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Polyphenols as Natural Food Pigments: Changes During Food Processing

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Abstract: The colors of the foods and beverages contribute not only to their appearance but also to their agreeableness, which linked to both taste and flavor. Polyphenols, particularly flavonoids, are important pigments for determining food color, similar to carotenoids and chlorophylls. Foods and beverages contain a mixture of intact pigments, which are derived from raw materials and artificial pigments that are generated by chemical and enzymatic reactions during processing and storage. This study provides an overview of the characteristics and contributions of native polyphenol pigments from plants materials, as well as those generated by chemical and enzymatic reactions during food and beverage production.

Key words: Flavonoids, anthocyanins, flavanols, artificial pigments, food colors

INTRODUCTION

The colors of foods and beverages contribute not only to their appearance but also to their agreeableness, which is linked to both taste and flavor. Foods and beverages contain intact pigments derived from raw materials, as well as artificial pigments that are generated by chemical and enzymatic reactions during processing and storage (if food additives are not employed). Polyphenols, carotenoids and chlorophylls are natural pigments that are derived from raw materials. Various other compounds, such as proteins, amino acids, lipids and sugars, are altered by the complex chemical reactions that occur during processing and storage; the products of these reactions can also contribute to the colors of foods and beverages.

Polyphenols derive from the plant materials, such as fruits and vegetables, have important organoleptic properties on taste and flavor, similar to those of color (Cheynier *et al.*, 1998). Polyphenols have previously been investigated in terms of their contents and compositions in plants, foods and beverages. They have also been studied with respect to their effects on the food and beverage qualities. Since red wine was reported to protect low-density lipoproteins against the oxidation and to prevent cardiovascular disease through the so-called French Paradox (Renaud and Lorgeril, 1992), the polyphenols in a variety of other foods and beverages have been investigated and demonstrated to have a range of physiological functions. In fact, interest in polyphenols has increased over a broad range of subject areas, including agriculture, botany, pharmacognosy, pharmaceutics, nutritional science and organic chemistry. Many researchers have focused on the correlation between polyphenols in foods and beverages and their physiological functions. Consequently, the numbers of reports on polyphenols, their antioxidants properties and their physiological functions have been increasing since 1995 (Scalbert *et al.*, 2005).

Polyphenols are reactive both among themselves and with other compounds, including the artificial polymeric pigments generated during food and beverage manufacture. The main purpose of the current review is to summarize the contribution of intact polyphenols, as natural pigments, to the colors of foods and beverages. The contributions of artificial polymeric pigments, such as those in black tea, red wine and rosé cider, are also reviewed.

POLYPHENOLS IN FOODS

Polyphenols are widely common secondary metabolites of plants and are found in a range of foodstuffs (for example, legume seeds and cereal grains) and beverages (for example, tea, wine, cocoa and cider). All polyphenols contain some phenolic hydroxyl groups and hydrophilic properties. The astringent properties and bitter taste of polyphenols are thought to be caused by precipitation with salivary proteins (Sarni-Manchado *et al.*, 1999). Polyphenols are classified according to their structure as either non-flavonoids (Fig. 1) or flavonoids (Fig. 2). The non-flavonoids include phenolic-acid derivatives, stilbenes (e.g., resveratrol [1]), gallotannins and lignins. These compounds are colorless or slightly brownish and rarely contribute directly to the color of foods. The flavonoids have been identified more than 4,000 from various plants and new compounds of this type, even presently, are still being discovered (Harborne and Gryer, 1994). They can be subdivided into several further groups, such as flavonols and anthocyanidins, on the basis of the hydroxylation of phenolic rings, glycosylation, acylation with phenolic acids and the existence of stereoisomers (Harborne and Gryer, 1988).

Polyphenol compositions and contents vary greatly between different plant species and cultivars, as well as according to the growth or maturation stage, season, region and yield. In apples, for example, polyphenols are localized mostly in the skin and core, rather than in the pulp (Mayr *et al.*, 1995; Guyot *et al.*, 1998). Chlorogenic acid [2], phloretin glycosides (e.g., phloridzin [3]) and quercetin glycosides (e.g., rutin [4]) are most common in the younger fruiting stages and decrease markedly during maturation. Flavanols and procyanidins reach the maximum levels in July and decrease thereafter until the fall. By contrast, anthocyanidins (such as cyanidin-3-galactoside [5]) are not detectable in immature fruits and their levels increase in the skin according to the temperature and the number of hours of sunlight (Lister *et al.*, 1994).

