic bond, either acid or enzyme catalyzed, severely reduces the stability of the pigments and may finally result in degradation. After hydrolysis, C ring opening may lead to chalcone formation and eventually to cleavage of the anthocyanidin, yielding aldehydes and phenolic acids (Sadilova, Stintzing, & Carle, 2006). Oxidation may also lead to polymeric material with indefinable structures, which will also include other phenolic compounds (Cheynier et al., 2006; Li, Guo, & Wang, 2008). The brown polymeric pigments formed by either chemical or enzymatic oxidation may be considered the end of a complex reaction cascade. Initial adduct formation of anthocyanins and other phenolic and non-phenolic compounds leads to more or less unstable derivatives with different color tonalities. These reactions have well been studied especially during wine making, and aging and those of them not involving ethanol might very well occur during juice production (Fulcrand, Dueñas, Salas, & Cheynier, 2006).

Similar to the hydration, the anthocyanin moiety can form an adduct with HSO_3^-, which may be used during fruit processing (Jurd, 1964). This adduct formation also results in colorless forms of the anthocyanins. Interruption of the conjugated π-electron system by addition of HSO_3^- at position 2 results in discolorization. Copigments are able to prevent the formation of this colorless from caused by the addition of water and bisulfite. These non-colored compounds additionally increase the absorption (hyperchromic shift) and change the color tonality (bathochromic shift) by stacking of the π-electron systems (Boulton, 2001; Escribano-Bailon & Santos-Buelga, 2012).

3. Juice processing

3.1. Pre-press procedures

Berry fruits are commonly produced from individually quick frozen (IQF) fruits, thawed before processing. The effects of freezing, storage effects, and the influence of thawing on the anthocyanin concentration of the juices have to be taken into consideration since they affect tissue integrity (Van Buggenhout, Sila, Duvetter, Van Loey, & Hendrickx, 2009). These influences are closely related to oxidation and extraction effects that are discussed later. Initial freezing and thawing resulted in increased anthocyanin concentrations found in grapes, presumably due to enhanced extraction (Rio Segade et al., 2014). Prolonged frozen storage leads to a slow but considerable decrease in anthocyanins. Poiana, Moigradean, Raba, Alda, and Popa (2010) monitored anthocyanin concentration over ten months at − 18 °C and observed losses of 13% in blueberries, 19% in blackberries, and 16% in raspberries. Another study reported considerable differences in frozen storage stability regarding raspberry varieties ranging from 18% loss of total anthocyanins to an increase of 17% (de Ancos, Ibañez, Reglero, & Cano, 2000). They also observed variety dependent differences in the stability of individual anthocyanins that were explained by differences in pH and the content of soluble solids. The effect of thawing procedure was evaluated for strawberries and revealed that faster thawing at 20 °C (12% loss) preserved anthocyanins better than longer thawing at lower temperatures (21% loss at 4 °C) (Holzwarth, Korhummel, Carle, & Kammerer, 2012). The procedure for the production of pomegranate juice differs due to the different raw material. Processing of whole fruits led to juices with 30% lower anthocyanin concentrations compared to juices produced from hand-peeled sacs (Turfan, Turkyilmaz, Yemis, & Ozkan, 2011).

3.2. Mashing and pressing

Juice production necessitates the disintegration of fruit cells to release the liquid which is commonly achieved by mechanical crushing of the fruits. The subsequent pressing or sieving separates coarse residues from the juice. Since anthocyanins are predominantly located in the skins that are robust and hard to grind, especially those of berries like blackcurrant and blueberry, a major part of fruit anthocyanins is lost into the press cake. Hager and co-workers observed 14% loss of anthocyanins into the press-cake for blackberry juice production (T.J. Hager, Howard, & Prior, 2008) and 16% loss during the production of juice from black raspberries (A. Hager, Howard, Prior, & Brownmiller, 2008). At industrial scale of black mulberry juice production, Tomas et al. (2015) observed 6% loss of anthocyanins due to milling and 28% increase after pressing. This might be explained by a prolonged