

Authentication of saffron (*Crocus sativus* L.) using ^1H nuclear magnetic resonance (NMR) spectroscopy

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Saffron, the dried stigmata of the plant *Crocus sativus* L., is a spice used for colouring and flavouring food. It is considered to be the most expensive spice, which can be explained by the laborious way the stigmata have to be harvested. There is a considerable profit to be made by adulterating saffron, e.g. by mixing it with other saffron plant materials such as flower petals and styles or other colouring plants such as safflower or turmeric. Another way of adulteration is to use artificial colours to falsify deteriorated natural material or complete imitation using coloured paper. Fifteen saffron samples (mostly from internet trade) were analysed using ^1H nuclear magnetic resonance (NMR) spectroscopy for the saffron-specific colouring agents. Thirteen samples were found by NMR to consist of natural saffron material (as validated by microscopic analysis), but one sample was additionally (and illegally) coloured with tartrazine (E102). Two samples were complete frauds (coloured paper), and 8 samples had to be objected because of offences against mandatory food labelling requirements. NMR has been proven to be of higher versatility and specificity to detect saffron adulteration compared to traditionally applied techniques such as UV/VIS spectrophotometry or thin-layer chromatography. The nontargeted spectral “fingerprinting approach” is specifically advantageous to detect food fraud with previously unexpected substances. Due to the high quota of non-compliance, the importance to check saffron spices for authenticity must be stressed.

Introduction

Saffron is considered to be one of the oldest and the most expensive spice in the world and it is used for yellow-colouring but also for flavouring food including hot dishes and bakery products. Saffron consists of the dried stigmata of the plant *Crocus sativus* L. (Iridaceae). Its high price can be explained by the laborious way the stigma have to be harvested.

Saffron contains 0.4–1.3% essential oils with safranal (2,3-dihydro-2,2,6-trimethylbenzaldehyde) as major compound, which is also responsible for the typical flavour of saffron. Its bitter taste is caused by picrocrocin, a glucoside of safranal. The intensive colouring of saffron is caused by crocin, which is a water-soluble gentiobiose diester of crocetin (8,8'-diapocarotenedioic acid). Other colouring agents are α - and β -carotene, lycopene and zeaxanthin.¹

Due to its high price, there is a considerable profit to be made by adulterating saffron, e.g. by mixing it with other saffron plant materials (besides the stigmata) such as flower petals and styles, with old or deteriorated saffron materials or with other colouring plants such as safflower or turmeric. Adulterations with marigold leaves (*Calendula officinalis* L.), arnica (*Arnica montana* L.) or colouring wood (sandal wood fibres) were also described.² Another rather crude way of adulteration is to use artificial colours to falsify deteriorated natural material or by complete imitation using coloured paper and wood.^{3–5} For this reason, saffron must be authenticated in food control, e.g. by analysing saffron-specific compounds using nuclear magnetic resonance (NMR) spectroscopic techniques. NMR has been proven to be of higher versatility and specificity to detect saffron adulteration compared to traditionally applied techniques such as ultraviolet-visible (UV/VIS) spectrophotometry or

thin-layer chromatography. The nontargeted spectral “fingerprinting approach” is specifically advantageous to detect food fraud with previously unexpected substances.⁶ As suggested in several previous studies^{3–5}, NMR is a highly appropriate technique for saffron authentication. Based on these literature methods, we have developed our measurement protocol and evaluated authentic samples from official food control.

Materials and methods

All NMR measurements were performed on a Bruker Ascend 400 spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a 5-mm broadband observe (BBO) probe with Z-gradient coils. For NMR measurement, 10 mg saffron was mixed with 1 mL methanol- d_4 and placed on a shaker (300 U/min) for 8 minutes. Afterwards, the saffron solution was filtrated and 600 μL were added to a NMR tube. The same procedure was applied to crocin and safranal standards. Additionally to conventional 1D ^1H experiments, J-resolved experiments were also conducted. NMR pulse programs and measurement procedures were similar to our methodology for measurement of lovastatin in red rice products.⁷ Fifteen saffron samples (mostly from internet trade) presented to the Chemical and Veterinary Investigation Agency (CVUA) Karlsruhe, Germany, in 2015 were analysed using ^1H NMR for the saffron-specific colouring agents, but also using nontargeted principal component analysis (PCA). PCA was performed using the Unscrambler X software (CAMO Software AS, Oslo, Norway). Bucketing of spectra data was done with Amix 3.9.14 (Bruker BioSpin, Rheinstetten, Germany) with a 0.01 ppm width as previously described for alcoholic beverages⁸.