Stilbenes
1, Resveratrol (colorles)
Hydroxycinnamic acids
2, R1 = Quinic acid: Chlorogenic acid (Colorless)
Hydroxybenzoic acids
Gallic acid (Colorless)

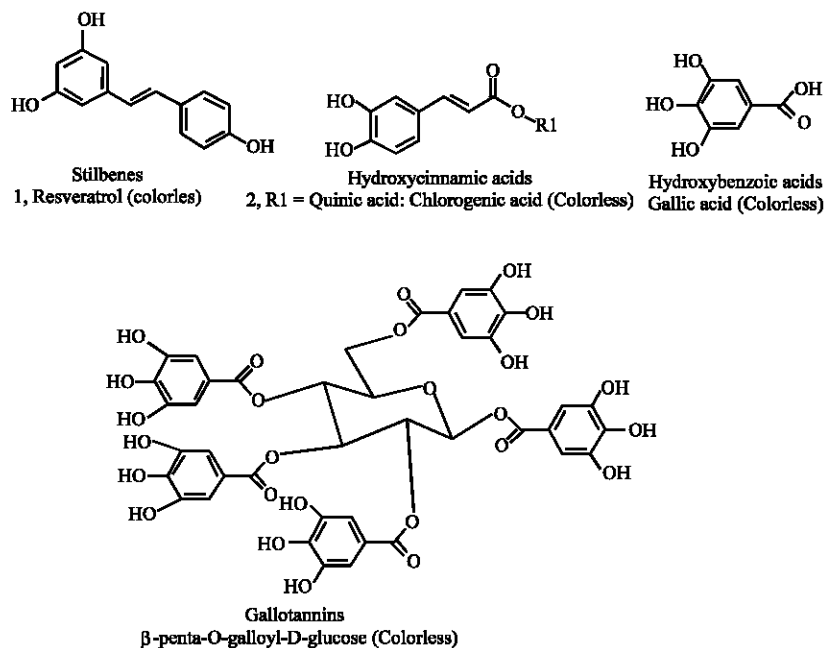**Gallotannins**
β-penta-O-galloyl-D-glucose (Colorless)

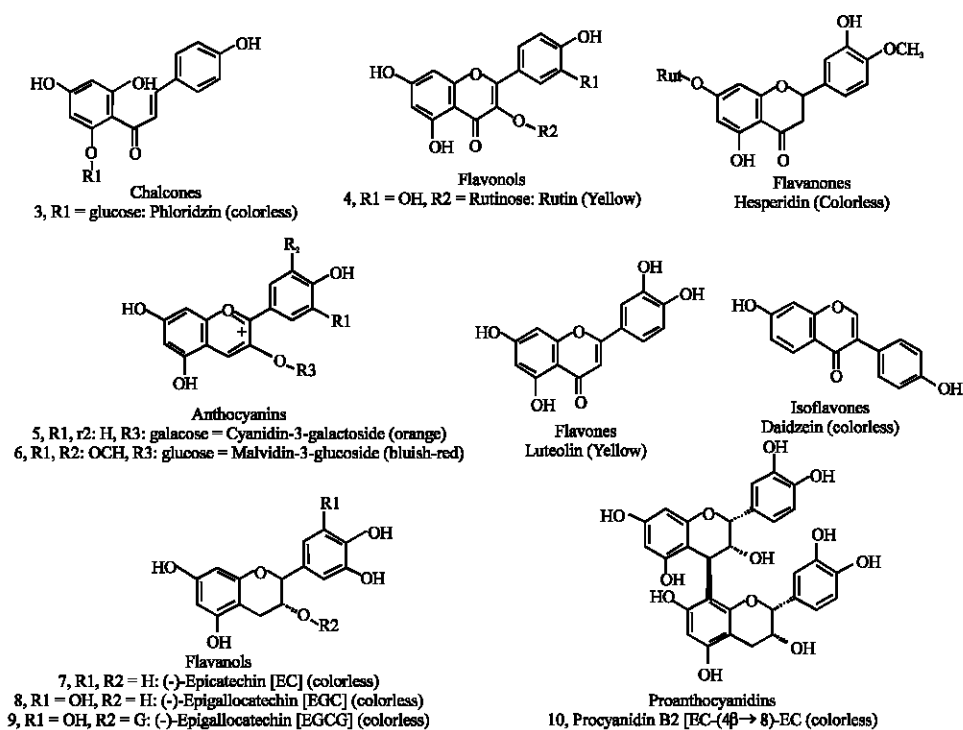Fig. 1: Chemical structures and colors of non-flavonoid polyphenols

