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A RAPID MALDI MS/MS BASED METHOD FOR ASSESSING SAFFRON (*Crocus sativus L.*) ADULTERATION

D. Aiello[1], C. Siciliano[2], F. Mazzotti[1], L. Di Donna[1], C.M. Athanassopoulos[3], A. Napoli[1]

[1]*Department of Chemistry and Chemical Technologies, University of Calabria, Italy*
[2]*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Italy*
[3]*Department of Chemistry, University of Patras, Patras, Greece*

Saffron, the red dried stigmas of *Crocus sativus* L., is the world's most expensive spice and thereby is considered within the major candidates for economically motivated fraud. Saffron authentication through established methodologies is a challenging task, as saffron of higher quality may intentionally be blended with plant-derived adulterants. The most frequently used adulterants are saffron stamens, safflower, calendula, turmeric rhizomes or dried gardenia fruits [1,2]. Fruits of *Gardenia jasminoides* Ellis represent a bio-adulterant which is difficult to detect by classical methods, because it contains crocins (C-1÷C-3) and flavonoids as does saffron itself. The quality of saffron and its commercial value are determined by specifications described within the ISO/TS-3632 standard that established spectrophotometric (for picrocrocin and safranal) and chromatographic (for crocins and polar dyes) measurements. According to the ISO/TS-3632 standard, the maximum mass fraction of foreign matter permitted in the third-class products is 1% (w/w). The standard UV–vis spectrophotometric method of ISO 3632-2 for grading saffron may not reveal saffron adulteration with amounts lower to 20% (w/w) of safflower, turmeric, or calendula [3]. Many analytical methods have been developed for authentication of saffron, including strategies based on the use of NMR and LC-MS [4]. Mass spectrometry is a powerful tool for the high-throughput detection and quantitation of metabolites, several studies have shown that MALDI (Matrix-assisted laser desorption ionization) can be used as an alternative to LC-ESI for the highly sensitive analysis of low molecular weight compounds in complex matrices [5]. This MS technique is extremely advantageous due to short analysis times, high sensitivity, tolerance to contaminants, and the ability to detect different components in highly complex mixtures. MALDI MS/MS provided quantitation of target compounds and small sets of analytes in a complex matrix with great sensitivity, dynamic range, and precision. The common workflow requires the construction of a calibration curve with standard solutions containing the same (fixed) amount of the stable isotope internal standard, and variable amounts of the single specific analyte of interest. This approach is suitable only when stable isotope internal standards are available for each analyte of interest, and the analyte concentration levels to be measured are known. Synthetic isotope labeled markers of saffron are not available, therefore, to overcome this drawback, we evaluated the use of whole extracts obtained from sets of standard sample (w/w), with the addition of a non-isotopic isobaric internal standard (IIS). The method reported is a sensitive and fast quantitative MALDI-MS/MS method used to assess saffron authenticity by direct

analysis through the determination of picrocrocin as the saffron authenticity marker, and using curcumin as the non-isotopic isobaric internal standard. The internal standard curcumin yielded good linearity (R^2 = 0.994), and with confidence intervals at 95% for intercept. The detectable maximum adulteration percentage (99.0%) was estimated interpolating the limit of detection (LOD) for the isobaric internal standard in linear regression. The LOD was 47.63 ppm, and LOQ was 56.53 ppm. The capability of the MS approach to monitor analytes in a specific, selective fashion was used to obtain a semi-quantitative adulteration percentage and to establish the adulterant by additional experiments. Finally, the detection of gardecin and its derivatives in commercial samples indicated that *Gardenia jasminoides* Ellis was used as adulterant.

References

[1] Carmona M., Zalacain A., Sanchez A.M., Novella J. L., Alonso G.L., J. Agric. Food, 2006, 54, 973.
[2] Petrakis E. A., Cagliani L. R., Polissiou M. G., Consonni R., Food Chem., 2015, 173, 890.
[3] Sabatino L., Scordino M., Gargano M., Belligno A., Traulo P., Gagliano G., Nat. Prod. Commun., 2011, 6, 1873.
[4] Guijarro-Díez M., Castro-Puyana M., Crego A. L., Marina M. L., Food Chem., 2017, 228, 403.
[5] Persike M., Zimmermann M., Klein J., Karas M., Anal. Chem., 2010, 82, 922–929.