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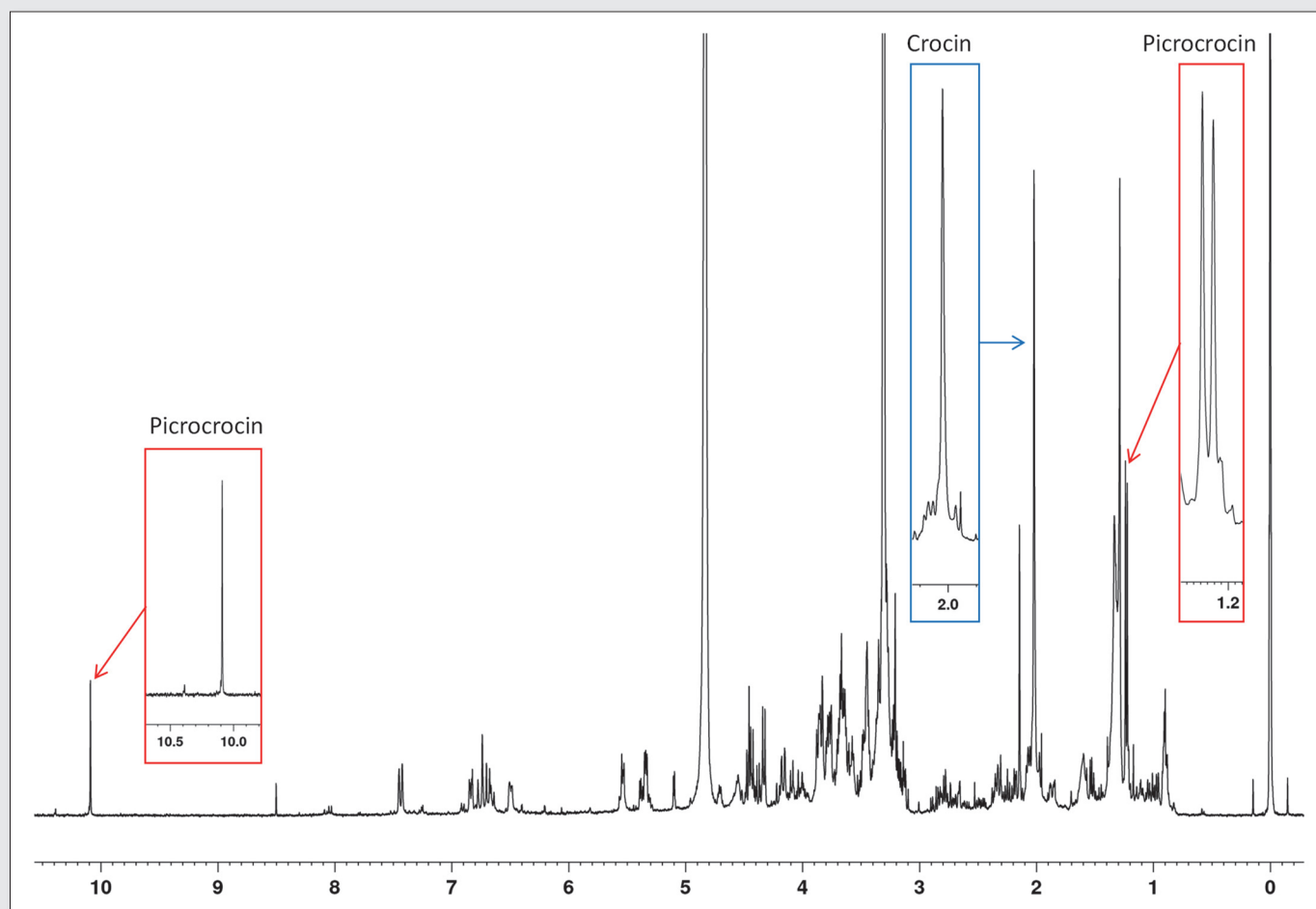


Figure 1. ^1H NMR spectrum of an authentic saffron sample with marked and magnified characteristic resonances of picrocrocin and crocin

Results

The complete NMR spectrum of a saffron sample is shown in Figure 1. The saffron signals can be seen at δ 10.09 ppm, δ 7.44 ppm, δ 2.14 ppm, δ 2.02 ppm and δ 1.23 ppm, some other peaks can be found in the spectrum as well. Most likely these are fatty acids and saccharides from saffron.

