



Ultra-high-performance liquid chromatography/tandem high-resolution mass spectrometry analysis of sixteen red beverages containing carminic acid: Identification of degradation products by using principal component analysis/discriminant analysis



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ABSTRACT

The study investigates the sunlight photodegradation process of carminic acid, a natural red colourant used in beverages. For this purpose, both carminic acid aqueous standard solutions and sixteen different commercial beverages, ten containing carminic acid and six containing E120 dye, were subjected to photoirradiation. The results show different patterns of degradation, not only between the standard solutions and the beverages, but also from beverage to beverage. Due to the different beverage recipes, unpredictable reactions take place between the dye and the other ingredients.

To identify the dye degradation products in a very complex scenario, a methodology was used, based on the combined use of principal component analysis with discriminant analysis and ultra-high-performance liquid chromatography coupled with tandem high resolution mass spectrometry. The methodology is unaffected by beverage composition and allows the degradation products of carminic acid dye to be identified for each beverage.

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1. Introduction

The final colour of food and drinks greatly affects consumer choice. Many colourants are therefore added to commercial products to avoid colour losses caused by exposure to light, temperature and/or moisture, to correct natural colour variation and to enhance weak colours (Gloria, 2004). A wide variety of natural and synthetic colourants is available for these purposes.

Natural colours derive from natural sources like vegetables, minerals and animals and include for example carotenoids, caramel, turmeric, annatto and carminic acid (CA). Synthetic dyes are generally preferred to natural ones, since they are more stable, give the expected colour to food and drinks, guarantee a better uniformity and are generally less expensive. While for a long time they have also been considered safer because more stable and inert, nowadays many reports are denouncing their potential negative influence on health. Synthetic dyes may contain intermediate or synthesis side products, often much more dangerous than the principal product and can undergo degradation reactions with

formation of potentially toxic products. In particular, azo-dyes can be decomposed by natural intestinal flora to aromatic amines, which can cause headaches, asthma and allergic reactions (Shimada, Kano, Sasaki, Sato, & Tsudua, 2010).

The present legislation lists the colourants permitted in food and drinks and gives the maximum amounts allowed, but it does not contain information on dye inertness towards sunlight action, thermal treatments and possible cross-reactions with other compounds also present in food and drinks (European Commission Directive 2008/128/EC, 2008).

Recently, some studies have dealt with dye thermal instability during process treatments or conservation at inappropriate conditions (Castellar, Obón, Alacid, & Fernández-López, 2003; Jenshi Roobha, Saravanakumar, Aravindhan, & Suganya devi, 2011; Ma et al., 2012; Vlase, Vlase, Modra, & Doca, 2007). Recent studies from our laboratory showed that some synthetic dyes contained in beverages undergo degradation reactions induced by sunlight irradiation (Gosetti et al., 2004, 2008, 2012, 2013; Gosetti, Gianotti, Polati, & Gennaro, 2005). Degradation was clearly proved by colour variations, which were observed by the naked eye. These studies showed that under sunlight irradiation, some dyes are quite stable in aqueous solution but undergo degradation when

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present in beverages. The effect of sun irradiation on beverages has not been considered sufficiently, although it is not a trivial problem, as beverages are often exposed to sun irradiation during transport and storage or on the shelf. The authors studied the degradation promoted by sunlight irradiation of Brilliant Blue FCF, Sunset Yellow FCF, Carmoisine and Allura Red (Gianotti, Angioi, Gosetti, Marengo, & Gennaro, 2005; Gosetti, Gianotti, Mazzucco, Polati, & Gennaro, 2007; Gosetti et al., 2004, 2005, 2008, 2012, 2013). As a consequence of degradation, a plethora of compounds might form, some of which are potentially dangerous for consumer health, as in the case of aromatic amines identified in the degradation of Allura Red (Gosetti et al., 2012, 2013).

It has also been shown that dye degradation pathways often involve other ingredients contained in drinks, such as additives, stabilizers and preservatives, resulting in cross-reactions that can lead to potentially toxic species (Gianotti et al., 2005; Gosetti et al., 2004, 2005, 2007, 2008, 2012, 2013). The beverage recipe often exerts its effect on the formation of degradation products that are generally different from those formed in simplified model solutions prepared to simulate the beverages (Gosetti et al., 2012, 2013). After the well-known Southampton study (McCann et al., 2007), which showed a link between hyperactivity in children and six food dyes, and according to Regulation (EC) No. 1333/2008 on food additives, the labelling of food commercial products that contain the azo dyes, listed in Annex V of the above reported legislation, must include the sentence: “the dye (specify name or E number) may have an adverse effect on the activity and attention in children” (European Community Regulation (EC) No. 1333/2008, 2008). After this regulation, several beverage manufacturers preferred the use of natural colours.

