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Detection of saffron adulteration with gardenia extracts through the determination of geniposide by liquid chromatography–mass spectrometry

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ABSTRACT

A new and sophisticated saffron adulteration method with gardenia was recently discovered in the European saffron market. In this work, an analytical methodology using liquid chromatography-(quadrupole-time of flight)-mass spectrometry has been developed for the detection of the adulteration of saffron samples with gardenia through the determination of geniposide as an adulteration marker. A fused-core C18 column was employed using an isocratic elution with water:acetonitrile (85:15 v/v) containing 0.1% formic acid. After optimization of the mass spectrometry conditions, the analytical characteristics related to the determination of geniposide in negative electrospray ionization mode were evaluated. Then, it was possible to detect up to 10 ng/mL geniposide after a dilution step of 50-fold of the saffron extract (LOD of 41.7 μg of geniposide per gram of sample analysed (i.e up to 0.004%)). The developed LC–MS methodology was applied to the analysis of different authentic and suspicious saffron samples.

1. Introduction

Food safety involves no risk to consumer health. However, this property is impossible to ensure when food adulteration by food producers, manufacturers, processors, distributors, or retailers occurs because in any case it results in a change of the identity and/or purity of the original food using physical or chemical means. One of the risks gaining attention in food safety is the possibility of food poisoning when adulterated with chemical extracts (Moore et al., 2012).

Saffron has been described as one of the most commonly adulterated food ingredients due to its high price and limited quality assurance (Moore et al., 2012; Petrakis et al., 2015). It is produced from the dried stigmas of *Crocus sativus* L. being considered as one of the most expensive spice in the world because of the direct labor required for growing, harvesting and handling as well as its limited production. This spice has been employed for a long time as a flavoring and colorant in food preparation; however, it is also known for a wide range of health benefits, such as offering some protection against heart disease and cancer, and having a high potential as a memory enhancer (Rios et al., 1996; Karimi et al., 2001; Abdullaev, 2002; Hosseinzadeh and Younesi, 2002; Melnyk et al., 2010; Papandreau et al., 2011). In addition to its three main secondary metabolites, crocins (crocin and its derivatives are responsible for coloring strength), picrocrocin (responsible for the saffron taste), and safranal (responsible for the flavor), saffron also contains flavonoids, proteins, sugars, vitamins, amino acids, mineral materials, gums, and other chemical compounds (Winterhalter and Straubinger, 2000; USDA Food composition Database).

Common fraudulent practices aimed to saffron adulteration include the addition of different plant materials with similar color and morphology in order to increase its weight and/or to improve its colour properties (or enhance its colour when foreign matter has been added) using natural or synthetic components (Melnyk et al., 2010).

To certificate saffron quality in the international trade market, it is classified by its aroma, flavor, and color strength using the ISO 3632-1: 2011 method, which combines spectrophotometric measurements of picrocrocin and safranal, and chromatographic profiles of pigments (crocins) and apolar dyes that can be toxic (as

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Sudan dyes) (ISO 3632-1; ISO 3632-2). Nevertheless, this legislation is being revised due to the fact that it is not able to detect saffron adulterations by plant foreign matter with similar color and morphology. In fact, it has recently been demonstrated that saffron adulterants (safflower, marigold or turmeric) up to 20% (w/w) were not detected by the ISO normative (Sabatino et al., 2011).

Several analytical methodologies have been developed to detect plants adulterants in saffron samples. Chromatographic (Sampathu et al., 1984; Alonso et al., 1998; Lozano et al., 1999; Haghighi et al., 2007; Sabatino et al., 2011) and molecular techniques (Ma et al., 2001; Javanmardi et al., 2011; Marieschi et al., 2012; Babaei et al., 2014; Torelli et al., 2014) have been employed with this purpose and have originated encouraging results. For instance, the use of DNA markers enabled the detection of low amounts (up to 1%) of various materials including safflower and turmeric (Javanmardi et al., 2011; Marieschi et al., 2012). Some non-targeted metabolomic studies have also been carried out to discover new authenticity saffron markers but the proposed markers do not allow the identification of the type of the plant used for saffron adulteration (Yilmaz et al., 2010; Cagliani et al., 2015; Guijarro-Díez et al., 2015).

None of the above-mentioned methods enabled to detect saffron adulterations with chemical extracts of *Gardenia jasminoides Ellis* L. (gardenia), a new and more sophisticated type of adulteration than those previously used and difficult to detect because this plant shares with saffron a large number of crocins and flavonoids (responsible for the yellow color) (Pfister et al., 1996; Van Calsteren et al., 1997; Carmona et al., 2006;). In fact, a large number of saffron adulterations using gardenia extracts has been discovered in the European market. Due to the morphological differences of gardenia and saffron stigmas, adulteration mainly occurs when saffron is in powder form since gardenia extract can be more easily hidden (Guijarro-Díez et al., 2015). Recently, a metabolite fingerprinting strategy based on the use of NMR (using chemometric strategies for classification of samples) has shown to be able to differentiate authentic saffron samples from saffron samples adulterated with 20% of gardenia, turmeric, safflower, and saffron stamens (Petrakis et al., 2015). However, there is an ongoing demand for the development of rapid, simple and sensitive analytical methodologies enabling the detection of saffron adulteration with low amounts of plant adulterants.

