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Co-pigmentation of pelargonidin derivatives in strawberry and red radish model solutions by the addition of phenolic fractions from mango peels

Judith Müller-Maatsch [a], Lena Bechtold [a], Ralf M. Schweiggert [a,*], Reinhold Carle [a,b]

[a] University of Hohenheim, Institute of Food Science and Biotechnology, Chair of Plant Foodstuff Technology and Analysis, Garbenstrasse 25, 70599 Stuttgart, Germany
[b] King Abdulaziz University, Faculty of Science, Biological Science Department, P.O. Box 80257, Jeddah 21589, Saudi Arabia

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ABSTRACT

Pelargonidin-based colors suffer from notorious instability. A phenolic mango peel extract and defined phenolic fractions thereof were shown to effectively modulate the visible absorption of anthocyanins from strawberry (*Fragaria* x *ananassa* Duch.) and red radish (*Raphanus sativus* L.) by intermolecular co-pigmentation. Consistently, non-acylated pelargonidin derivatives from strawberry exerted significantly greater hyper- and bathochromic spectral shifts than their acylated counterparts from red radish. The addition of low molecular-weight co-pigments such as gallic acid and monogalloyl glucoses to strawberry anthocyanins led to strong hyperchromic shifts from 30% to 48%, while gallotannins (>six galloyl units) exerted smaller co-pigmentation effects (36 ± 2%; $\Delta\lambda_{max}$ 13 nm), possibly due to steric hindrances. In contrast, penta- and hexa-*O*-galloyl-glucose induced greatest and most stable co-pigmentation effects (53 ± 2%; $\Delta\lambda_{max}$ 13 nm). Irrespective of the underlying mechanisms and the responsible compounds, phenolic mango peel extracts might represent suitable color enhancers for coloring foodstuff, particularly for those containing non-acylated pelargonidin derivatives.

1. Introduction

Anthocyanins are a sub-class of secondary plant metabolites belonging to the flavonoids. They are water-soluble pigments imparting bright colors from red to blue hues to all kinds of plant tissue including flowers, fruits, leaves, roots, and stems. During the past decades, they have been subject of extensive botanical and chemical research and, to date, over 500 naturally occurring anthocyanins have been found in nature. Their structural diversity is based on more than 30 monomeric anthocyanidins, mostly diversified by methoxylation, glycosylation, and acylation. The naturally most abundant anthocyanidins are cyanidin, delphinidin, and pelargonidin followed by malvidin, petunidin, and peonidin (Trouillas et al., 2016). Several pelargonidin derivatives are the main coloring principles in strawberry (*Fragaria x ananassa* Duch.) fruits and red radish (*Raphanus sativus* L.) roots (Giusti et al., 1998; Holzwarth, Korhummel, Carle, & Kammerer, 2012). Strawberries are among the most important fruits for industrial food processing, therefore being of high economic relevance (BMEL, 2015). Besides its limited use on fresh markets, red radish is currently used for the production of pigment rich extracts or coloring model solutions to be used as a valuable natural food colorant (Giusti et al., 1998). Although both strawberry and red radish contain pelargonidin derivatives, those of strawberry are burdened by high instability during processing and subsequent storage. In contrast, those of red radish have been shown to be more stable. Their susceptibility to degradation is primarily determined by their molecular structure and the surrounding matrix, i.e. pH, water activity, hydrocolloid content, and concomitant phenolic compounds. These factors determine their stability against light exposure, temperature, oxygen, and thus, losses during storage (Markakis & Jurd, 1974). While the influences of pH, water activity, and hydrocolloids on anthocyanin stability have been extensively studied, knowledge about stabilizing and color enhancing effects of phenolic compounds is poor. The protective effect is believed to be based on a close molecular association of the phenolics with the aromatic chromophore of the anthocyanins in aqueous solutions, being known as co-pigmentation. Although van der Waals forces, ionic interactions, and hydrophobic effects may play a certain role, π–π interactions are believed to be the main mechanistic driving force for intermolecular co-pigmentation, leading to vertical or sandwich-type stacking of anthocyanins and phenolics