Fig. 2: Chemical structures and colors of flavonoid polyphenols

Polyphenol compositions and contents also vary according to the methods of elution and the manufacture processing used to produce foods and beverages. In particular, the various maceration and pressing methods employed in wine and cider making process can affect the tone and strength of the color, as well as the types and contents of polyphenols within the products.

The flavonoids are descried more fully below, with a focus on anthocyanins, flavanols and proanthocyanidins.

Flavonoids

Flavonoids (excluding anthocyanins, flavanols and proanthocyanidins) are yellow or brown color depending upon their structures. Briefly, flavones and flavonols tend to be yellow, whereas flavanones and isoflavones are generally colorless, white or slightly brownish (Fig. 2). Generally, flavonoids are present in foods and beverages as glycoside, rather than as aglycones. They are relatively low solubility in water, so their contributions to food color might be reduced compared with those of anthocyanins, flavanols and proanthocyanidins. Numerous flavonoids have been described in previous reviews on fruits (Macheix *et al.*, 1990), processed foods (Shahidi and Naczk, 1995) and plants (Harbone, 1988, 1994).

Various xanthones and quinones are also known to exist in plants as minor pigments and to act as important food additive. However, as their contributions to the color of foods and beverages are relatively minor, they are not discussed further in the current review.

Anthocyanins

Anthocyanins are major polyphenol pigments that largely determine the colors of flowers and fruits. They can be orange, red, blue or purple in color. For example, elderberry (*Sambucus caerulea*),
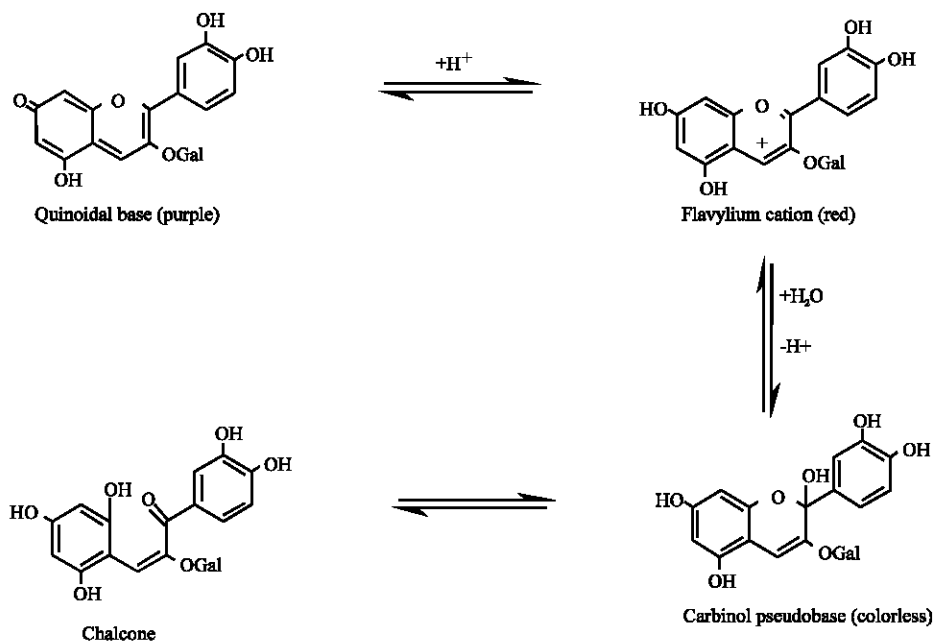
Fig. 3: Equilibrium forms of anthocyanin, cyanidin-3-galactoside

purple corn (*Zea mays*) and red cabbage (*Brassica oleracea*) all contain abundant anthocyanin pigments and are utilized as natural food additives. Anthocyanins can equilibrate with some forms in water according to the pH and the temperature. They are generally present in a red flavylium form or colorless carbinol pseudo-base form, depending upon the pH conditions (Brouillard and Delaporte, 1977). The colorless carbinol pseudo-base form changes into a yellow chalcone form as the pH approaches neutral, while the red color tends to fade (Fig. 3).

