

REVIEW

Advances in adulteration and authenticity testing of turmeric (*Curcuma longa* L.)

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Abstract

Synthetic colorants such as metanil yellow, lead chromate, Acid orange 7, Sudan Red; rhizomes of related *Curcuma* sp. besides spent turmeric, starch, chalk and yellow soapstone are the main adulterants in traded turmeric while synthetic curcumin is an adulterant of natural curcumin. Both branded products as well as the produce from the unorganized sector are found adulterated. The adulterants, added either to increase the bulk, improve the colour and appearance or enhance the profit margin, often result in corroding the biological efficacy of the commodity and eroding the public impression besides posing health risks to the consumers. Various physical, chemical and PCR based methods are available to detect the adulterants in traded turmeric. While chemical methods are suited to detect the synthetic adulterants and spent turmeric, DNA based methods are the best options for detecting the biological adulterants (except spent turmeric) in the commodity. Along with adopting a supply chain system and quality linked pricing in turmeric trade, commercial adulteration diagnostic kits, if they can be developed and deployed, will be a very convenient way to ensure the quality of the traded produce.

Keywords: adulterants, detection, food safety, methods, supply chain, turmeric

Introduction

Spices are high value, export-oriented commodities and are extensively used for flavouring food and beverages as well as in medicine, cosmetics and perfumery. Traded forms of spices include dried or fresh whole commodity, powdered forms, pastes, dehydrated material, oils, oleoresin and extractives. Good quality spices are very relevant for the perceived biological efficiency of these commodities, their flavour or aroma.

The health-conscious public all over the world is increasingly looking for quality spices, be it for health, culinary or cosmetic uses. However, spices are often adulterated with inferior, similar-looking entities leading to erosion of the perceived biological value and public faith in these products.

Turmeric [*Curcuma longa* L. (Zingiberaceae)], already well known as a spice, a colouring agent for food, and cosmetic, is becoming increasingly important as a medicinal herb for its anti-

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inflammatory, anti-cancerous, anti-oxidant, antimicrobial, and anti-viral properties; as an antiseptic; and in the treatment of diabetes and Alzheimer's disease (Sasikumar 2005; Bejar 2018). Turmeric has a history of 5000 years as a herb in folk medicine as well as in Indian and Chinese systems of medicine. India is the largest producer, consumer, and exporter of turmeric, which is traded mainly in the form of whole-dried rhizomes, as powder and as valued-added forms. In the powder form, which is mainly used in commerce and by the food, cosmetic and pharmaceutical industries, turmeric consists of particles approximately 0.2–0.25 mm in size (60–80 mesh). The powdered form has the highest share in exports, constituting about 42% of the world trade in turmeric. Many branded turmeric powders, besides the produce from the unorganized sector, are available in India and these products constitute the bulk of the domestic consumption of turmeric.

Recent reports on the medicinal value of turmeric in treating a variety of ailments have further increased the demand for turmeric all over the world. Major importers of turmeric powder are USA, UAE, Saudi Arabia, UK, Australia, and Canada. Consumer preference for natural /organic products has also spurred the demand for turmeric. Unfortunately, as common to other powdered spices, turmeric powder too is being adulterated, with filler materials, synthetic dyes, inert or biological entities, that go visually undetected while synthetic materials are the sole adulterants of the whole commodity (Singhal *et al.* 1997; Dhanya & Sasikumar 2010) and the natural curcumin with the synthetic product. These adulterants/extraneous matters besides adding bulk and increasing appearance, result in diluting the main product and thereby making it less effective, which, in turn, erodes consumer confidence besides posing health hazards (synthetic colorants).

The Bureau of Indian Standards suggests a minimum of 3% curcumin for powdered turmeric, whereas the mandatory Prevention of Food Adulteration (PFA) Act of 1954 does not specify any minimum curcumin limit (Dixit *et al.*

2009). Despite the regulations in place in India, the quality of turmeric products in the Indian market is highly variable owing to a variety of reasons such as genotype, location and cultural practices (Sasikumar 2001). Adulteration is another reason for the variation in curcumin content of traded turmeric powder (Ali *et al.* 2019).