* Corresponding author at: University of Hohenheim, Institute of Food Science and Biotechnology, Chair of Plant Foodstuff Technology and Analysis, Garbenstrasse 25, D-70599 Stuttgart, Germany.
E-mail addresses: j.mueller-maatsch@uni-hohenheim.de (J. Müller-Maatsch), lena.bechtold@t-online.de (L. Bechtold), ralf.schweiggert@uni-hohenheim.de (R.M. Schweiggert), reinhold.carle@uni-hohenheim.de (R. Carle).

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(Goto & Kondo, 1991; Kunsági-Máté, Szabó, Nikfardjam, & Kollár, 2006; Trouillas et al., 2016). As a result of their close association, the anthocyanidin core becomes sterically shielded against nucleophilic attacks. Furthermore, the levels of the prevailing anthocyanin form at a given pH may be drastically altered due to co-pigmentation, and thus a strong influence on the observed color intensity and stability of the respective anthocyanins is often also observed. Under slightly acidic conditions (pH 3.65), Brouillard, Mazza, Saad, Albrecht-Gary, and Cheminat (1989) suggested a shift from the hemiketal and chalcone forms towards the red-colored flavylium cations due to the complexation of the latter by co-pigments. Thus, co-pigmentation may increase the overall ratio of color-intense and stable flavylium cations at the expense of the pale hemiketal or chalcone forms, ultimately leading to notable intensification of color and increased stability (Pina, Melo, Laia, Parola, & Lima, 2012). In addition, the delocalized electrons of the chromophore may interact with those of the neighboring phenolic co-pigments, potentially leading to a shift of the color hue (Brouillard & Dangles, 1994). Several organic acids, phenolic acids, flavonoids, and specific alkaloids such as caffeine have been described to act as effective co-pigments. Previously, chlorogenic acid, caffeic acid, ferulic acid, sinapic acid, rosmarinic acid as well as tannic acids have been shown to serve as co-pigments for pelargonidin 3-glucoside, indicating a maximum co-pigmentation effect from pH 3.2 to 4.0 (Davies & Mazza, 1993; Wilska-Jezka & Korzuchowska, 1996; Eiro & Heinonen, 2002). When performing co-pigmentation experiments with non-purified pelargonidin 3-glucoside-rich strawberry model solutions, the observed effect was higher than with purified pigments, leading to the conclusion that genuine co-pigments might synergistically enhance the effect (Mazza & Brouillard, 1990; Wilska-Jezka & Korzuchowska, 1996). Besides purified co-pigments, various plant extracts obtained from quince, rhubarb, chokeberry, and rose petals were found to fortify the color of strawberry products (Mollov, Mihalev, Shikov, Yoncheva, & Karagyozov, 2007; Wojdyło, Oszmiański, & Bober, 2008; Shikov, Kammerer, Mihalev, Mollov, & Carle, 2008). In the present study, a phenolic extract of dried mango peels (*Mangifera indica* L. cv. Kaew) was shown to substantially modulate the color of anthocyanin-rich strawberry and red radish model solutions. To identify the responsible compounds, the mango extract was fractionated by preparative HPLC, and the co-pigmentation effect of the collected fractions was investigated. Besides pelargonidin derivatives from strawberry, those from red radish acylated with different hydroxycinnamic acids were used to provide insights into the structural prerequisites of anthocyanins to effectively interact with phenolic co-pigments. To date, mango peel extracts have not yet been proposed as color enhancers for anthocyanin solutions, although mango peels are inexpensively available in large amounts, being a by-product of industrial mango processing (Geerkens et al., 2013). The usage of this inexpensive source of co-pigments might help the industry to enhance the color stability of strawberry-based products such as jam, spreads, and smoothies. Moreover, it may foster the application of acylated anthocyanins, e.g. from red radish, as colorants. The high economic pressure on finding solutions to improve the stability of anthocyanins may be illustrated by our recent detection of fraudulent adulterations of anthocyanin-based coloring foodstuffs with an extraordinarily stable textile dye, namely Reactive Red 195 (Müller-Maatsch, Schweiggert, & Carle, 2016).