Although anthocyanidins have relatively limited aglycone types, a single compound can produce various tones and strengths of colors in different flowers. This suggests that anthocyanins are affected by the co-existent of other polyphenols and metal ions. For example, anthocyanins can be stable, show enhanced absorbance, or undergo a shift to a higher maximum absorbance wavelength (λmax) as a results of their stacking and co-pigmentation with anthocyanins and co-pigments that have little or no inherent color, such as other flavonoids, phenolic acids and metal ions (Mistry *et al.*, 1991; Boulton, 2001).

Anthocyanins can also react with other compounds (such as flavanols and proanthocyanidins) in foods and beverages during processing and storage. These reactions and the resulting products, are described in the following sections.

Flavanols and Proanthocyanidins

Proanthocyanidins, which are also known as condensed tannins, have higher molecular weights than other flavonoids. They are originated from the resulting to generate anthocyanidins, when they are heated under acidic conditions and degraded. They are classified as procyanidins or prodelphinidins according to their flavanol units. They are then joined by the interflavanoid linkages of 4–8 or 4–6 (B-type) of flavanols (Fig. 2), such as those in (+)-catechin and (−)-epicatechin [7] or (+)-gallocatechin and (−)-epigallocatechin (EGC [8]), respectively (Porter, 1988). These flavanols can also be esterified with gallic acid to form 3-O-gallates. Procyanidins are the most common proanthocyanidins found in

plant and their average degree, nature and composition can vary widely. Structural proanthocyanidin variants can also occur as a result of the formation of a second interflavanoid linkage by C–O oxidative coupling, resulting in the A-type bond.

Both flavanols and proanthocyanidins have a slight brownish color. However, they tend to contribute to the colors of foods and beverages through their reactions with other polyphenols, particularly anthocyanins and by enzymatic reactions, rather than directly contributing as native pigments.

REACTIONS OF POLYPHENOLS DURING PROCESSING

Polyphenols are reactive compounds, which can be degraded and polymerized through both enzymatic and non-enzymatic reactions during processing. Polyphenols can be oxygenated and degraded by various enzymes (e.g., polyphenoloxidases, peroxidases, glycosidases and esterases) that are released when plant cells are broken down. Glycosidase and esterase enzymes catalyze polyphenol degradation during hydrolysis reactions. Oxidation reactions caused by polyphenol oxidase and peroxidase enzymes tend to produce black pigments, thereby having negative effects on fresh vegetables and fruits during transport and fruits juice and wines before fermentation. These enzymatic reactions are, however, necessary to improve the quality of both black tea and oolong tea.

Flavanols, proanthocyanidins and anthocyanins can also react among themselves and with other compounds, including the high molecular weight artificial polymeric compounds generated during processing and storage, via non-enzymatic reactions. These reactions are important for changing the color tones and strengths of beverages, such as rosé cider and red wine. Changes in polyphenols can also affect the taste of the latter during maturation and aging.

The above mentioned reactions can significantly influence food quality through the development and degradation of polyphenols. It is therefore important to analyze the compositions and contents of intact polyphenols, as well as the products resulting from these reactions. However, although various spectrometric and chromatographic methods (such as Folin-Ciocalteu and reversed-phase High-performance Liquid Chromatography [HPLC]) can be generally used for the analysis, detection and fractionation of polyphenols, they have restricted abilities to separate and detect polymeric tannin-like compounds. Moreover, various combinations and types of proanthocyanidins and anthocyanins are present in foods and beverages and the reaction products vary continuously and randomly. The structures of the resulting products have thus remained poorly understood.

