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Adulteration of anthocyanin- and betalain-based coloring foodstuffs with the textile dye 'Reactive Red 195' and its detection by spectrophotometric, chromatic and HPLC-PDA-MS/MS analyses

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ABSTRACT

A wide range of natural food colorants and coloring foodstuffs is available for the food industry to meet current costumer trends. Most natural pigments are more sensitive towards heat, light, and pH changes compared to their synthetic counterparts. Additionally, high dosages are often required to attain desired color hues and intensities. In this research article, we report on the broad and worldwide incidence of a fraudulent practice to overcome these disadvantages by adding a non-approved azo-dye preparation originating from the textile dye Reactive Red 195 to natural pigment extracts. Since the respective products and their derivatives have been widely distributed, we present a rapid method allowing the differentiation of the fraudulent azo-dye from *Hibiscus sabdariffa* (roselle) flower and *Beta vulgaris* (red beet) root extracts, the two coloring foodstuffs that are most frequently adulterated. Furthermore, detailed HPLC-PDA-MS/MS data is presented for the unambiguous identification of Reactive Red 195 and its derivatives.

1. Introduction

During the past two years, red beet- and roselle flower-based coloring foodstuffs appeared on worldwide markets, instantly gaining an enormous interest of the food industry due to their apparently exceptional processing and storage stability. These preparations or extracts solely containing natural plant pigments would be highly desired by the food industry producing savory meat and neutral dairy products. Most of the currently available, highly stable colorants permitted for these foods are either synthetic or animal origin (e.g. carmine). However, both synthetic dyes and carmine have clear disadvantages limiting their application. For instance, several synthetic food dyes such as carmoisine, allura red, tartrazine, and ponceau 4R have been related to an increased hyperactivity in 3-year-old and 8/9-year-old children (McCann et al., 2007), thus requiring a warning. Deriving from the insect *Dactylopius coccus* Costa, carmine was previously related with allergenic and toxicological risks, furthermore being nauseating to many customers. In addition, the presence of aluminium in carmine lakes has been frequently criticized, often hampering its use for coloring foods (Müller-Maatsch & Gras, 2016). In contrast, plant-based natural colorant preparations often comprise potential health benefits beyond their tinctorial strength, such as relevant levels of health-promoting constituents like pro-vitamin A and phenolic antioxidants (Delgado-Vargas, Jiménez, & Paredes-López, 2000; Stintzing & Carle, 2004). Since consumers are widely aware of these facts, they increasingly reject artificial and carmine-based colorants. Hence, the production of food, colored with expensive and less effective natural colorants, puts pressure on the market. Consequently, committing fraud is driven by considerable incentives (Carocho, Morales, & Ferreira, 2015).

The above mentioned specific coloring foodstuffs, labelled as red beet and roselle flower extracts, as well as further plant-based colorant preparations appeared on several markets within Europe and worldwide. Although doubts about the authenticity of such extracts have been raised, and adulterations with a synthetic food colorant have been suspected by several food companies and state agencies, the fraudulent pigment remained unknown. In the

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present communication, we elucidate the identity of the pigment in question. Besides presenting a newly developed HPLC-PDA-MS/MS method for its unambiguous detection, we present a methodology to distinguish the anthocyanins from roselle and the betalains from red beet from this fraudulent textile colorant by simple spectrophotometric, chromatic, thermal, and adsorptive methods.

2. Materials and methods

2.1. Reagents and materials

Methanol and formic acid were purchased from VWR International (Leuven, Belgium) and Merck (Darmstadt, Germany), respectively. Polyamide (particle size 0.05–0.16 mm, CAS 63428-84-2) was purchased from Carl Roth (Karlsruhe, Germany). Ultrapure water was used throughout and all chemicals were of analytical purity. The textile dye Reactive Red 195 was provided by Yorkshire Farben (Krefeld, Germany). Dried roselle flowers (*Hibiscus sabdariffa* L.) were provided by Martin Bauer (Vestenbergsgreuth, Germany) and red beet extract (*Beta vulgaris* L. ssp. *vulgaris* var. *conditiva* Alef.) by Colin Ingrédients (Mittelhausen, France). Three adulterated preparations from Turkey (Sample 1), France (Sample 2), and Germany (Sample 3) were included in this study, being obtained from undisclosed providers.

2.2. Sample preparation and chromatic analyses

Red beet extract, roselle flowers, and Reactive Red 195 were dissolved in citric acid sodium phosphate buffer (McIlvaine buffer) at seven different pH values (pH 2, 3, 4, 5, 6, 7, and 8) and filtered using cellulose filter paper (Whatman grade 2) prior to further analyses (Herbach, Maier, Stintzing, & Carle, 2007; Malien-Aubert, Dangles, & Amiot, 2001). The solutions were diluted with the respective buffer to adjust an absorbance of 1.0 ± 0.1 AU at the wavelength of the absorption maximum, further named standard solutions. CIE-L*a*b* values of the standard solutions were recorded in transmission by an UltraScan Vis spectrophotometer (Standard illumination D65/10°, HunterLab, Murnau, Germany) in 1 cm path length disposable cuvettes. All treatments and measurements were carried out in duplicate.