World organizations like the International Organization for Standardization (ISO), American Spice Trade Association (ASTA), The Food Safety and Standards Authority, India (FSSAI), impose strict regulations on the quality of spices and herbs imported and exported. The globalization of food trade requires the development of integrated approaches, such as traceability of origin, quality and authenticity to ensure food safety and quality (Barbuto *et al.* 2010). In the post-WTO era, importing countries, as well as the consumers, pay more and more attention to food quality, demanding clearer product traceability as well as the use of detailed and accurate product labels.

Adoption of a supply chain system in turmeric trade and quality linked pricing coupled with developing and deploying easy and fast adulteration detection kits are sure shots to ensure the quality of the traded turmeric.

Adulterants in turmeric

Adulteration may be defined as mixing or substituting the original material with other spurious, inferior, defective, spoiled, useless parts of the same or different plant, harmful substances or synthetic chemicals which do not conform with official standards. Adulteration can be in two ways- direct/intentional adulteration and indirect/unintentional adulteration. Direct/intentional adulteration includes practices of substitution partially or fully with inferior materials owing to their morphological resemblance or chemicals or inert materials in order to attain financial gain. Unintentional adulteration results mainly from the absence of a proper evaluation method (Preethi *et al.* 2014; Bharathi *et al.* 2018) and clerical errors (Zhao

et al. 2006). Though adulterants in turmeric are reported since the 1970s, adulterant detection in commercial turmeric products is of recent origin (Salmén *et al.* 1987; Sasikumar *et al.* 2004). The common adulterants in traded turmeric are given in Table 1.

Techniques for adulterant detection

Many techniques have been developed to detect adulteration in turmeric owing to the increased consumer awareness on food safety and quality control.

Physical methods

The physical methods involved microscopic observation and other parameters such as solubility, bulk density, texture etc.

Microscopic analysis

Details on the microscopic features of turmeric rhizome and other *Curcuma* species such as *C. aromatica*, *C. xanthorrhiza*, and *C. zedoaria* have been reported (Upton *et al.* 2011; Tandon *et al.* 2008; Eschrich 1999). However, the microscopic methods, in general, suffer from subjectivity, phenological variation, expressivity, lack of distinguishing markers, low throughput etc.

Analytical methods

Analytical techniques mainly use the chemical composition or organic components present

in the plant for their identification and authentication. Depending on this basic principle, the techniques can be grouped into different types.

Chromatographic techniques

Thin-layer chromatography (TLC) is the simplest, most versatile and economical way of obtaining the chemical fingerprints of multiple herbal samples. Sen *et al.* (1974) described a method to detect the adulteration of *Curcuma longa* with *C. zedoaria* and *C. aromatica* that involves a three-step colour sequence for the detection of camphor and camphene, the active principles of these adulterants, which are absent in turmeric. Raghuvver *et al.* (1979) reported a thin layer-gas chromatographic method to detect *C. aromatica* admixture with common turmeric (*C. longa*). More recently, the HPTLC Association published a method to distinguish *C. longa* and *C. xanthorrhiza* (Anonymous 2017). The same method was earlier used to detect the adulteration of turmeric with *C. aromatica* (Booker *et al.* 2014).

Dixit *et al.* (2008) reported turmeric adulteration with synthetic dyes and detected the presence of organic dyes, such as metanil yellow (1.5–4.6 mg g⁻¹), Sudan I (4.8–12.1 mg g⁻¹), and Sudan IV (0.9–2 mg g⁻¹) in loose turmeric and chilli samples from city markets across India. The curcumin content in turmeric and mixed curry powder samples ranged from 6.5 to 36.4 mg g⁻¹ and from 0.3 to 1.9 mg g⁻¹, respectively. In a more detailed study by the same group, 712

Table 1. Common adulterants in traded turmeric/curcumin

| Commodity | Synthetic/chemical and non chemical adulterant | Biological adulterant |
|-----------------------|---|--|
| Turmeric whole/powder | Metanil yellow Lead chromate Acid orange Sudan Red G Aniline Yellow soap stone Chalk powder | Wild <i>Curcuma</i> sp. (<i>C. zedoaria</i> or <i>C. malabarica</i> , <i>C. aromatica</i>) Starch from cheaper source Sawdust Spent turmeric powder |
| Curcumin | Synthetic curcumin | - |

commercial samples in India were tested using a two-dimensional high-performance thin-layer chromatography (HPTLC) method. None of the branded samples (N=100) showed the presence of artificial color, but 105 (17.2%) of the non-branded samples (N=612) of turmeric powders were dyed with metanil yellow (Dixit *et al.* 2009). Jaiswal *et al.* (2016) analysed 15 turmeric samples for synthetic adulterants by TLC and found that 10 out of 15 turmeric samples collected from Allahabad (now Prayag) were adulterated with metanil yellow, Sudan III and artificial colour.