Polymeric Compounds in Oolong and Black Tea

Unfermented green tea is rich in flavanols (e.g., EGCG [9]), which make up about 30% of the dry leaves weight. Enzymatic oxidation reactions during the fermentation are essential for the production of semi-fermented oolong tea and fermented black tea. Colorless low molecular weight flavanols within the fresh leaves are converted into polymeric compounds (e.g., theaflavins [11] and thearubigins), which are characteristic dark-brown pigments with high molecular weights (Davies, 1999; Horie and Kohata, 2000; Haslam, 2003). The fermentations of oolong tea and black tea induce flavanol oxidation reactions by polyphenoloxidases, following the non-enzymatic reaction of quinones. A series of theaflavins (Fig. 4), which are yellow/orange pigments, results from the oxidative coupling of the B-rings of gallocatechins. These reactions in fermentation are in conflict with one another and the polyphenols and theaflavins are continuously and randomly polymerized. Hashimoto *et al.* (1992) showed that the flavanols EGC [8] and EGCG [9] were transformed by polyphenoloxidases into theasinensins [12], rather than into theaflavins and that oolongtheanin [13] could be obtained from EGCG and theasinensins. Moreover, Haslam (2003) proposed that the structures and mechanisms of the thearubigins, which remain poorly understood, are similar to those of oolongtheanins. However, these reaction products remain to be explored fully in oolong tea and black tea.
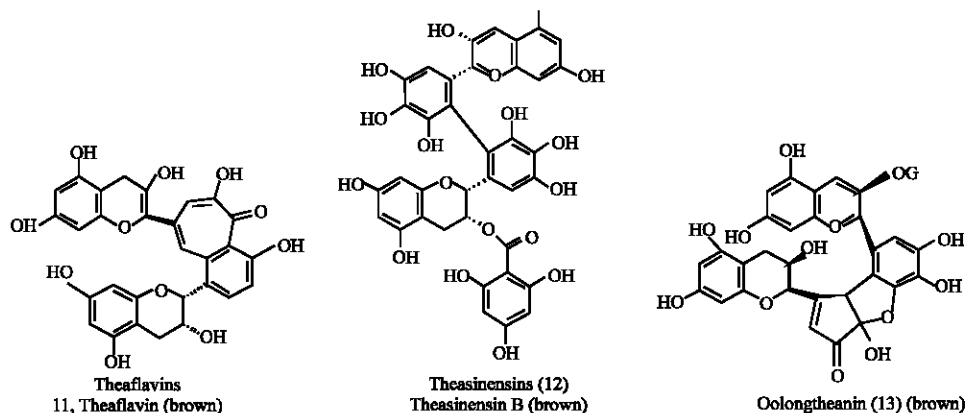
Theaflavins
11, Theaflavin (brown)

Theasinensins (12)
Theasinensin B (brown)

Oolongtheanin (13) (brown)

Fig. 4: Chemical structures and colors of tea pigments

Polymeric Anthocyanins in Red Wine

The color evolution of red wine is of great interest, as the changes from bluish red to reddish orange is related to organoleptic properties, such as astringency, during storage and aging. Co-pigmentation can account for 30-50% of the color of young red wines and plays a crucial role, similar to those in fruits and flowers (Liao *et al.*, 1992; Mirabel, 1999; Boulton, 2001). Moreover, co-pigments, such as (–)-epicatechin [7], can reportedly enhance the color of red wine.

The changes in the color of red wine are related to the formation of new, more stable, polymeric pigments through reactions between anthocyanidins or other polyphenols and wine components. Some conflicting reactions are known to be important role during this process and for determining the resulting pigments. Polymeric products in wine can react with anthocyanins, flavonols, or proanthocyanidins, either directly (Santos-Bugelga *et al.*, 1995; Remy *et al.*, 2000; Remy-Tanneau *et al.*, 2003; Salas *et al.*, 2003; Vidal *et al.*, 2004) or indirectly through various other wine components (Timberlake and Bridle, 1976; Baranowski and Nagel, 1983; Bakker *et al.*, 1993; Garcia-Viguera *et al.*, 1994; Rivas-Gonzalo *et al.*, 1995; Dallas *et al.*, 1996a; Francia-Aricha *et al.*, 1997; Es-Safi *et al.*, 1999a; Atanasova *et al.*, 2002).