Thirteen samples were found by NMR to consist of natural saffron material (as validated by microscopic analysis), but one sample was additionally (and illegally) coloured with tartrazine (E102) as confirmed by high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC).^{9,10} The saffron-specific resonances in this sample (Figure 2) were of much lesser intensity than in the other authentic saffron samples.

Two samples, which were privately submitted after having been bought from a bazar in Egypt, were complete frauds (coloured paper) showing no saffron-specific NMR resonances at all (Figure 2). HPLC detected brilliant crocein (CI 27290), tartrazine (E102) and ponceau 4 R (E124) in these two samples. Eight samples were non-compliant because of offences against food labelling requirements.

The PCA analysis of all spectra shown in Figure 3 confirmed the analyses of univariate spectra evaluation. The two counterfeited samples can be easily distinguished. However, the one sample of natural material coloured with E102 was very near the grouping of authentic samples.

Discussion

Based on the evaluation of NMR signals, we can postulate that NMR is a suitable method to easily and quickly distinguish between saffron samples and fraudulent material. The qualitative differentiation between genuine, pure saffron samples and adulterated material is easily possible by comparing signal intensity of the saffron-specific compounds. This can be achieved by visual investigation of the sample spectra, because the resonances are baseline-separated and matrix interferences were not observed in any of the cases. Multivariate data analysis (such as principal component analysis) is possible but was unable to uncover any additional information in this case. Nevertheless, we believe that the nontargeted PCA might be suitable for automated analyses of larger sample series as well as to uncover completely counterfeited samples. Both approaches – the targeted or nontargeted evaluation of NMR spectra – might also be suitable in an industrial context, e.g. for the confirmation of authenticity in quality control of incoming goods or for the importers of spices. Due to the complexity of the NMR technique this would, however, rather be conducted at central industrial laboratories than at each plant. Due to the prevalence of saffron for food fraud, the incoming goods should be blocked until quality inspection is complete. For example, this could be managed within the hazard analysis critical control point (HACCP) system. Counterfeited saffron can be well seen as hazard for the consumer, e.g. due to allergies from