A detailed study on the quality of 39 commercial turmeric samples for food, dietary supplement and cosmetic uses sold in supermarkets and retail stores in the United Kingdom (27), India (8), the Netherlands (2), Iceland (1), and Greenland (1) labeled to contain *C. longa* (34), *C. amada* (1), *C. aromatica* (2), *C. xanthorrhiza* (1), and *C. kwangsiensis* (1) by HPTLC showed that three products did not contain turmeric, one turmeric product was adulterated with *C. aromatica*, and one product from India contained merely curcumin, with little to no demethoxy- and bisdemethoxy curcumin (Booker *et al.* 2014).

Gas chromatography can also be used to detect the presence of other *Curcuma* sp. in turmeric as many commercial turmeric dietary supplements contain essential oil in addition to the curcuminoids. There are substantial differences in the composition of the sesquiterpene fractions and lower amounts or absence of turmerones in some of the adulterating species (Raghuvver *et al.* 1979; Sasikumar 2005).

A number of HPLC methods have been used for the detection and estimation of curcuminoids as a tool for the evaluation of the quality of commercial ingredients and products. The methods include a variety of detection systems (UV, diode array, mass spectrometric, and fluorescence) and chromatographic techniques (HPLC, GC, CE) (Hong *et al.* 2017; Mudge *et al.* 2016; Rohman 2012; Lee & Choung 2011). For typical turmeric extracts, HPLC chromatograms showing a characteristic fingerprint of the three curcuminoids in a

consistent ratio (~77% curcumin (Curcumin I) ~17% demethoxycurcumin and ~3% bisdemethoxy curcumin) has been, for many years, the approach to determine the product identity and quality (Li *et al.* 2011; Rohman 2012; Lee & Choung 2011; Wichitnithad *et al.* 2009; Jayaprakasha *et al.* 2002). Bisdemethoxy curcumin is reportedly absent in *C. aromatica* and *C. xanthorrhiza*, allowing for a distinction from *C. longa* based on this compound (Booker *et al.* 2014; Anonymous 2017). *Curcuma zedoaria* has demethoxy curcumin as the main curcuminoid, contrary to *C. longa* where curcumin I is the most abundant curcuminoid (Avula *et al.* 2012; Thomas *et al.* 2011; Paramapojn *et al.* 2009). However, adulteration detection based solely on the curcuminoid profile may not be appropriate due to varietal, location and seasonal variations besides the solvent used in the curcuminoid profiling (Li *et al.* 2011).

Spectroscopic analysis and chemo metrics

Spectroscopy, the study of the interaction between electromagnetic radiation and matter, includes techniques like UV, visible, mid or near infrared (MIR, NIR), Raman, fluorescence, and nuclear magnetic resonance (NMR) that allow non-destructive testing and the use of small samples to achieve identification (Meuren 2010; Bharathi *et al.* 2018). Tiwari *et al.* (2013) using Laser-Induced Breakdown Spectroscopy (LIBS) analysed four commercial samples of whole dried rhizomes of turmeric collected randomly from four different areas of the spice market of Allahabad (Prayag), India, for possible adulteration. The analysis demonstrated that one of the four samples had spectral signatures corresponding to lead (Pb) and chromium (Cr), suggesting they might contain lead chromate as an adulterant providing color to make them more attractive to consumers.

¹H NMR spectroscopy-metabolomics has been used to identify *Curcuma* species and authenticate turmeric samples. Using this method it was possible to differentiate *C. longa* from *C. aromatica* and *C. xanthorrhiza* based on Principal Component Analysis (PCA). A

contribution plot also allowed determination of the main curcuminoid differences among the *Curcuma* species (the absence of bisdemethoxy curcumin in *C. aromatica* and *C. xanthorrhiza* being one of the main distinguishing traits) and among *C. longa* extracts made with different solvents (Booker *et al.* 2014).