Many researchers have investigated the abovementioned reactions and pigments (Somers, 1971; Timberlake and Bridle, 1976). However, various polyphenols, (particularly anthocyanidins and proanthocyanidins) are present in grapes and wine and numerous vinification process (e.g., maceration, pressing, fermentation, yeast metabolism, maturation and aging) and techniques (e.g., maceration carboniqe) are used in wine making. In addition, the reaction products continuously change from monomeric to polymeric compounds during these stages. It is therefore difficult to analyze and separate the reaction products within red wines. Recent developments have allowed the products formed by direct and indirect condensation reactions to be characterized and identified by HPLC/diode array detection (DAD), liquid chromatography/mass spectrometry (LC/MS) and nuclear magnetic resonance (NMR) analyses (Bakker and Timberlake, 1997; Bakker *et al.*, 1997; Fulcrand *et al.*, 1997, 1998, Saucier, 1997; Remy-Tanneau *et al.*, 2003; De Villiers *et al.*, 2004).

As shown in Fig. 5, anthocyanins, flavanols, or proanthocyanidins can directly react in order to generate two red pigments: flavanol-anthocyanin [14]; anthocyanin-flavanol (Salas *et al.*, 2003). Two possible pathways have been proposed to explain these observations: first, anthocyanins might react with carbonium ions from proanthocyanidins as nucleophiles and second, the electrophilic C4-position of anthocyanins might be attacked by flavonols. A colorless product [15], which linked by both carbon-carbon (C4–C8) and ether (C2–O–C7) bonds, has also been reported to be present in red wines (Remy-Tanneau *et al.*, 2003).
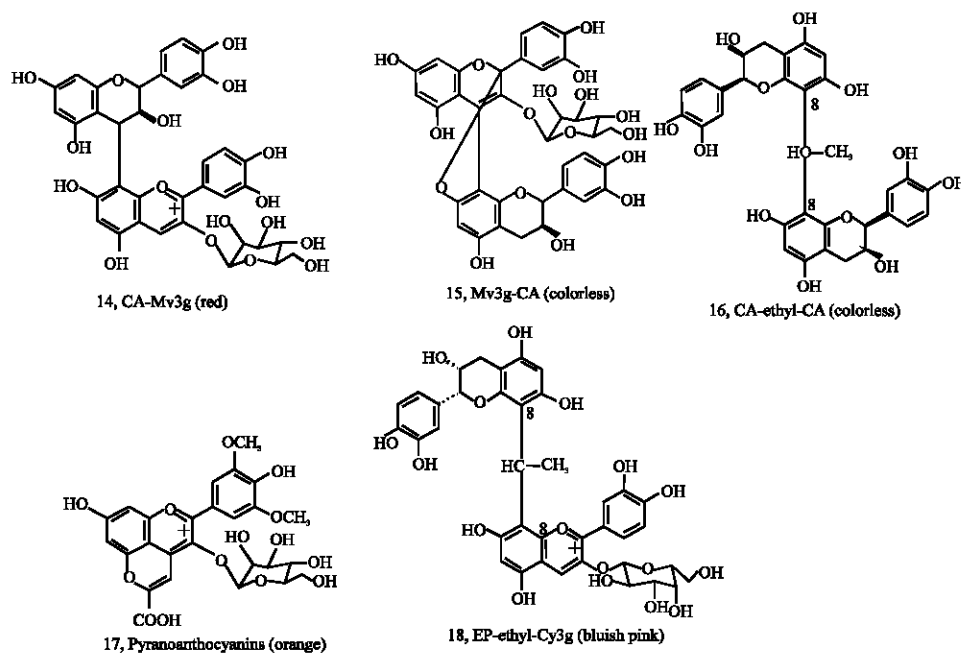
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Fig. 5: Chemical structures and colors of red wine pigments. CA, (+)-catechin; Cy3g, cyanidin-3-galactoside [5]; EP, (-)-epicatechin [7]; Mv3g, Malvidin-3-glucoside [6]