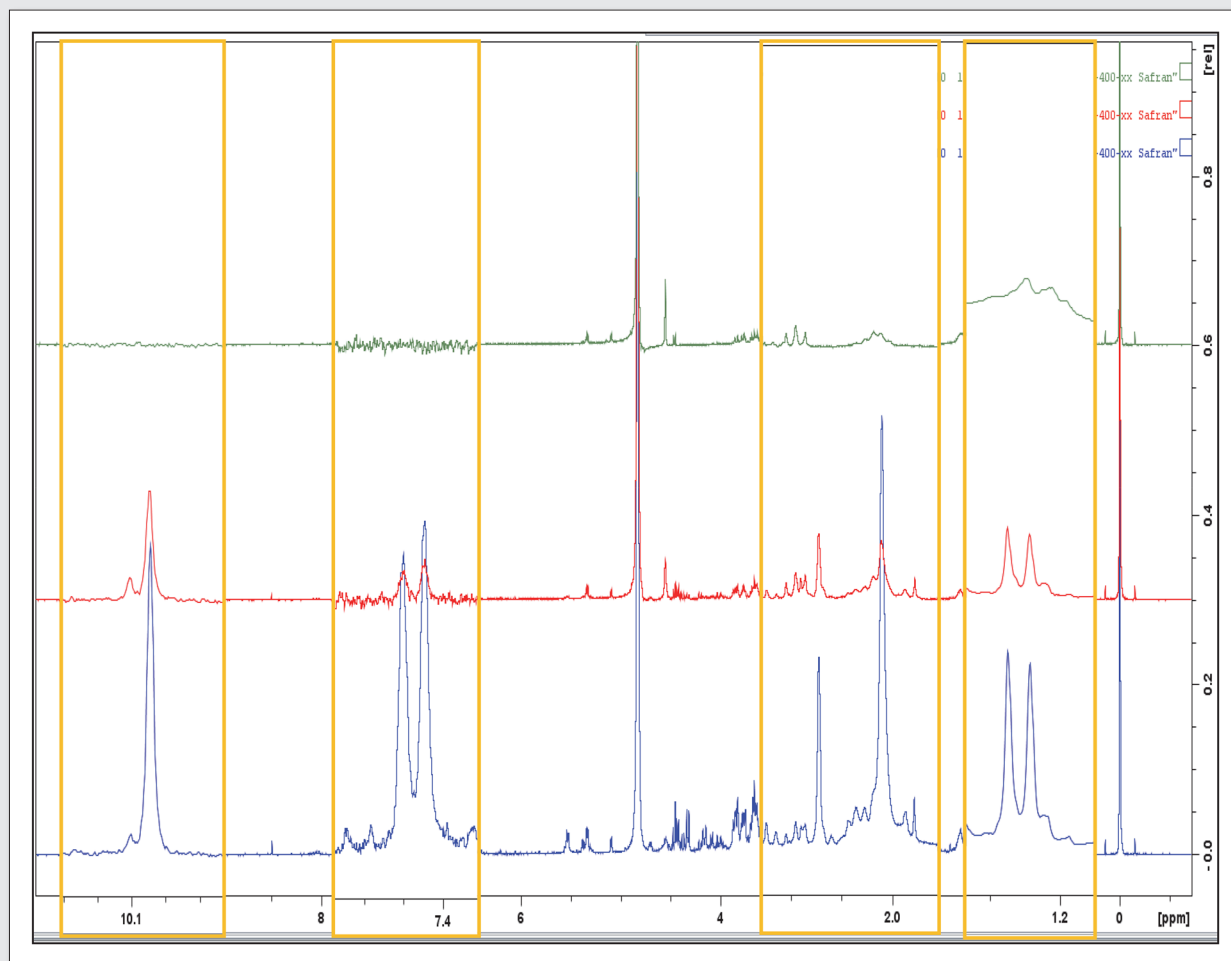


Figure 2. Magnification of saffron-specific signals in several samples (blue line: authentic sample; red line: adulterated sample (low concentration of saffron-specific compounds); green line: completely fraudulent sample, no saffron signals detectable)

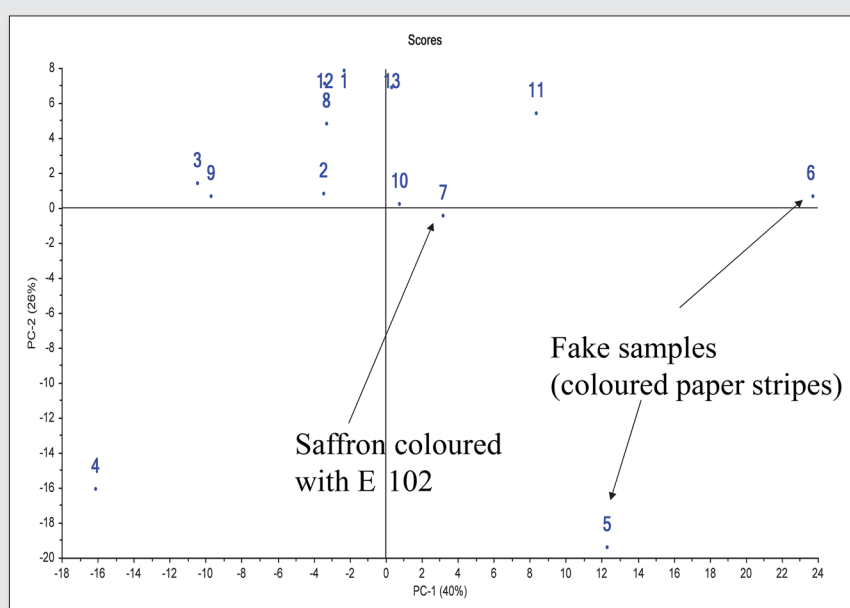


Figure 3. Principal component analysis (PCA) of saffron NMR spectra. The two completely faked samples (samples 5 & 6) can be directly distinguished by extreme values on PC1. If the content of natural saffron constituents is low such as in sample 7, further analyses such as for artificial colours may be necessary. The deviation of sample 4, which contained the typical resonances of saffron in univariate analysis, could not be established.

non-labelled colours or from non-labelled plant materials (such as safflower).

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