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A stepwise approach for the detection of carminic acid in saffron with regard to religious food certification

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7-β-D-Glucopyranosyl-9,10-dihydro-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-2-anthracenecarboxylic acid (Carminic acid, PubChem CID: 10255083)

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ABSTRACT

The stepwise approach takes advantage of simple, versatile, low-cost screening tools that can be applied to several posts of the saffron trade chain to specifically detect adulteration with carminic acid (CA). This natural dye is of insect origin and should not be present in Kosher and Halal foods such as saffron. For gross adulteration levels (>25.0%, w/w) reaction with diphenylamine-sulfuric acid was found adequate to indicate the presence of extraneous matter but not its identity. FT-IR analysis of the dry material combined with chemometrics served to rapidly sort out samples containing >10.0% CA without any sample pretreatment except grinding. Aqueous extracts prepared according to ISO 3632-2 were then examined by tristimulus colorimetry and derivative UV–Vis spectrometry to detect adulteration down to the level of 2.0% (w/w). Determination of CA down to 0.2%, w/w was achieved by RP-HPLC-DAD using aqueous acetonitrile elution solvent (pH = 2.8).

1. Introduction

The market for foods with religious certification (mainly kosher and halal) is constantly growing and expands from the Middle East, North Africa and South Asia to the Europe and the USA. This is not only the consequence of ethnic mixing due to mass population movement but it is also related to the recognition of certain quality characteristics of the certified products. The dietary guidelines for Jews and Muslims are described in their respective holy books (Torah and Koran) where the acceptable type(s), preparation or processing of food to be consumed as well as the non-acceptable ingredients or procedures involved can be found. Kosher and Halal certification of internationally traded products is provided by expert bodies that are authorized to check for the absence of non-kosher (treife) or haram ingredients that may derive from non-certified animal (mammals, birds), insects or even plants (grapes) (Al-Mazeedi, Regenstein, & Riaz, 2013). Given the technological development in the food processing area, authorities need to continuously update their control measures in order to detect prohibited constituents or procedures and furthermore to make jurisdictions for new generation products such as genetically engineered or irradiated foods.

Cochineal, a deep-purple powder derived from the dried, crushed bodies of the female insect *Dactylopius coccus* by extraction with water, alcohol or their mixtures is a characteristic case of a non-kosher and haram ingredient. This material is the source of carminic acid (CA, Fig. 1). The latter is a natural colorant that has been widely appreciated in diverse applications (cosmetics, textiles, plastics, pharmaceuticals, food and beverages) for its stability against heat and oxygen-induced decomposition (Müller-Maatsch & Gras, 2016). Stability under light exposure is debatable (Gosetti et al., 2015; Gosetti, Chiuminatto, Mazzucco, Mastroianni, & Marengo, 2015). Along with its salts (ammonium, calcium, sodium, potassium) and aluminum lakes (carmines), CA is permitted as a food additive not only in the European Union (as E120) but also in many other countries (C.I. No 75470). It is most often used to enhance the color of meat, dairy and confectionery products, where it can be added at variable levels depending on the application (EC, 2011; Müller-Maatsch & Gras, 2016). Due to the diverse range of foodstuffs where it can be added, there are several reported methods of analysis that differ mainly in the sample

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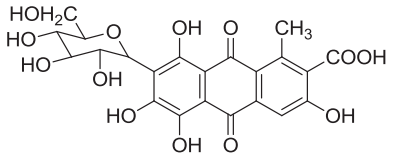

Fig. 1. Chemical structure of carminic acid (CA).

preparation step. Extraction conditions generally involve acid hydrolysis with or without preconcentration (Scotter, 2011). Most research has been carried out using HPLC with UV–Vis or fluorescence detection (Carvalho & Collins, 1997; González, Méndez, Carnero, Lobo, & Afonso, 2002; Jalón, Peña, & Rivas, 1989; Lancaster & Lawrence, 1996; Lim, Choi, Song, & Kim, 2014; Merino, Edberg, & Tidriks, 1997) although other methods based on chemiluminescence (Mokhtari, Keyvanfard, & Emami, 2015), differential pulse polarography (Yilmaz, Ergun, & Yilmaz, 2014), nuclear magnetic resonance spectroscopy (NMR) (Sugimoto et al., 2010), spectrophotometry (Samari, Hemmateenejad, & Shamsipur, 2010) or stripping voltammetry (Alghamdi, Alshammery, Abdalla, & Alghamdi, 2009) have been also proposed recently. Apart from religious laws that prohibit the consumption of insect-based foods and ethical constraints due to the animal slaughter, health issues related mainly with allergic reactions and aluminum exposure have recently raised serious public concerns against the use of this particular additive (Müller-Maatsch & Gras, 2016). The European Food Safety Authority (EFSA) panel on *Food Additives and Nutrient Sources Added to Food* that re-evaluated the safety of cochineal and its products suggested that the main food contributors to the exposure of adults and elderly people to E120 are herbs, spices and seasonings (EFSA, 2015). Interestingly, only a few spices may impart color to foods, with paprika, turmeric and saffron being the most important. These plant materials do not contain cochineal and related products unless the latter have been added illegally to enhance the appearance or coloring strength upon use.