Wine components (such as acetaldehyde) that are generated by yeast metabolism or ethanol oxidation might be involved in indirect reactions between anthocyanins, flavanols and proanthocyanidins. Such a mechanism was proposed by Timberlake and Bridle (1976) and many researchers have demonstrated the presence of ethyl-bridged anthocyanins-(epi)catechin dimers in wine both theoretically and experimentally (Fulcrand et al., 1996b; Saucier et al., 1997). New pigments, in which the anthocyanins are self condensed through acetaldehyde, have also been reported in the model wine and red wine (Atanasova et al., 2002). Other aldehydes such as glyoxylic acid originated from tartaric acid in grape (Es-Safi et al., 1999b, c) and furfural extracted from barrel (Es-Safi et al., 2000, 2002) instead of acetaldehyde also concern with indirect polymerization reaction. In addition, anthocyanin-glyoxylic acid adducts [17], anthocyanin-pyruvic acid adducts (Bakker and Timberlake, 1997; Fulcrand et al., 1998; Mateus et al., 2001), anthocyanin-vinyl phenol adducts (Fulcrand et al., 1996a) have been characterized in red wine.

The above mentioned pigments are generally present as a colorless hemiketal form in wine, depending upon the pH conditions, following the decrease and stabilization of the color. These pigments generated during the vinification process are more stable than intact anthocyanins from grape (Fig. 5). However, although some reactions and the resulting products have been investigated, the phenolic components of wine remains largely ambiguous. This is because the reactions and pigments are affect not only by the grape polyphenols themselves, but also by various other wine components (e.g., acetaldehyde and SO_2), as well as the temperature, pH and oxygenation levels. New reaction pathways and their resulting products are expected to be reported in the future.

Polymeric Anthocyanins in Rosé Cider

The changes of color in rosé cider during the vinification process differ considerably from those in red wine, even though the two are made from similar fruits. Rosé cider is produced using red apple (such as Starking or Red Delicious) and progresses from colorless to orange/red during the vinification

Fig. 6: Change of color in rosé cider during vinification. (A), apple juice; (B), young rosé cider after fermentation and (C), rosé cider after maturation

process (Fig. 6). The anthocyanidins (for example, cyanidin-3-galactoside [5]) extracted from apple peel combine with SO_2, which is added in order to extract them stably and the pre-fermented juice is colorless (Somers and Evans, 1977; Brouillard and Duabois, 1980). By contrast, the juice used in the production of red wine is reddish purple, owing to grape anthocyanins (for example, malvidin-3-glucoside [6]). The color of young rosé cider change to orange/red during fermentation, as the acetaldehyde, α ketoglutaric acid and pyruvic acid produced by yeast metabolism combine with SO2, thereby causing the linked colorless anthocyanins to become free red anthocyanins. The intensity of color is affected by the ability of yeast to generate these products. The color of rosé cider further changes to bluish pink from orange/red color during maturation in preservation tank, as novel pigments that are present in neither juice nor young rosé cider are generated. Size-exclusion chromatography using a new solvent system confirmed that the anthocyanins in rosé cider changed into higher molecular weight polymeric compounds (Shoji *et al.*, 1999). Furthermore, some compounds from rosé cider were shown to have the λmax values that had shifted to a higher range, as well as molecular ion peaks $[M+H]^+$ at m/z 765 and 1,053, using reversed-phase HPLC/DAD and LC/MS, respectively. Among these, four of the pigments with $[M+H]^+$ peaks at m/z 765 corresponded to cyanidin-3-galactoside [5] and flavanols linked by CH_3-CH bridge [18]. Moreover, three of the pigments with $[M+H]^+$ peaks at m/z 1,053 corresponded to cyanidin-3-galactoside and procyanidin dimers linked by CH_3-CH bridges (Fig. 7). We demonstrated that two of the anthocyanin pigments with $[M+H]^+$ peaks at m/z 765 in rosé cider were linked at the C8-position of (–)-epicatechin and cyanidin-3-galactoside by CH_3-CH bridges [18]. These diastereomers differed in the configuration of the asymmetric methine carbon, according to 1H- and 13C-NMR analyses (Shoji *et al.*, 2002). Furthermore, two of the pigments with $[M+H]^+$ peaks at m/z 1,053 were found to be procyanidin B2 (epicatechin 4β-epicatechin 8) and cyanidin-3-galactoside linked by CH_3-CH bridges.