Fourier Transform-Raman (FT-Raman) and Fourier (FT-IR) spectroscopy are very useful in detecting metanil yellow in turmeric powder. Dhakal *et al.* (2016) demonstrated the application of FT-Raman and FT-IR spectroscopy in detecting metanil yellow in turmeric. These authors utilized Fourier Transform-Raman (FT-Raman) and Fourier Transform-Infrared (FT-IR) spectroscopy as separate but complementary methods for detecting metanil yellow adulteration of turmeric powder. Simulated samples of turmeric powder and metanil yellow were prepared at concentrations of 30%, 25%, 20%, 15%, 10%, 5%, 1%, and 0.01% (w/w). FT-Raman and FT-IR spectra were acquired for these mixtures as well as for pure samples of turmeric powder and metanil yellow. Spectral analysis showed that the FT-IR method could detect the metanil yellow at 5% concentration, while the FT-Raman method appeared to be more sensitive and could detect the metanil yellow at 1% concentration. Relationships between metanil yellow spectral peak intensities and metanil yellow concentration were established using representative peaks at FT-Raman 1406 cm^{-1} and FT-IR 1140 cm^{-1} with correlation coefficients of 0.93 and 0.95, respectively. The potential of a 1064 nm Raman chemical imaging system for the identification of azo color contamination in turmeric and curry powders were further studied by this group (Dhakal *et al.* 2018). Metanil yellow and Sudan-I, both azo compounds, were mixed separately with store-bought turmeric and curry powder at the concentration ranging from 1% to 10% (w/w). Each mixture sample was packed in a shallow nickel-plated sample container (25 mm \times 25 mm \times 1 mm). One Raman chemical image of each sample was acquired across the 25 mm \times 25 mm surface area using a 0.25 mm step size. A threshold value was applied to the

spectral images of metanil yellow mixtures (at 1147 cm^{-1}) and Sudan-I mixtures (at 1593 cm^{-1}) to obtain binary detection images by converting adulterant pixels into white pixels and spice powder pixels into the black (background) pixels. The detected number of pixels of each contaminant is linearly correlated with the sample's concentration ($R^2 = 0.99$). This study demonstrates the 1064 nm Raman chemical imaging system as a potential tool for food safety and quality evaluation.

The use of HPLC-MS provides even lower sensitivity with a limit of detection of as little as 100 pg mL^{-1} metanil yellow in turmeric powder (Feng *et al.* 2011). Fourier Transform Near-Infrared (FT-NIR) spectroscopy coupled with chemometrics was also used to detect corn starch illegally added to turmeric powder, using simulated samples (Kar *et al.* 2019a). In this work, the pure turmeric powders were blended with corn starch to generate different concentrations (1-30%) (w/w) of starch-adulterated turmeric samples. The reflectance spectra of a total of 224 samples were taken by FT-NIR spectroscopy. The exploratory data analysis was done by Principal Component Analysis (PCA). The starch related peaks were selected by Variable Importance in Projection (VIP) method and were explored by examination of original reflectance spectra, 1st derivative spectra, PCA loadings and β coefficients plot of the Partial Least Square Regression (PLSR) model. The coefficient of determination (R^2) and root-mean-square error of partial least square regression (PLSR) models were found to be 0.91-0.99 and 0.23-1.3%, respectively, depending on the pre-processing techniques of spectral data. The Figure Of Merit (FOM) of the model was found with the help of the Net Analyte Signal (NAS) theory. These authors recently estimated the potential of Near-Infrared (NIR) spectroscopy coupled with chemometrics as a rapid and non-destructive tool for the detection as well as quantification of Sudan dye I adulterated turmeric powder using simulated samples. The concentrations of the adulterants were 0.05%, 0.1%, 0.2%, 0.5%, 1%, 1.5%, 2%, 5%, 10%, 15%, 20%, 25% and 30% (w/w), respectively. Exploratory data analysis

was done for the visualization of the adulterant classes by Principal Component Analysis (PCA). In the classification approach, Principal Components (PCs) extracted by PCA were fed as the inputs of the Support Vector Machine (SVM) classifier. The average accuracy of the adulterants classes noted was greater than 90% (Kar *et al.* 2019b).