2. Materials and methods

2.1. Reagents

Citric acid, disodium hydrogen phosphate dihydrate, and methanol were purchased from VWR International (Leuven, Belgium). Acetone and formic acid were from Merck (Darmstadt, Germany). Mangiferin (⩾98% purity) and gallic acid (⩾98% purity) were obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Ultrapure water was used throughout and all chemicals were of analytical purity.

2.2. Strawberry and red radish plant material preparation

Individually quick frozen strawberries were purchased from Mainfrucht (Gochsheim, Germany), stored at −20 °C, and processed according to Buchweitz, Speth, Kammerer, and Carle (2013). Briefly, the defrosted (4 °C) fruits were pressed using a rack and cloth press (Wahler, Stuttgart, Germany). The filtrate obtained was subjected to enzymatic maceration (0.01% v/v, Fructozym Color, Erblöh, Geisenheim, Germany) at 35 °C for 1 h. Then, the enzymes were inactivated by heating for 2 min at 75 °C. The obtained macerate was stored at −30 °C until further use and will be termed "initial strawberry model solution" in the following. The initial strawberry model solution was thawed for 12 h at 7 °C and subsequently filtered through a 20 μm cellulose filter (Macherey-Nagel, Düren, Germany) prior to its use for the co-pigmentation experiments described below. Red radish extract powder (containing citric acid as acidity regulator) was provided by Diana Naturals SAS (Antrain, France). The powder was dissolved in citric acid sodium phosphate buffer at a ratio of 1:10 (w/v), obtaining the "initial red radish model solution" with a pH of 3.5.

2.3. Spectrophotometric determination of anthocyanins

The contents of monomeric anthocyanins were measured after 1:5 (v/v) dilution of the above mentioned initial model solutions by the pH-differential method, according to Giusti and Wrolstad (2003). Results were expressed as pelargonidin equivalents (PE), applying the molar absorptivity of pelargonidin 3-glucoside (15,600 L cm^{-1} mol^{-1}) and pelargonidin 3-(feruloyl)-diglucoside 5-glucoside (24,140 L cm^{-1} mol^{-1}) for strawberry and red radish, respectively. Anthocyanin degradation in the initial model solutions was described by the browning index, being the ratio of polymeric color and color density (Giusti & Wrolstad, 2003. Spectral measurements were performed on a Perkin Elmer UV/Vis Spectrophotometer Lambda 35 (Überlingen, Germany) using 1 cm path length disposable cuvettes. Quantification was performed at 497 nm and 510 nm for the initial strawberry and red radish model solution, respectively. Reference wavelengths (420 nm and 700 nm) were used as described previously (Giusti & Wrolstad, 2003).

2.4. HPLC-DAD-MS/MS analyses of individual anthocyanins

Prior to their HPLC separation, the samples were membrane-filtered (cellulose, 0.45 μm, Macherey-Nagel, Düren, Germany). The separation of individual anthocyanins in the initial strawberry model solution was carried out according to a previously published method (Holzwarth et al., 2012), while anthocyanins of the initial red radish model solution were separated using a newly developed method. Both separations were performed on an Agilent 1100 series HPLC (Agilent, Waldbronn, Germany), using aqueous formic acid (5%, v/v) and formic acid in methanol (5%, v/v) as eluents A and B, respectively. For the separation of anthocyanins from strawberry, the used column, solvent gradient, and flow rate were identical to those reported by Holzwarth et al. (2012).

Anthocyanins from the initial red radish model solution were separated at 40 °C on a Phenomenex (Torrance, CA, USA) Kinetex C_{18} column (250 × 4.6 mm i.d., 5 μm particle size, 100 Å pore size) combined with a guard column (4.0 × 2.0 mm i.d.) of the same material. The solvent gradient program was as follows: 25% B to