The novel pigments in rosé cider were cyanidin-3-galacotoside and flavanols or procyanidin dimers, linked by the CH_3-CH bridges from the acetaldehydes, as proposed by Timberlake and Bridle (1976). Our NMR results and those of Es-Safi *et al.* (1999a) showed that only the C8-position of the anthocyanins was involved in the connection of the CH_3-CH bridges. As described above, anthocyanin-flavonol adducts linked by CH_3-CH bridges from the acetaldehyde were detected in red wine (Fulcrand *et al.*, 1996b; De Villiers *et al.*, 2004). However, pigments in which anthocyanins and
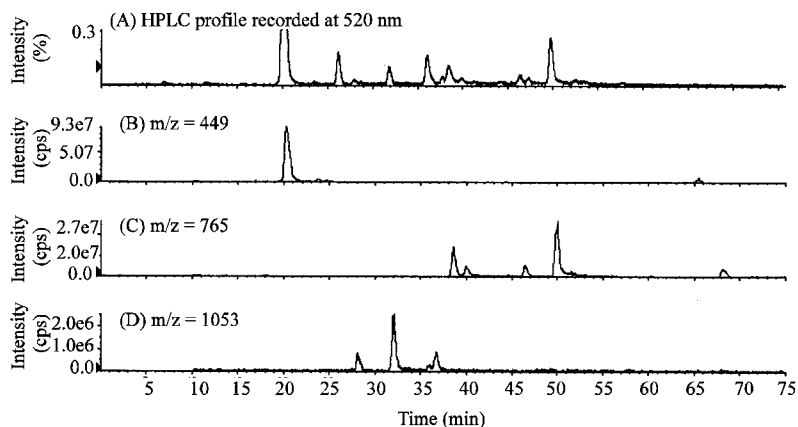
Fig. 7: Anthocyanin profile of rosé cider recorded at 520 nm using reversed-phase HPLC. (A), HPLC profile of rosé cider recorded at 520 nm; (B), [M+H]⁺ peaks at m/z 449, (C), [M+H]⁺ peaks at m/z 765 and (D), [M+H]⁺ peaks at m/z 1053

flavonols or procyanidins directly condensed, or anthocyanins and flavonols were indirectly linked by other aldehydes (excluding acetaldehyde, anthocyanin-pyruvic acid adducts and anthocyanin-vinyl phenol adducts) were not detected in rosé cider. These differences in the pigments generated by red wine and rosé cider might have been caused by the raw materials (this is, apples and grapes) and the vinification process employed. In particular, the preservation temperature needed for rosé cider (2-4°C) was lower than that for red wine and the SO_2 dose required for rosé cider was higher than that for red wine; thus, anthocyanins might have been more stable and the reaction rate might have been slower in the former.

CONCLUSIONS

Polyphenols, particularly flavonoids (such as flavanols, proanthocyanidins and anthocyanins), play crucial roles in determining the colors of foods and beverages, as well as elements of their taste (e.g., astringency and bitterness) and flavor. During processing, polyphenols are converted into new pigments by oxidation via enzymatic reactions and by direct and indirect condensation through non-enzymatic reactions. These resulting products are important in determining the organoleptic properties (for example, the appearance and taste) of foods and beverages. These compounds have been detected and characterized in various products using HPLC/DAD, LC/MS and NMR. However, few data have quantified their contents in foods and beverages.

The above mentioned products are predicted to possess various physiological functions. Recently, physiological functions such as anti-oxidative activity of red wine have been reported and polyphenols are expected to protect against food-style disease. However, these effects have not been confirmed, because of the difficulties involved in isolating such products in large quantities. Intact polyphenols in red wine, which can be obtained commercially as reagents, have only been evaluated in terms of their anti-oxidative activities and minor compounds such as resveratrol [1] have been reported to physiological functions as active compounds (Frankel *et al.*, 1993). The products generated during processing remain to be isolated on a large scale and their effects on physiological functions remain to be evaluated. Thorough investigations into the activities, distributions and bioavailability of these products will be needed to assess their physiological functions in foods and beverages.

ACKNOWLEDGMENTS

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