Using Atomic Absorption Spectroscopy (AAS), Quratey & Kwarkey (2018) reported the highest level of chromium in turmeric samples amongst ten spices collected from the Ghana market. Turmeric recorded the highest mean Cr concentration (0.42 ± 0.03 mg kg⁻¹). Nallappan *et al.* (2013) used terahertz spectroscopy, a non-intrusive method, to effectively identify adulteration of turmeric with chalk powder in packed produce.

PCR based molecular methods

Polymerase Chain Reaction (PCR) has a high potential in biological adulterant detection and authentication of commodities due to its simplicity, sensitivity, specificity as well as rapid analysis time and low cost (Sasikumar *et al.* 2016; Swetha *et al.* 2016; Dhanya & Sasikumar 2011; Mafra *et al.* 2008; Vidal *et al.* 2007).

PCR based adulteration detection in turmeric has been started by our group as early as 2004. Sasikumar *et al.* (2004), analysed three popular market samples of branded turmeric powder from the Indian market using Random Amplified Polymorphic DNA [RAPD] analysis and revealed the presence of *C. zedoaria* in the samples though the curcumin levels of the samples met the quality standards. Dhanya *et al.* (2011) developed RAPD based Sequence Characterized Amplified Region (SCAR) markers to detect plant-based adulterants in traded turmeric. Six samples of branded turmeric powder procured from a local market at Calicut (Kozhikode), Kerala, India were analyzed using the two SCAR markers and both markers detected the presence of adulteration with *C. zedoaria* or *C. malabarica* in four out of six market samples and in simulated mixtures, i.e.,

samples of turmeric powder and the adulterants made at different concentrations. Parvathy *et al.* (2015) successfully used the DNA barcoding locus *ITS* to detect the plant-based adulterants in commercial samples of branded turmeric powder. Though band level discrimination of the adulterants and the genuine sample was not possible, single nucleotide polymorphisms (SNPs) related to the adulterants and genuine product was observed (Table 2). One out of the 10 samples analysed was found adulterated with *C. zedoaria*.

Radiotracer techniques

After the first report of adulteration of natural curcumin with synthetic curcumin in 2011, research on using radiocarbon dating techniques to analyze curcumin products on the market to determine the percentage that contained synthetic versus natural curcumin, or a combination of both gained momentum (Rafi 2016; Watson 2011; Krishnakumar & Sanandakumar 2011). The ¹⁴C testing of five commercial samples of curcumin showed that four of the materials contained curcumin that was 32-45% synthetic, while the fifth sample was 100% natural (Press release –Sabinsa, 2015). Using the same testing approach other commercial samples too were analysed for synthetic curcumin (Anonymous 2017).

Miscellaneous techniques

Sen *et al.* (2017) developed physical and chemical methods to detect yellow lead salt chalk, metanil yellow, anilue dye and starch in turmeric powder.

Future perspectives and conclusion

Turmeric powder and turmeric extracts are valued both for their medicinal properties and as a culinary spice. Turmeric-based dietary supplements (which also include standardized extracts with high concentrations of curcumin) have seen a steady increase in popularity globally. In the United States, the largest market for turmeric supplements, sales have almost

Table 2. SNPs that discriminate between *C. longa* and *C. zedoaria*

| Species | Position of SNP and nucleotide substituted | | | |
|--------------------|--|-----|-----|-----|
| | 293 | 388 | 410 | 439 |
| <i>C. longa</i> | G | G | G | G |
| <i>C. zedoaria</i> | A | A | T | C |

Source: Parvathy *et al.* (2015)

tripled from 2013 to 2016, totaling over US \$69 million in 2016. Unfortunately this high value, low volume commodity, has been subjected to deliberate, economically-motivated adulteration leading to reduced perceived biological value and posing health risks besides eroding public faith. Adulteration is also a major economic fraud involving public health. Reliable, easy, sensitive and high throughput traceability and authentication methods coupled with quality standards thus assume significance. Adoption of a supply chain practice in turmeric trade and quality linked pricing in addition to commercial adulteration diagnostic kits, if they can be developed and deployed, will be a sure shot to ensure the quality of the traded produce.

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