2.3. Adsorption on polyamide

The adsorption experiment for the separation of synthetic and natural pigments followed the instructions of Lehmann and Hahn (1968) with slight modifications. Aliquots of 10 mL of the respective standard solutions of red beet, roselle flowers, and Reactive Red 195 (all at pH 4) were combined with 1 g polyamide. After adsorption of the pigments, the adsorbent was transferred into glass columns and washed with 50 mL water, 50 mL acetone, and 50 mL methanol containing 10% ammonia in the respective order. All eluates were collected and visually inspected. Adsorption experiments were carried out in duplicate.

2.4. Thermal degradation

Standard solutions diluted in McIlvaine buffer (pH 3) were subjected to a heat treatment at 80 °C in sealed glass tubes (Pyrex), as previously described by Dyrby, Westergaard, and Stapelfeldt (2001). Samples were taken at 30 and 60 min and subsequently cooled to room temperature (25 °C). UV/Vis absorption spectra from 200 to 700 nm were recorded prior to and after heating using a Perkin Elmer UV/Vis spectrophotometer Lambda 35 (Überlingen, Germany) and 1 cm path length disposable cuvettes. All treatments and measurements were carried out in duplicate.

2.5. HPLC-PDA-MS/MS analyses of individual compounds

The developed HPLC-PDA-MS/MS method was based on a previously reported HPLC method for the separation of anthocyanins and betalains (Stintzing, Trichterborn, & Carle, 2006) with several modifications. Prior to the separation, the aqueous extracts were membrane-filtered (cellulose, 0.45 μm, Macherey-Nagel, Düren, Germany). Separation of individual compounds in the aqueous extracts was performed on an Agilent 1100 series HPLC (Agilent, Waldbronn, Germany), using aqueous formic acid (1%, v/v) and formic acid in methanol (1%, v/v) as eluents A and B, respectively. Pigments were separated at 40 °C on a Phenomenex (Torrance, CA, USA) Synergi Hydro-RP 80A C_{18} column (150 × 3.0 mm i.d., 4 μm particle size, 80 Å pore size) combined with a guard column (4.0 × 2.0 mm i.d.) of the same material. The gradient program was as follows: 0% B to 40% B (20 min), 40% B to 100% B (5 min), 100% B isocratic (5 min), 100% B to 0% B (1 min), 0% B isocratic (4 min). Total run time was 35 min at a flow rate of 0.6 mL/min. Pigments were monitored at 520 nm, additionally recording UV/Vis spectra from 200 to 600 nm. For mass spectrometric analyses, the HPLC system described above was coupled on-line to a Bruker (Bremen, Germany) model Esquire 3000 + ion trap mass spectrometer applying an electrospray ionization (ESI) interface. Positive ion mass spectra of the column eluate were recorded in the range of m/z 50-2000 at a scan speed of 13,000 m/z s^{-1}. Nitrogen was used both as drying gas at a flow rate of 9 L/min and as nebulizing gas at a pressure of 40 psi.

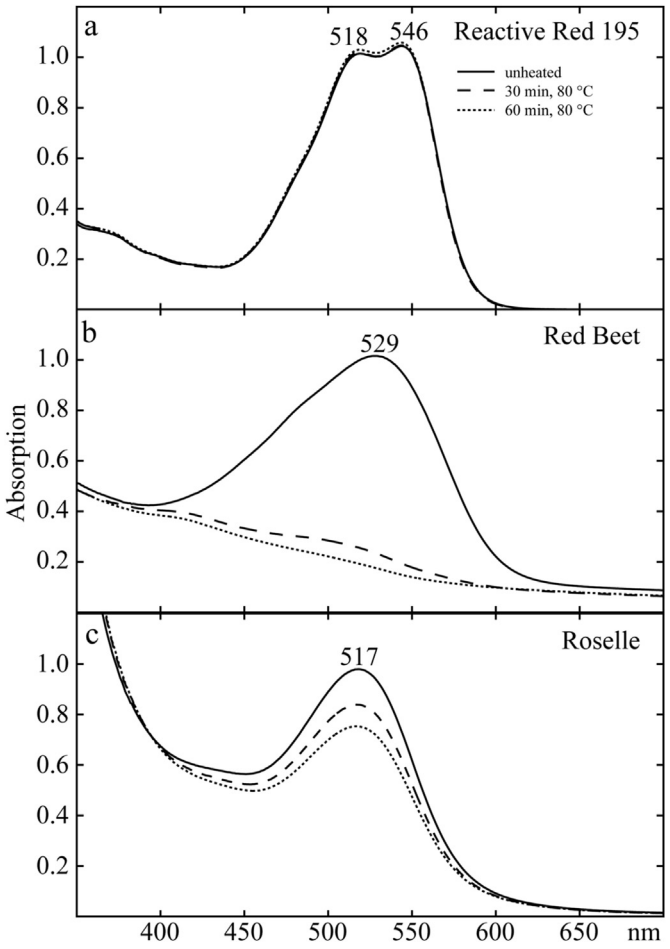

Fig. 1. Visible absorption spectra of aqueous solutions (pH 3) of Reactive Red 195 (a), red beet extract (b), and roselle extract (c) monitored before heating (solid line) as well as after 30 min (dashed line), and 60 min (dotted line) of heating at 80 °C.