Several analytical methods were proposed to find out the fingerprint of Gardenia fruit including HPLC and GC (Yan et al., 2006; Zhou et al., 2010; Ding et al., 2010; Yang et al., 2011; Li et al., 2015; Han et al., 2015). The principal active constituents of gardenia are the iridoid glycosides: geniposide, gardenoside, genipin-1-β-gentiobioside, geniposidic acid, acetilgeniposide, and gardoside (Wang et al., 2004). Among them, geniposide has been recognized as the major iridoid component. Carmona et al. described the presence of geniposide in gardenia and its lack in saffron when they studied differences in the chromatographic profile of both samples (Carmona et al., 2006). This fact is of high relevance since it points out the possibility of using this compound as a marker of adulteration of saffron with cheaper gardenia extracts. Even though different HPLC and CE methodologies have been developed to determine geniposide in the Gardenia fruit (Tsai et al., 2002; He et al., 2006; Hou et al., 2007; Bergonzi et al., 2012; Gao et al., 2013; Coran et al., 2014; Lee et al., 2014; Wang et al., 2015), no studies have been reported until now on the determination of geniposide in saffron samples which could enable to propose geniposide as a novel adulteration marker of saffron with gardenia extracts.

The aim of this work was to develop a sensitive LC–MS methodology enabling the determination of geniposide as adulteration marker of saffron with gardenia extracts which could be a powerful tool to be applied in the routine quality control to detect adulterations of saffron with gardenia extracts.

2. Materials and methods

2.1. Chemicals and samples

Acetonitrile, ethanol, and formic acid of HPLC grade were purchased from Scharlab (Barcelona, Spain), while water was purified through a Milli-Q system (Millipore, Bedford, MA). Geniposide standard (purity ≥98%), sodium tetraborate, ammonium formate, and ammonium acetate were obtained from Sigma (St. Louis, MO, USA).

A total of eight samples (stigmas and powered) of authentic saffron from Iran and Spain were provided by "Carmencita" (Alicante, Spain). All these samples were of Commercial Category I and their quality and authenticity were checked according to ISO 3632. The low number of these samples can be explained by the fact that they were supplied with the guarantee of their origin and authenticity (lack of adulteration). One powdered gardenia extract (with an estimated geniposide content of 37.5 mg/g extract) and ten saffron samples (stigmas and powered) suspected of being adulterated according to the criteria of the market based on their low cost and/or questionable origin were also provided by "Carmencita" company.

2.2. Standard and sample preparation

A stock standard solution of geniposide was prepared by dissolving it in acetonitrile up to a final concentration of 1 mg/mL. This solution was stored at 4 °C and different aliquots were diluted in Milli-Q water to get solutions with different concentrations of geniposide.

Saffron stigmas were finely ground in a mortar with stainless balls Ultra Turrax (IKA, Staufen, Germany) for 2 min 0.3 g of ground or powdered saffron samples and gardenia extract were extracted under optimized conditions with 25 mL of ethanol:borate buffer at pH 9.0 (50:50 v/v) by using an ultrasonic-assisted solid-liquid extraction for 15 min at room temperature. After centrifugation (15 min, 4000*g* and 25 °C) the supernatant fraction was diluted 1/50 with Milli-Q water and 4 mL of this solution were ultra-filtered through a 3 kDa cut-off filter (Amicon Ultra Filters, Merck, Darmstadt, Germany) to remove carbohydrates and proteins. These solutions were stored at 4 °C and warmed at room temperature before use.

2.3. LC–MS analysis

LC analysis were carried out in a 1100 series LC system (Agilent Technologies, Palo Alto, CA, USA) coupled to a mass spectrometer via an orthogonal electrospray ionization source (ESI) with Jet Stream thermal focusing technology (6530 series, Agilent Technologies, Palo Alto, CA, USA). MS detection was performed in a quadrupole time offlight (QTOF) series 6530 (Agilent Technologies, Palo Alto, CA, USA). MS control, data acquisition, and data analysis were performed by using the Agilent Mass Hunter software (B.040.00).

Two different columns supplied by Sigma (Sigma, St. Louis, MO, USA), namely an Ascentis Express Fused-core C18 column and an Ascentis Express Fused-core Cyano column, both 100 mm × 2.1 mm, fused-core® particles with 0.5 μm thick porous shell and 2.7 μm particle size, were tested. Both separation columns were protected using C18 and cyano pre-columns, respectively (Ascentis Express guard column (5 × 2.1 mm) from Sigma).

LC analyses with gradient elution were carried out by using a mobile phase of water containing 0.1% formic acid (solvent A) and