In the case of red dyes, in place of E122, E124 and E129, extensive use of E120, which is extracted from cochineal insects, has occurred. The label E120 comprises the whole family of cochineal Red, such as CA, carmine, amino-carmine, etc. Cochineal is the crude material derived from the dried bodies of female cactus insects, CA is the active colour ingredient of cochineal, and carmine is a metal coordination complex involving aluminium and CA (Dapson, 2007). An insoluble pigment sold as carmine is carmine, as just defined, precipitated with calcium or other metal ions (Dapson, 2007). Aminocarmine acid, or acid-stable carmine is a derivative of CA that imparts a deep red colour to acidic food (Dapson, 2007). The problem of dye impurity and variability is due to different causes, such as geopolitical history, agricultural practices and divergent manufacturing methods, as reported in detail in Dapson (2007).

The chemical structure of CA consists of a core anthraquinone structure linked to a glucose sugar unit. Its safety was evaluated by the Scientific Committee for Food in 1984 (Reports from the Scientific Committee for Food (14th series), 1983) and is currently being re-evaluated by the European Food Safety Authority (EFSA). The re-evaluation shall be completed by 31 December 2015 and also cover the aspects concerning children health and possible allergic reactions.

The stability of natural colourants to irradiation, pH and temperature is quite poor with respect to synthetic dyes (Ghidouche, Rey, Michel, & Galaffu, 2013; Sari, Wijaya, Sajuthi, & Supratman, 2012; Wissgott & Bortlik, 1996) and so natural colourants can undergo degradation in food and beverages (Socaciu, 2008). In the present article, the effect of sun irradiation on CA dye in aqueous solution and in sixteen different beverages has been investigated, to mimic the action of uncontrolled sunlight as it can be encountered during the transport, distribution and storage of beverages and to identify the photodegradation products. As the formation of degradation products strongly depends on the other ingredients present in the beverage, a chemometric method (based on principal component analysis coupled with discriminant

analysis, PCA-DA), independent of the beverage composition, is proposed here.

2. Materials and methods

2.1. Reagents

Methanol (LC-MS Ultra CHROMASOLV, >99.9%), 2-propanol (LC-MS Ultra CHROMASOLV, >99.9%), CA (>96%), acetic acid (eluent additive for LC-MS, >99.9%) were purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure water was obtained through a Millipore Milli-Q system (Millipore, Billerica, MA).

The 1000.000 mg L⁻¹ stock standard solution of CA was prepared in ultrapure water, diluted as required in ultrapure water and preserved at 4 °C in dark glass vials.

The ten beverages containing CA and six containing E120 dye were bought in supermarkets. Their composition, as declared on the labels, is different (Table 1 of Supplementary Materials). Eleven beverages were contained in polyethylene terephthalate bottles, whereas five were contained in glass bottles. The beverages were degassed by sonication, if necessary, and filtered before UHPLC-MS/MS analysis through 0.45 µm and then 0.2 µm PTFE filters (VWR International, Darmstadt, Germany).

2.2. Apparatus

To simulate sunlight irradiation, a CoFoMeGra solar box 3000e (Milan, Italy) was used, that assures, with respect to natural sunlight, continuous availability and constant intensity. The irradiation density of the xenon lamp (λ range 280–800 nm) was set at 600 W m⁻² and the temperature at 35 °C. An outdoor UV glass filter was employed. The instrumental conditions were chosen on the basis of the sun average irradiation and temperature in the period May–September of Alessandria city, as monitored by the meteorological station of our Department.

The UHPLC-MS/MS analyses were performed by a UHPLC 1290 Infinity (Agilent, Waldbronn, Germany) system that included a 1290 Infinity binary pump with solvent selection valves and a programmable autosampler. The system was interfaced with a 5600 TripleTOF™ system (AB Sciex, Concord, Canada) equipped with DuoSpray™ Ion Source. The 5600 TripleTOF™ data were processed by PeakView™ 1.2.0.3 software (AB Sciex, Concord, Canada), exploring and interpreting mass spectral data with special tools for processing accurate mass data and structural elucidation. Data analysis and graphical representations were performed by MarkerView™ 1.2.1.1 (AB Sciex, Concord, Canada).

2.3. Chromatographic and mass spectrometry conditions

The stationary phase was an Acquity BEH phenyl column (2.1 × 100 mm, 1.7 µm; Waters, Milford, MA). The mobile phase was a mixture of a water/acetic acid 99/1 (v/v) solution (A) and a methanol/isopropanol/acetic acid 97/2/1 (v/v/v) solution (B), eluting at flow-rate of 0.350 mL min⁻¹ using the following gradient conditions: 0.0–0.7 min 5% B, 10–11 min 100% B, 11.1–15.0 min 5% B. The injection volume was 2.0 µL. Oven temperature was set at 60 °C.

The DuoSpray™ ion source worked in negative ion (NI) mode. The instrumental parameters were set as follows: curtain gas (N₂) at 25 psig, nebuliser gas GS1 and GS2 at 55 and 50 psig, respectively, desolvation temperature (TEM) at 500 °C, collision activated dissociation gas (CAD) at 6 units of the arbitrary scale of the instrument and ionspray floating voltage (ISFV) at –4500 V. As concerns the compound parameters, during TOF MS experiments the declustering potential (DP) and the collision energy (CE) were set at –80

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