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Comparative evaluation of an ISO 3632 method and an HPLC-DAD method for safranal quantity determination in saffron

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ABSTRACT

The aim of this work was a comparison of the ISO 3632 (2011) method and an HPLC-DAD method for safranal quantity determination in saffron. Samples from different origins were analysed by UV–vis according to ISO 3632 (2011) and by HPLC-DAD. Both methods were compared, and there was no correlation between the safranal content obtained by UV–vis and HPLC-DAD. An over-estimation in the UV–vis experiment was observed, which was related to the *cis*-crocetin esters content, as well as other compounds. The results demonstrated that there was no relationship between ISO quality categories and safranal content using HPLC-DAD. Therefore, HPLC-DAD might be preferable to UV–vis for determining the safranal content and the classification of saffron for commercial purposes. In addition, HPLC-DAD was adequate for determining the three foremost parameters that define the quality of saffron (crocetin esters, picrocrocin and safranal); therefore, this approach could be included in the ISO 3632 method (2011).

1. Introduction

Saffron, one of the most expensive spices used in the food industry, comes from the dried stigmas of *Crocus sativus* L. This spice is highly valued for colour, taste and aroma. Although saffron's colour is highly appreciated, its unmistakable aroma and pleasant bitter taste are what differentiate saffron from other natural or synthetic colorants, such as safflower, curcumin, gardenia and tartrazine.

With regard to the saffron aroma, safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) is the major compound (Carmona, Zalacain, Salinas, & Alonso, 2007; Maggi et al., 2009; Tarantilis & Polissiou, 1997). Safranal is soluble in apolar solvents and poorly soluble in polar ones (Maggi et al., 2011). García-Rodríguez et al. (2014) studied the safranal water solubility; these researchers determined that a saffron aqueous extract prepared according to the extraction method of ISO (2011) could be used for safranal determination.

Currently, the quality of saffron in international commercial agreements is determined according to ISO 3632 (2011), where the safranal content is measured by absorbance in an aqueous extract at 330 nm using UV–vis spectrophotometry. This determination is not the best since the crocetin esters (mainly *cis*-isomers) absorb at 330 nm (Carmona, Zalacain, Sanchez, Novella, & Alonso, 2006; Hadizadeh et al., 2007; Tarantilis, Polissiou, & Manfait, 1994), thereby interfering with the determination of safranal, although to the best of our knowledge, the extent of this interference has not been estimated to date.

Additionally, the ISO 3632 method (2011) does not classify saffron based on safranal content, the range values of safranal for the three different categories is the same (20–50); this means one of the main properties is not being valued. Saffron is only classified for its content of crocetin esters and picrocrocin.

Many methods have been developed for the determination of safranal, such as UV–vis spectrophotometry (Maggi et al., 2011; Sanchez et al., 2008), gas chromatography (GC) (Aliakbarzadeh, Sereshti, & Parastar, 2016; Amanpour, Sonmezdag, Kelebek, & Selli, 2015; Bononi, Milella, & Tateo, 2015; Cullere, San-Juan, & Cacho, 2011; Maggi et al., 2009) and liquid chromatography (HPLC) (García-Rodríguez et al., 2014; Lage & Cantrell, 2009; Rubert, Lacina, Zachariasova, & Hajslova, 2016; Tarantilis, Tsoupras, & Polissiou, 1995).

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HPLC is the most efficient analytical technique for the analysis of sensitive compounds in complex extracts of natural products (Alonso, Salinas, Garijo, & Sanchez-Fernandez, 2001). The three foremost parameters used to define the quality of saffron are colour, taste and aroma (Carmona, Zalacain, & Alonso, 2006; Tarantilis et al., 1994), and a method is already published that allows the evaluation of these three parameters though the determination of crocetin esters (colour), picrocrocin (taste) and safranal (aroma) by HPLC-DAD, using only a water solution (García-Rodríguez et al., 2014).

The aim of this work was to compare the ISO 3632 (2011) method and an HPLC-DAD method for safranal quantity determination in saffron. This comparison was undertaken to demonstrate that the data produced by specific HPLC-DAD analysis are more suitable for quality control of saffron.

2. Materials and methods

2.1. Saffron samples and reagents

Samples: A total of 390 samples from different countries were analysed in duplicate. The geographical distribution of the samples was: 115 samples from Greece, 154 from Iran, 57 samples from Italy and 64 samples from Spain. All samples have an origin certificate; although their certificate is not decisive for research purposes. Iranian samples have been supplied by different international saffron trade companies, which ensure that the samples were obtained directly from different saffron producers and also from different harvesting years. Although all Greek samples came from "Cooperative of saffron, Krokos Kozanis," they are obtained from different farmers. About half of the Spanish samples came from the P.D.O. "La Mancha Saffron," and the rest were supplied by the different Spanish saffron producers located mainly in the Castilla-La Mancha region. Italian samples came from various Sardinian producers.