Saffron, the most expensive spice in the world that consists of the red stigmas of the flower of the plant *Crocus sativus* L., is particularly popular in Middle Eastern countries, both producing (Iran, Afghanistan, India) and consuming [Kingdom of Saudi Arabia (KSA), The Emirates]. Quality specifications of this spice that are described in the ISO 3632 trade standard (ISO 3632-1, 2011) preclude any kind of artificial coloring. Still, the product is quite susceptible to fraudulent practices of economic motivation (Everstine, Spink, & Kennedy, 2013). The use of azo-dyes or natural dyes (e.g., turmeric, cochineal) for enhancement of the appearance or of the coloring strength of saffron extracts is considered as one of the most frequent types of fraud (Moradi-Khatoonabadi, Amirpour, & AkbariAzam, 2015; Sanchez, Maggi, Carmona, & Alonso, 2011).

The combat of the illegal use of carminic acid in kosher and halal foods through the potential use of adulterated saffron was the objective of this study that was undertaken within the COST ACTION FA1101 (Saffron-OMICS) frame (http://www.saffronomics.org). UV–Vis spectrophotometric detection of relatively high amounts (15.0%, w/w) of this dye in saffron has been reported in the past (Carmona, Carrion, Zalacain, & Alonso, 2004). At that time, detection of lower amounts of CA could not be achieved even after a tedious pre-concentration stage, due to irreversible retention of the dye on polyamide SPE cartridges (Zalacain et al., 2005). The same drawback appears when the ISO 3632-suggested methods for the detection of illegal artificial coloring of saffron are considered. The latter methods, based on chromatographic separation of exogenous colorants after their pre-concentration on polyamide cartridges are only applicable to the detection and quantification of selected acidic water-soluble synthetic colorants (e.g. azo-colorants) at the concentration levels listed therein, but not to carminic acid (ISO 3632-2, 2010). Since there is no provision in the current ISO standard for the detection of CA, it is not possible to satisfy those buyers who would possibly request additional information, e.g., religious certification directly or on a label on each shipping container (ISO 3632-1, 2011). In the present study, a stepwise approach was developed to detect CA in saffron at various addition levels. The workflow started with high addition levels corresponding to gross adulteration (75.0%, w/w) and ended up with the estimation of very low concentrations of the potential adulterant. An array of different analytical tools was used to cover the needs of the various levels of adulteration, involving macroscopic examination, colorimetric tests, FT-IR fingerprinting, CIEL*-a*b* tristimulus colorimetry, derivative UV–Vis spectrophotometry and RP-HPLC-DAD. Our approach is expected to be considered in a future revision of the relevant ISO standard.

2. Materials and methods

2.1. Saffron samples

The saffron samples used were: Greek PDO "Krokos Kozanis ($n = 7$), Spanish PDO "Azafrán de La Mancha" ($n = 2$), Italian PDO "Zafferano di Sardegna" ($n = 1$) and three ones from Gonabad, Torbat-e Heydariyeh and Ghaen regions (Khorasan, Iran). All of them were obtained directly from the producers to guarantee origin and authenticity.

2.2. Standards, reagents and solvents

Carminic acid (*ca.* 90.0%), amaranth (*ca.* 80.0%), erythrosine B (solvent Red 51) and FT-IR grade potassium bromide (KBr >99.0%) were from Sigma-Aldrich Chemie (Steinhem, Germany). Diphenylamine (p.a.) was from Schering-Kahlbaum A.G. (Berlin, Germany). Tartrazine (≥85.0%) was from Riedel-de Haën (Seelze, Germany). The solvents used were of the maximum required purity. Acetonitrile (Chem Lab, Zedelgen, Belgium) and acetic acid (Fluka Chemie, Buchs, Switzerland) used were HPLC grade. Ultra-high-purity water (pH = 5.9) was produced using a SG 2002 v.1.01 system (Barsbüttel, Germany). All other reagents used were of analytical grade.

2.3. Preparation of artificially adulterated saffron

Two saffron samples belonging to category I according to ISO 3632-1 quality specifications were used as the base for adulteration as follows: a certain amount of ground saffron stigmas and CA (total weight of 0.1 g) was transferred to Eppendorf tubes and homogenized by vortex. Twelve (12) different weight ratios of CA to saffron (0.2, 0.5, 1.0, 2.0, 3.0, 5.0, 10.0, 15.0, 20.0, 25.0, 50.0 and 75.0%, w/w) and also two (2) weight ratios of amaranth, erythrosine and tartrazine to saffron (15.0 and 20.0%, w/w) were prepared in the same way.

2.4. Examination of the dry material

2.4.1. FT-IR analysis

Each test sample was mixed with KBr at a 1:180 ratio (w/w), homogenized and the mixture (0.181 g) was then compressed under a pressure of *ca.* 200 MPa for 1 min to form a thin KBr disc (in triplicate). FT-IR spectra were obtained using a Shimadzu IRAffinity-1 (Shimadzu Europa GmbH, Duisburg, Germany) spectrometer that operates in the region 4000–400 cm^{-1} at conditioned room temperature (25 °C), with a total of 64 scans and 4 cm^{-1}