Standards: safranal with a purity ⩾88% was obtained from Sigma-Aldrich (Madrid, Spain).

Solvents: Super-gradient HPLC grade acetonitrile was purchased from Scharlau (Barcelona, Spain), while water was purified through a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Saffron extract preparation

The saffron aqueous extracts were prepared according to ISO 3632 (2011). A total of 500 mg of powdered saffron, previously passed through a sieve of 0.5 mm pore diameter, was placed in a 1 L volumetric flask, and 900 mL of Milli-Q water was added. The solution was stirred using a magnetic stir bar at 1000 rpm for 1 h while being kept away from light. The flask was filled to the 1 L mark, and the solution was homogenized through agitation. The solution was filtered through a filter made of hydrophilic polytetrafluoroethylene (PTFE) with a pore size of 0.45 μm (Millipore, Bedford, MA, USA).

2.3. UV–vis analysis

Saffron extracts, after proper dilution (1:10, v/v), were monitored by scanning from 190 to 700 nm using a Perkin-Elmer Lambda 25 spectrophotometer (Norwalk, CT, USA) with UV WinLab 2.85.04 software (Perkin-Elmer). All of the analyses were performed in duplicate, and two measurements were taken for each replicate. For the safranal quantification, two series of safranal standard solutions in water with concentrations of 4, 2, 1, 0.5, and 0.25 mg/L were prepared and analysed in duplicate by UV–vis. A calibration curve was constructed for the safranal concentration, c (mg/L), as a function of its absorbance a, at 330 nm, with the equation; $c = 20.836a - 0.1023$ and R value = 0.999.

2.4. Moisture and volatile matter content

Determination of the moisture content and the volatile matter content of saffron was carried out according to ISO 3632-2 (2011).

2.5. HPLC-DAD analysis

Twenty microliters of each sample (saffron extracts) were injected into an Agilent 1200 HPLC chromatograph (Palo Alto, CA) equipped with a 150 mm × 4.6 mm i.d., 5 μm Phenomenex (Le Pecq Cedex, France) Luna C18 column that was equilibrated at 30 °C. The eluents were water (A) and acetonitrile (B) with the following gradient: 20% B, 0–5 min; 20–80% B, 5–15 min; and 80% B, 15–20 min. The flow rate was 0.8 mL/min. The DAD detector (Hewlett Packard, Waldbronn, Germany) was set at 250, 330, and 440 nm for picrocrocin, safranal and crocetin ester detection, respectively. All of the analyses were performed in duplicate, and two measurements were taken for each replicate. The identifications of crocetin esters were carried out using the UV–Vis spectrum, the retention time by the HPLC-DAD method at 440 nm and by the parameter %III/II (Mínguez Mosquera, 1997) of their standards. Picrocrocin identification was carried out by combination of its UV–Vis spectrum and its retention time by HPLC-DAD at 250 nm. For its quantification, picrocrocin was isolated as described by Sánchez, Carmona, Ordoudi, Tsimidou, and Alonso (2008). Safranal identification was carried out using the UV–Vis spectrum and the retention time of the safranal standard by HPLC-DAD at 330 nm. Their quantification was based on calibration curves (García-Rodríguez et al., 2014).

Table 1
Quality characteristics of the saffron samples according to ISO 3632 (2011) by origin.

Origin	ISO category	Moisture and volatile matter content (%)	Range of $E^{1\%}_{1\,cm}$ 440 nm	Range of $E^{1\%}_{1\,cm}$ 330 nm	Range of $E^{1\%}_{1\,cm}$ 257 nm
Greece (115)	I (105)	5.42–10.50	205.92–281.97	29.81–45.55	70.45–101.03
	II (7)	7.65–10.50	170.96–195.92	40.18–43.00	71.23–77.61
	III (3)	9.73–10.47	167.05–168.21	39.59–41.91	71.40–72.66
Iran (154)	I (81)	5.96–10.29	201.00–285.91	30.00–43.64	67.70–100.60
	II (46)	6.61–10.52	170.55–199.66	34.42–46.42	62.45–87.58
	III (27)	6.60–9.18	129.07–129.90	29.04–39.83	53.57–71.19
Italy (57)	I (55)	7.03–10.42	202.23–301.08	30.39–41.95	80.11–120.32
	II (2)	8.14–8.45	192.96–197.73	37.05–37.94	75.96–78.58
Spain (64)	I (60)	5.49–10.43	209.40–299.35	28.58–46.07	73.78–104.94
	II (2)	6.26–6.39	175.70–181.45	20.76–38.33	68.58–72.54
	III (2)	6.03–7.68	165.21–167.75	27.04–38.87	69.89–71.04